LUNG HOST DEFENSES IN THE SURGICAL PATIENT: THE ROLE OF THE ALVEOLAR MACROPHAGE

Submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

Dr. Hani A. Fouad Shenouda,
Department of Surgery,
Division of Surgical Research,
McGill University, Montreal,
July, 1983

Alveolar macrophage function in the surgical patient

TO MY FATHER,

WHO HAS TAUGHT ME MOST.

PREFACE

Three years ago, I had commenced work on this thesis. The experiments included in this research were carried out in the McGill University Surgical Clinic at the Montreal General Hospital.

The area of research in this thesis evolved around the efficiency of the alveolar macrophages as primary antibacterial defenses. A variety of experiments were performed to test such an immune mechanism.

The preliminary results on the effect of splenectomy and splenic auto-implantation on alveolar macrophages were presented at the 15th Annual meeting of the Association of Academic Surgery (Chicago, Ill., Nov. 1981). The complete work was later presented at the American College of Surgeons, Committee on Trauma, 62nd Annual meeting (Washington, D.C., March 1982). At this latter meeting, during the residents' paper competition, the paper won an award. The full paper was published in the Journal of Trauma 23:7-12, 1983.

In June of 1982, the results of the study on pulmonary bacterial clearance and alveolar macrophage function in septic shock lung were presented at the Shock Society 5th Annual meeting (Stowe, Vermont, June 1982).

In October of 1982, the effects of starvation and refeeding on alveolar macrophage function was selected as one of the finalist papers to compete for the Cecile Lehman Mayer Award, and was presented at the 48th Annual Scientific Assembly of the American College of Chest Physicians.

The results of the work on the effect of atelectasis and re-expansion of the lung on alveolar macrophage function was presented at the 14th World Congress on Diseases of the Chest (Toronto, Ont., October 1982).

The results on the effect of Methylprednisolone on the lung host defenses was included in a study which was presented in the Transplantation Section of the 68th Annual Clinical Congress of the American College of Surgeons (Chicago, Ill., October 1982).

The paper was published in Surgical Forum 33:338, 1982 and was quoted in What's New in Surgery, both at the meeting and in the Bulletin of the American College of Surgeons (January 1983).

Dr. Ray Chiu, my project director's influence and input on this study and me is beyond acknowledgement. I consider myself one of the few fortunate to be taught and advised by him during the execution of this work. His experience as a mentor and researcher which he had kindly shared with me, has reshaped my surgical interest and led me to seek academic roots for my surgical practice. I will ever be indebted to him.

Dr. David Mulder formulated the basic plans for these studies and made possible the execution of this whole project. Over the last three years, these studies seemed like his own child. Even though his busy schedule kept him at times away, his direction and encouragement and overall advice certainly matured this work. I am thankful for his support.

Dr. A. Hope McArdle spent considerable time providing valuable advice on the performance of biochemical assays used in this study. Dr. Emil Skamene gave many helpful suggestions in relation to the experimental methods. I would like to thank both of them.

The technical assistance of Ms. Daniéle Richer who spent many hours with me during the performance of the neck aching phagocytic assays and Maureen Smith in providing excellent operating room facilities are deeply appreciated.

I would finally like to thank Mrs. Emma Lisi for the considerable amount of energy and time spent in preparing and typing this thesis.

Hani Shennib
16.5.83
Montreal, Que.

ABSTRACT

The basic mechanisms underlying susceptibility to lung infection in certain clinical states, frequently complicated with pulmonary infections, remains uncertain. In this study, a thorough review of pulmonary immune functions was undertaken. The role played by the alveolar macrophage in maintenance of immune competence was emphasized.

Alveolar macrophage functions were examined after establishing models of respiratory distress syndrome, lung atelectasis, starvation, administration of pharmacological doses of corticosteroids, and splenectomy; the latter condition recognized to predispose to pneumonia and overwhelming fatal bacteremia.

Broncho-alveolar lavage cells were analyzed and alveolar macrophages were examined with particular interest in their morphological and functional characters. Methods for studying their phagocytic and bactericidal activities were developed.

Alveolar macrophage dysfunction was identified in septic shock lungs and in atelectic lung segments. Mechanical expansion of the lungs and supply of oxygen resulted in functional recovery of these cells.

Splenectomy resulted in age, bacterial species and type specific alteration of alveolar macrophage phagocytic ability. Autogenous splenic peritoneal implantation reversed this macrophage dysfunction.

Starvation depressed alveolar macrophage phagocytic activity and refeeding was associated with a delay in their recovery. Finally, short-term high dose corticosteroids did not affect the macrophages up to a dose of 30 mg/kg body weight for 48 hours.

These studies will not only help understand the etiology of lung susceptibility to infection under similar clinical circumstances, but will suggest basic concepts in its prevention.

RÉSUMÉ

Les mécanismes de base prédisposant à l'infection pulmonaire dans certains états cliniques fréquemment compliqués par de tels infections demeurrent inconnus. Dans cette étude, une révision complète des fonctions immunitaires pulmonaire a été conduite. Le rôle joué par le macrophage alvéolaire dans la maintenance de cet état immunitaire a surtout été considéré.

Les fonctions du macrophage alvéolaire ont été étudiés après avoir établi des modèles pour le syndrome de détresse respiratorie, atélectasie pulmonaire, jeûne, administration de doses pharmacologigues de corticostéroides et splénectomie; cette dernière condition étant reconnue pour son association fréquente avec les pneumonies et bactériémies fatales.

Des cellules de lavage broncho-alvéolaire ont été étudiées et les macrophages alvéolaires furent examinés avec un intérêt particulier quant à leurs caractères morphologigues et fonctionnels. Des méthodes permettant d'étudier leurs activités phagocytiques et bactéricides furent développées.

Un malfonctionnement du macrophage alvéolaire a été identifié dans les poumons de choc septique et dans les segments pulmonaire atélectasiques. L'expansion mécanique de ces poumons, de même que l'apport d'oxygène résulta en le recouvrement fonctionnel des ces cellules.

Selon l'âge de l'individu, la splénectomie résultat en l'altération de l'abilité phagocytaire du macrophage, cette fonction étant spécifique pour chaque espèce et type de bactériés. L'implantation péritoneale de fragments splénique autogène renversa cette dis-fonction du macrophage.

Le jêune diminua l'activité phagocytique du macrophage alvéolaire et l'apport nutritionnel fut associé à un délai quant à leur récupération. Finallement, l'administration à court-terme de corticostéroides n'affecta pas les macrophages, et ce jusqu'a une dose de 30 mg/kg de poids corporel pour une période de 48 hrs.

Ces études ne vont pas seulement aider quant à la compréhension de l'étiologie de la susceptibilité pulmonaire à l'infection en des circonstances cliniques similaires, mais permettront d'envisager des concepts de base quant à sa prévention.

TABLE OF CONTENTS

CHAPTER III

THE EXPERIMENTS

CHAPTER I

LUNG SUSCEPTIBILITY TO INFECTION IN THE SURGICAL PATIENT

LUNG SUSCEPTIBILITY TO INFECTION IN THE SURGICAL PATIENT

There is no doubt that the mere admission of surgical patients to the hospital renders them susceptible to infection (155). Hospitals, frequently containing threatening reservoirs of pathogenic microbes, render its population at a higher risk of infection than that of the community at large.

At the Center for Disease Control, an adjusted rate of all types of nosocomial infections was 3.5% (304). The lower respiratory tract was found to be involved in 15% of nosocomial infections in 80 NNIS Hospitals (84). In another study with similar results (304), 53% of nosocomial lower respiratory tract infections were caused by aerobic gram-negative bacilli. The latter finding was again demonstrated in the study done at the St. Vincent Hospital and Medical Center (1975), where gram-negative bacilli such as Escherichia coli, Klebsiella pneumonia and Pseudomonas aeruginosa were the common pathogens in nosocomial respiratory tract infections (155).

In the intensive care units, the incidence of lower respiratory tract infection is even much higher. A detailed analysis of an intensive care unit experience with pneumonia at the Beth Israel Hospital in Boston showed that in a 30 month period, 158 episodes of pneumonia occurred among 711 patients admitted to the Intensive Care Unit, an incidence of 21.6% (472).

It is now doubted that nosocomial pneumonia is primarily a problem seen on the medical services. A report from the Center for Disease Control (1978) indicated that the rate of nosocomial pneumonias on the medical and surgical services were almost identical, that is, between 75 to 80 per 10,000 patient discharges (86). In another analysis by Wenzel et al from the University of Virginia, the percent (1.84) of patients who developed nosocomial pulmonary infections on their general surgical services exceeded that (0.96) of patients on the medical services (508). Perhaps the fact that a large number of surgical patients undergo surgery, and of those who do 15% will develop post-operative pulmonary complications (301), attributes largely in maintaining the incidence of infection in the hospitalized patients at such reported levels.

SOURCE AND CHANNEL OF INFECTION IN THE SURGICAL PATIENT

Inhaled Bacteria

The pulmonary alveolar surface with some 90 m² exposed to the environment is vulnerable to injury by all manner of airborn agents (60). The surgical patients by nature of their illness and its management are not infrequently exposed to otherwise unusual sources of infection; these add to the ordinary ways of exposure to pathogenic organism in the patients in general.

Oropharyngeal Inhabitants

It is believed that most bacterial pneumonias are usually due to the aspiration of micro-organisms that make up oropharyngeal flora (133). Perhaps the work of Johanson et al presents a most convincing evidence of this theory. Studying the relationship between colonization of the oropharynx and the development of pneumonia in his medical intensive care patients, they showed that 95 patients (45%) became colonized with gram-negative bacilli, and that 26 developed gram-negative pneumonia. Out of the latter, 22 were already colonized with the same organisms. The study demonstrated that 23% of colonized patients developed nosocomial respiratory tract infection in comparison to only 3% of the non-colonized patients (237).

Ventilatory Support Equipment

Severe outbreaks of gram-negative bacillary pneumonia associated with the use of contaminated nebulizer unequivocally demonstrates the importance of recognizing such source of infection. Epidemics of pneumonia caused by Klebsiella pneumoniae, Pseudomonas aeruginosa and serratia marcescens have been found to result from contamination of ultrasonic and reservoir nebulization equipment (185, 397, 402). Reinarz et al demonstrated a relationship between pathological evidence of necrotizing pneumonia at autopsy and the use of nebulizer therapy (390). Pierce and co-authors reported that aerosol reservoirs were frequently, extensively contaminated with aerobic gram-negative rods and that the routine rinsing of nebulizers with acetic acid had led to a decline in the incidence of necrotizing gram-negative bacillary pneumonia noticed at autopsy (366).

It is unfortunate that with the awareness of the hazards of extrinsic contamination of ventilatory support equipment errors and breaks in decontamination procedures persist.

Aspiration

Aspiration as a possible cause of bacterial availability in the lungs is evident by tracing the leakage of sensitive markers such as Evans blue and radio-isotope into the lower respiratory tract. Of interest is the finding that 69% of 61 patients with tracheostomies (79) and 40% of 100 patients with endotracheal tubes in place (35a) do aspirate; and perhaps more interesting is the observation by Huxley et al which shows that even normal volunteers (45%) aspirate while in a deep sleep (229).

Such prevalence of a leakage phenomenon when considered together with the fact that bacteria are not cultivable from the normal lungs (283), reflects the efficiency of the mechanisms by which clearance of the invading micro-organisms is performed. It appears that it is not the quality and quantity of the bacterial inoculum per se that determines the outcome of any aspiration. Factors related to the amount, composition and characters of the aspirate frequently determine the fate of the pathogen host interreaction (283).

From the microbiological point of view, bacterial pneumonias differ strikingly according to whether aspiration has occurred within or outside the hospital; the causative organism in each of the cases reflects the oropharyngeal inhabitants. While it is the streptococcus pneumonia which prevales as an inhabitant of the oropharynx in the community acquired pneumonia patients (526), it is the gram-negative bacilli which are frequently isolated and found to be responsible for nosocomial pneumonias (237).

Indeed, it seems that the high frequency of nosocomial pneumonias due to gram-negative bacilli is but a probable reflection of the increased prevalence of such organisms as oropharyngeal inhabitants in hospitalized patients.

In the community in general, about 25% only of normal persons harbor gram-negative bacilli in their oropharynx at any given time (237), while 40 to 70% have streptococcus pneumoniae (526). The alteration of oropharyngeal flora noticed when the patient is hospitalized, in itself, seems to be very much determined by the state of the patient himself. In an extensive analysis by Johanson and his co-workers, the prevalence of gram-negative bacilli in the oropharyngeal flora was found to correlate directly with the severity of the underlying illness, but no correlation was detected with the type of therapy, whether this was inhalation therapy, antibiotics or others, nor with the duration of stay of the patient in the hospital (237). In agreement with this, MacKowiak et al reported a significantly higher prevalence of gram-negative bacilli in the pharyngeal flora of alcoholics and diabetics compared with that of normal controls. In an attempt to identify the origin and mechanism of alteration in the bacterial flora of the respiratory tract observed in the hospitalized patients, Atherton and White were able to trace the colonization of the respiratory tract in intubated or tracheostomized patients to gastric overgrowth (> 10^5/ml) of these organisms when ileus was present (17).

Distant Focus

If a distant focus of infection exists, the risk of developing pneumonia is certainly present. This becomes more obvious when the distant infection is that of gram-negative bacilli and is associated with bacteremia, such as in E. coli pneumonia during pyelonephritis or pseudomonas pneumonia in patients with serious burn wound sepsis (133).

With the recognition of the strong link between sepsis and pulmonary failure, the remote effects of a distant infection, particularly abdominal sepsis, became obvious. Eisman et al studied 42 patients recognized as having multiple organ failure (having significant failure of two or more critical organ systems including lungs, kidneys, liver, blood clotting system and gastro-intestinal tract), bacterial sepsis was present in 29, all of which except 7 were of intra-abdominal location (135).

THE HOST DEFENSES IN THE SURGICAL PATIENT

TRAUMA

In the critically injured patient in spite of the progress in knowledge of biomedical, supportive and anti-microbial management, infection still stands as the resilient major factor, maintaining the survival rates in those patients who develop bacteremia at the status quo figures. The cause of late death in such cases is usually progressive organ failure (423).

Host resistance to sepsis in patients after severe multiple trauma, burn injury, or major surgical procedures is of great clinical importance. The deterioration of host resistance in these patients if not indirectly responsible for death, is a major etiological factor in the high morbidity encountered in such conditions.

Review of literature to this date suggests that following a sublethal shock, the reticuloendothelial system undergoes phasic changes characterized by an early phagocytic depression and subsequent period of recovery which is then followed by transient stimulation (11,12,250,421,422). However, when shock is severe and irreversible, there is a progressive deterioration of the reticuloendothelial system.

Altura et al demonstrated a statistical correlation between recovery of the reticuloendothelial system and the survival rates after acute hemorrhage (12). In his experiment, animals that failed to manifest an early tendency towards recovery of its reticuloendothelial functions progressively deteriorated and died.

Saba et al (317) demonstrated that patients who did not survive trauma had a persistent humoral opsonic defect. Those who restored their plasma opsonic $\alpha 2$ SB glycoprotein levels in a few days went into stabilization of their clinical condition and recovery, even though the extent of their injury at the onset was indistinguishable from that of the non-survivors. while impairment of the reticuloendothelial phagocytic activity

has been well observed with different types of trauma, additional deterioration of host defenses can further be expected by the occurrence of sepsis itself (423).

While much of the research done to explain the high susceptibility to infection in the traumatized patient is directed towards examining the systemic host defenses, little is known on the effect of trauma and sepsis on local cellular defenses of different tissues. The local cellular immune response of the lung in trauma and sepsis has been either neglected or arbitrarily presumed to be of a similar nature to that of the other non-specific cellular immune response.

Of the few studies in this area is that of Grogan et al in which sublethal shock induced by tumbling rabbits in a modified Noble Collip drum had a depressing effect on the bactericidal activity of the alveolar macrophages. However, this depression was transient and was not associated with a similar change in the phagocytic activity (188). When the same group studied the same effect on rats, an increase in both the phagocytic and bactericidal activity was noticed one hour following trauma followed by a gradual return to baseline activity in 24 hours. Another report by Gudewicz et al working on their favorite guinea pig model demonstrated an opposite effect of shock on the bactericidal activity of the alveolar macrophages (192). This discrepancy in the response of the alveolar macrophage to experimental trauma and shock may be explained by the variation in both the animal species and the experimental design used in their experiments.

Studying the effect of blunt trauma on the host defenses, Christou et al detected an abnormality of polymorphonuclear leukocyte chemotaxis and adherence within 2 hours of injury. The depression in the neutrophil chemotaxis was found to be due to circulating blood factors of molecular weights distributed in at least 5 compartments ranging from 8000 to > 400,000 daltons (90). In a previously dated study, Meakins

and co-workers used skin testing with 5 standard recall antigens to assess the effect of trauma on cellular immunity. They observed that those patients who responded to less than 2 antigens had a significantly higher rate of sepsis and mortality. When they examined the chemotactic function of the neutrophils, they found it to be significantly lower than normal in the severely traumatized patients and had returned to normal with recovery of their patients (317).

Since recruitment of polymorphonuclear leukocytes is essential to the lung host defense against invading pathogens, it seems logical that such an observation of depressed recruitment may play a role in increasing the risk of lung infection early in the post-traumatic period. It may also be suggested that a delay in the recovery of polymorphonuclear leukocyte function may potentiate the development of a progressive lung lesion, such as in the shock lung.

ADULT RESPIRATORY DISTRESS SYNDROME

The most severe impact of trauma and sepsis on the pulmonary system is its failure manifested clinically as the Adult Respiratory Distress Syndrome (ARDS). Fulton et al (157) emphasized the significance of sepsis in the etiology and progress of ARDS. Studying 44 patients who developed post-traumatic pulmonary failure, he noted that sepsis singled other factors such as shock or massive fluid therapy in remaining a strong correlate to such failure. He also reported that in 45% of those patients with post-traumatic pulmonary failure, the lung was the primary site of infection and in another 40%, lung infection was secondary to intra-abdominal sepsis. Finally, it was noted that the critical time where a septic apex was often reached was the third to fifth day after injury which was also the time that pulmonary failure frequently manifests. A significant correlation between post-traumatic pulmonary insufficiency and hemorrhagic shock in the absence of sepsis was never found (157).

Experimentally, using a cecal ligation model of intra-abdominal sepsis, Clowes et al produced pulmonary insufficiency in one of

their dogs (92).

Endorsing the importance of sepsis in its association with ARDS, Vito and his co-workers (490) demonstrated that sepsis was present in 78% of their post-traumatic pulmonary insufficiency patients, and Horovitz et al added that thoracic injury as well as sepsis were major factors in the cause of post-traumatic pulmonary insufficiency in their group of patients (215).

For a period of time, the increase in the lung water content secondary to a crystalloid load observed in the ARDS patients was thought to be responsible for its development. However, this was noted to be a transient phenomenon and in the absence of sepsis, no patient receiving massive fluid loads required assisted respiration for more than 4 days (157).

The effect of bacteremia on the lung vascular permeability was studied by Brigham and co-workers who developed an excellent model for studying permeability edema. The slow intravenous infusion of 10^5-10^9 Pseudomonas aeruginosa organisms suspended in 100-500 ml of saline over 30 to 90 minutes resulted in an increased pulmonary vascular permeability to protein in sheep. However, the influx of fluid and protein, a result of the infusion of such a dose of Pseudomonas aeruginosa, was associated with an increased clearance via lung lymphatics. They also noted that 2 of their infused sheep died of pulmonary edema and another which was killed at 6 hours after Pseudomonas infusion had distinctly elevated lung water contents (70). While the authors emphasized that their model was that of a reversible membrane leakage and because of the variation in the log of bacteria infused, it may be possible to speculate that those sheep which developed marked edema and died were the ones which had a larger dose of the bacterial infusion, resulting in an extensive lung membrane damage, the same defect observed in ARDS.

Recently, Crocker et al demonstrated that continuous infusion of Pseudomonas aeruginosa into pigs causes dose-dependent pulmonary failure (101,132). When a bacterial infusion identical to the one which induced pulmonary failure in pigs was infused into dogs, they were unable to produce a similar effect (100).

Since this finding was associated with a maximum retention of bacteria in the lungs of the pigs compared to minimal retention in the lungs of the dogs, they concluded that both lung clearance of blood borne organisms and bacteremia-induced pulmonary failure were host dependent, a conclusion which will simply continue the argument in the literature in searching for the right animal model.

However, the fact that gram-negative bacteremia may produce ARDS is evident by the clinical observation of Kaplan et al (251) who identified 20 cases of ARDS in 86 patients with gram-negative bacteremia.

While many investigators have repeatedly demonstrated that circulating bacteria are predominently (60 to 90%) cleared by the liver and spleen with minimal or no clearance by the lung (31, 204,309,405,406); Crocker et al observed a relatively constant low pulmonary clearance rate of 20% of circulating bacteria in the dogs and an initially higher pulmonary clearance (56 to 63%) followed by a decline to 35% clearance in pigs during a 6 hour period of Pseudomonas aeruginosa infusion. These findings indicate a species variation in the ability of the lungs to clear circulating bacteria.

Although it may be feasible to conclude that the clearance ability of the liver and spleen is a reflection of its macrophage phagocytic function (420), such an assumption could not be justified in the case of the lung which does not constitute a major part of the reticuloendothelial system and has a relatively smaller number of macrophages compared to the liver and spleen.

Due to the multiplicity of factors which determine the amount of clearance of a circulating particulate by the lung or any other organ, it is difficult to conclude that phagocytosis by the mononuclear cells is the sole clearing mechanism. Other determining factors may include the amount, nature and properties of the circulating particulate, the general state of the animal examined and the anatomic, hemodynamic and cellular characterization of each of the clearing organs (31,70,100,204,309,405,420,423).

The mechanism by which the bacterium damages the membranes and produces membrane leakage edema which may progress further to

result in respiratory insufficiency is not yet clear.

Complement-Induced Pulmonary Leukostasis

A cardinal histological feature of early ARDS is the plugging of pulmonary microvasculature with granulocytes (429). This event seemed to be associated with activation of the fifth complement, the later in itself occurring with severe trauma, gram-negative bacteremia, acute pancreatitis and other pathological states which may induce ARDS (231).

Recently, Jacob et al demonstrated that brief bolus infusion of $C5_a$ in awake sheep in which pulmonary lymph efflux was harvested rapidly produced proteinaceous pulmonary edema as well as pulmonary hypertension and hypoxemia (ARDS). When the animals were rendered agranulocytic, they withstood complement challenge without pulmonary edema developing, indicating the importance of the granulocytes in the mechanism of endothelial injury (231). The fact that such a combination of $C5_a$ and granulocytes would produce a cytotoxic effect was demonstrated in a culture of umbilical vein endothelial cells. $C5_a$ was demonstrated to work both by promoting granulocyte adherence and its stimulation to produce toxic oxygen radicles. The addition of oxygen detoxifying enzymes to the culture prevented $C5_a$ induced endothelial damage (427).

In human, prolonged circulation of $C5_a$ was detected in the plasma of more than 30 recently studied patients with ARDS (231). In 9 patients, circulating $C5_a$ appeared at least 6 hours prior to the clinical detection of pulmonary dysfunction (505).

Moreover, the consumption of opsonins in leukostasis may result in the crippling of the reticuloendothelial phagocytic ability, increasing the susceptibility of the patient to a variety of stresses. Saba postulated that if the reticuloendothelial system failure co-exists with an excessive elevation in the blood level of potentially phagocytizable particulate matter, a prolonged retention of such substances in the blood and an accentuated pulmonary and peripheral circulatory localization may result (423), thus adding to the burden induced by $C5_a$ activated leukocyte aggregate on the lung endothelium.

Pulmonary Edema

Because bacterial pneumonia often complicates pulmonary congestive states (174,316), lung edema fluid was thought to predispose the lung to infection by depressing its defense mechanisms and/or by providing a nourishing medium for bacterial proliferation (278).

Inducing pulmonary edema in mice with graded doses of purified α-naphthyl thiourea (ANTU), and in rats by surgical constriction of the abdominal aorta, LaForce and his co-workers demonstrated a diminished pulmonary clearance of radio-labelled Staphylococcus aureus 4 hours afters its aerosolization. This depression of lung bacterial clearance was found to be directly related to the amount of accumulating intra-pulmonary fluid. They were also able to demonstrate that the in vitro phagocytic activity of alveolar macrophages against Staphylococcus albus had decreased significantly in the pulmonary edematous animals (278).

However, Harris et al presented evidence that proteinaceous intra-alveolar fluid, as in that induced by α-naphthyl thiourea, does not impair clearance as previously thought, but in fact, may enhance the clearance of certain bacterial species (202).

Whether pulmonary sepsis proceeds or follows the onset of pulmonary edema seems to vary from one clinical condition to another. A proper understanding of the type and pathophysiologic progression of pulmonary edema is of most importance in identifying when and how alterations in lung immune responses occur if any.

There is no doubt that our understanding of the mechanisms of the development of pulmonary edema has broadened significantly in the last decade. In typical cardiogenic pulmonary edema, recruitment of the capillary bed as well as an increase in the connective forces result in an increase in flux per unit surface area (69, 142). In the non-cardiogenic type of pulmonary edema, impairment of the normal molecular sieving function of the endothelial membrane results in flooding of the interstitium with oncotic active proteins (67, 495).

Carlson et al (81) analysed the pulmonary edema fluid in 37 patients with fulminant pulmonary edema. He noted that in 22 of these patients multiple causes of edema were implicated in each case. These included shock, aspiration, bacteremia, near drowning, coagulopathy drug overdose and pulmonary embolism; left ventricular failure was also a contributing factor in some of these patients. The data also suggested that when a high content of protein is found in the edema fluid, the pulmonary microvascular membrane would probably be damaged and the edema should be expected to be more severe. Hence, permeability edema has been identified experimentally by an increase in the lung lymph flow or lung water content which should contain plasma proteins at concentrations higher than expected (70,495).

As the water capacitance of the lung always exceeds its normal water content, considerable increase in the extra-vascular lung water can occur before flooding of the alveoli would result in disturbance of respiratory gas exchange (467). During this period, detection of such edema would allow early and aggressive therapy before the appearance of significant clinical symptoms.

Gorin et al described a non-invasive method for estimating trans-vascular protein flux in the lungs of experimental animals and recently in normal man (270). This method is based on the measurement of the net trans-vascular flux of (^{113}In) transferrin in the lungs using an externally placed scintillation detection unit. Of interest in this study is the finding that the pulmonary trans-capillary escape rate was found to be two-fold greater than the trans-capillary escape rate for the vascular bed as a whole in 9 normal human volunteers, indicating a greater porosity of the exchanging vessels in the lung than that for the average microvessel in the body.

ASPIRATION PNEUMONIA

In 1946, Mendelson described a syndrome in which aspiration of stomach contents during obstetric anaesthesia resulted in acute respiratory failure (319). Although this syndrome carries a high mortality rate (15, 27), the extent of lung damage appears

to relate to the amount and the acidity of the aspirated fluid (27). Those who survive the period of respiratory failure are at an increased risk of subsequent pulmonary infection (15, 527). Shellito and Murphy (444) investigated the effect of experimental acid aspiration on lung cellular immune functions in rabbits. They found that the aspirating animals alveolar macrophage against yeast was not affected, and that their adherence to glass had decreased. This adherence defect was reversed by the administration of corticosteroids, an empirical treatment frequently used in the treatment of such clinical conditions. Although it is difficult to determine the clinical implications of these findings, they presented a good model for inducing experimental aspiration pneumonia.

THE DIABETIC PATIENT

The association between diabetes mellitus and infection remains part of the clinical lore (8) in spite of the recognition by a few critical reviewers that the presumed cause and effect relationship between diabetes and infection is difficult to support with objective and controlled observations (254). Regardless of the question of the susceptibility to infection, there is little doubt that the presence of an infection in the diabetic may have more deleterious consequences than if present in a non-diabetic (112).

The fact that 1 to 10% of the general population depending upon age may be diabetics (112) presents the surgeon with a persistent challenge of successfully preparing for and carrying out surgical procedures in these patients with minimal morbidity. In addition, the surgeon is frequently required to deal with their infections.

Phycomycosis is the only infection reasonably believed to have a higher incidence of occurrence in the diabetic patients. While a smaller but definite percentage (10-20%) of patients with the pulmonary and disseminated forms of this infection were found to have diabetes mellitus, 80% of those who have the rhinocerebral form had diabetes (477). An extensive body of investigations in the area of the host immune responses in the diabetic has yielded

valid information. However, these investigations share certain characteristics with the blind man's assessment of the elephant: What you find may depend upon which part you feel.

Currently, available evidence provides no support for a deficiency in the B-cell function intrinsic to the diabetic state (8,139).

Studies on circulating T-lymphocytes revealed contradictory results; while MacCuish et al found no difference between the percentage of T-lymphocytes in the blood of normals and diabetics (303). Eliashiv et al demonstrated a significantly lower percentage in diabetics compared to non-diabetic controls, being 34% and 71% respectively (139). Back in 1933, Moen and Reimann examining the delayed type hypersensitivity function of the cellular immunity found that 44% of 65 patients with diabetes had a positive skin test of a 1:1000 dilution of old tuberculin compared to 24% of 65 control non-diabetic patients (328). On the other hand, a report from Mahmoud et al shows a poor cellular response as evident by a diminished pulmonary granuloma formation in response to Schistosomal ova injection in diabetic mice (305).

Berken and Sherman studied the in vivo ability of the reticulo-endothelial system to clear circulating radio-iodinated micro-aggregated albumin in normal and diabetic patients. They found no difference in the uptake between both groups (34). Drachman et al had a slightly different opinion about the phagocytic ability of the mononuclear phagocytes. They examined in detail the in vitro phagocytic function of peritoneal macrophages against pneumococci in normal and alloxan induced diabetic rats. When they used autologous serum as an opsonin, non-diabetic rat macrophages showed an average 77% phagocytosis in comparison to only 41% phagocytosis by diabetic macrophages.

However, they concluded that the observed defect was that of the serum and not the macrophage, as it was corrected by using normal serum rather than an autologous diabetic serum in the macrophage bacterial suspension. Finally, they were able to demonstrate a decrease in the actual intrinsic peritoneal macrophage phagocytic activity in diabetic rats when the blood sugar levels were as high as 800-900 mgm% (129).

While the previously described experiments have controversial results and may not necessarily reflect on the local lung host status, they present excellent models for the study of the effects of diabetes on the phagocytic functions which can be further applied to that of the alveolar macrophage.

Comparing the phagocytic function of PMN from untreated hyperglycemic non-ketotic uninfected diabetics with that of controls, Bagdade et al demonstrated a depressed activity in the diabetics which correlated with the levels of glucose in their blood (24).

When the effect of hyperosmolality on PMN functions was examined, a tremendous decrease from 100% to 32% of their phagocytic activity resulted from the elevation of the glucose concentration from 1% to 12.5% (88). This indicates a possible harmful effect of high levels of solutes, such as urea and glucose on the ability of the host to defend itself.

When the lung clearance of bacteria was examined by the intrabronchial inoculation of type 25 pneumococci in the diabetic rats, this was associated with a greater rate of pneumococcal proliferation when compared to control. Interestingly, this observed bacterial proliferation was not associated with an impaired inflammatory response (129); which may seem somehow contradictory to the finding by Ainsworth and Allison where impaired appearance of polymorphonuclear leukocytes existed in ear chambers applied over burn injuries of hyperglycemic and hyperosmolar rabbits (3).

In vitro, PMN chemotaxis in diabetic patients has frequently been reported to be depressed (66,335,372). However, this impairment may not be responsible on its own for the delayed appearance of PMN in the inflammatory site as diabetes is commonly complicated by additional vascular and circulatory defects.

It is true that the complications which are a frequent accompaniment of diabetes, will continue to provide difficulties in ascertaining that the susceptibility to infection and a defective in-

mune function observed in the diabetic patient is purely intrinsic to the diabetic state. Meanwhile, and until this argument is settled, it remains important to be aware of any such defects which are detected in the whole syndrome of diabetes and its complications.

MALIGNANCY, TRANSPLANTATION & IMMUNOSUPPRESSIVE THERAPY

The Systemic Defenses

Organ transplantation as a modality for the therapy of organ failure is now an accepted procedure. The current success of organ transplantation depends on good surgical technique, good histocompatability antigen (HLA) matching of donor and recipient and good immunosuppressive therapy to prevent graft rejection. In the absence of such rejection, the degree of restoration of function and overall health can be quite remarkable (138). However, prolonged immunosuppressive therapy frequently results in bacterial and fungal infections which may be fatal (208).

It has been found that 81% of patients who died after renal transplantation had significant pulmonary infection. All of those patients were on prednisone and azathioprine therapy, and all but two were receiving actinomycin C. Fungi, various or mixed bacteria and cytomegalovirus were the frequent pathogens (208).

In an excellent and detailed report by Elfenbein and Saral (138), the susceptibility to infection following bone marrow transplantation (BMT) was divided into an early period of aplasia which lasts near a month, followed by a post-aplasia period during which recovery of the immune system takes place; the latter period was further divided into early and late recovery periods, which ends or begins 3 months after BMT. In the period between 1968 and 1976, they reported a 31% incidence and 20% mortality rate from bacterial and fungal infections in the first month, following BMT, 90% of which were caused by bacterial and 10% by fungi. The bacterial organisms most frequently encountered were Pseudomonas aeruginosa (which had a 95% mortality rate if

bacteremia was detected), Klebsiella pneumonia (65% mortality), Escherichia coli (50% mortality) and Staphylococcus aureus (15% mortality); Candida species accounted for 80% of the fatal fungi infections and asparigillus species for 20%. Since the year 1976, their incidence of infection dropped to 13% and the mortality rate dropped to 4%. They emphasized the role of granulocyte transfusion, decontamination and isolation procedures and appropriate use of antibiotics in the reduction of such rates. They also demonstrated that one of the major and potentially drastic complications in the early recovery period was the development of interstitial pneumonia which frequently progresses to respiratory failure and death.

It appeared that the underlying disease (malignancy or anemia) did not affect the incidence of the interstitial pneumonia, but the preparative regimen used prior to transplantation did. Patients who received total body irradiation in addition to chemotherapy had a higher incidence (70%) than those receiving chemotherapy alone (37%) (138). In an endeavor to explain this susceptibility to infection, Elfenbein et al (136) examined both cellular and humoral immune responses in their post-BMT patients. They noted a measurable immune deficiency after BMT that improves with time towards normal, but may take up to 2 years before its complete reconstitution. They also noted that the kinetics of evolution for each immune function was different and that some functions were normal or nearly so as early as their first measurement following BMT. They noted, for example, that the MIF production by the patient cells in vitro was as vigorous as by normal cells as early as the first month after the transplantation. However, the proportion of T cells was lower than normal and the in vivo immune responses of both the T and B cells were impaired during the first 3 months after transplantation.

In another study, Elfenbein and Parrish (137) looked at some immune functions in vitro which they felt to be more relevant to the antimicrobial defense mechanisms in vivo. They found that the non-immune killer cells which participate in antibody

dependent cellular cytotoxicity were consistently present as early as one month after allogenic BMT. They also found that the MLC immunized T killer cells were present as early as one month after allogenic BMT and that Con-A inducible non-specific T suppressor cells were present as early as 1 1/2 months after allogenic BMT. Furthermore, after high dose chronic antigen exposure in vivo, it appeared that T helper function was present as early as 1-2 months after allogenic BMT, as not only IgM but also IgG antibodies were made to HLA antigens (from multiple transfusions) and to cytomegalovirus (CMV interstitial pneumonia). The study was concluded by the postulation that although the immune system appears weakened in general, it may be manipulated or stimulated under appropriate conditions to perform more effectively (138).

The Lung Defenses

The marked increase in infectious complications particularly pneumonia accompanying the increasingly aggressive use of cancer chemotherapy is well documented (55,226,408). Although interstitial pneumonia and fibrosis without definite identification of a causative organism have been reported to occur in patients receiving various chemotherapeutic regimens (408), the striking similarity in the pathology of interstitial pneumonia caused by pneumocystitis carinii and cytomegalovirus suggests a common pathogenesis (225,408,446).

Most of the literature on the direct relation between immunosuppressive therapy and lung susceptibility to infection is based on the observations obtained in animal experiments. Pennington described an animal model in which drug induced defects in the pulmonary host defense could be monitored. Following the subjection of guinea pigs to cyclophosphamide (15 mg/kg) plus cortisone acetate (100 mg/kg) daily for 7 days, he examined the number of the alveolar macrophages, their adherence and phagocytic activity against latex particles. He also tested the animals' response to skin sensitization and obtained peripheral blood differential leukocyte counts. Six weeks after the immunization of 6 guinea

pigs with Freund's adjuvent, after which they all became reactive to 5 tuberculin units of PPD given intra-dermally, the animals were subjected to the indicated therapy for one week. When the animals were retested for reactivity to intra-dermal PPD on the day of completion of the regimen, they all failed to respond. However, this disappearance of delayed type hypersensitivity lasted only for one week. When the alveolar macrophages were examined, he found that there was no change in their percentage in the broncho-alveolar lavage nor was there any significant difference in their viability, ability to phagocytize or adhere to plastic surfaces. However, he reported that both the leukocyte count and the absolute alveolar macrophage counts were significantly decreased and concluded that this may be the contributing factor in the increased incidence of pneumonia in patients undergoing immunosuppressive therapy (369).

In another experiment, Pennington compared the cellular response of animals treated with either cortisone acetate, cyclophosphamide or both to an endotracheal challenge of Pseudomonas aeruginosa. He noted that bacterial clearance from the broncho-alveolar compartment was inhibited only in animals treated with both cyclophosphamide and cortisone. He also demonstrated that combined therapy alone decreased both alveolar macrophage number and the delivery of polymorphonuclear leukocytes to the challenged lung (368). A further study indicated that the administration of either drug significantly inhibited the biosynthesis of the second and fourth component of complement by the alveolar macrophage (370). Not to preclude early inhibitory effects of these drugs on extrapulmonary complement synthesis, the impact of such a decrease in synthesis is almost certainly greater at sites such as broncho-alveolar fluid where minimal accumulation of fluid-phase occurs (395).

Corticosteroids

Corticosteroids are the most commonly implicated etiologic factor in the development of opportunistic pulmonary infections, particularly Pneumocystis carinii (408. Huber and co-workers aero-

solized radio-labelled (^{32}P) Staphylococcus aureus into the lungs of control and methylprednisolone acetate pretreated mice. Obtaining viable counts from the lungs of the challenged animals 6 hours after exposure to bacteria, they detected a three-fold depression in clearance only at a high dose of 50 mg/kg when given acutely (2 to 14 hr.). A significant depression of intra-pulmonary clearance was also detected when methyl-prednisolone acetate (5 mg/kg daily) and azathioprine (5 mg/kg daily) were given separately over 4 weeks (220). They also noted that the alveolar macrophages of steroid-treated animals were packed with large, electron-dense membrane-bound osmiophilic inclusions indicating an ultrastructural de-arrangement.

Very recently, Gudewicz studied the effect of cortisone therapy on the phagocytic function and glucose metabolism of guinea pig alveolar macrophages. The animals which were treated with 100 mg/kg of cortisone acetate for 7 days developed a profound leukocytosis, lymphocytopenia and monocytopenia in their peripheral blood, and their alveolar macrophages which were obtained by lung washings showed a 40% decrease in their number and a significant decrease in its in vitro phagocytic activity against Pseudomonas aeruginosa. This depression in the alveolar macrophage phagocytic ability of corticosteroid treated animals was associated with an inhibited stimulation in glucose oxidation normally occurring with macrophage phagocytosis, as well as a depression in the membrane transport of 2-deoxy glucose (191).

Contrary to these findings, Hunninghake and Fauci reported an increase in the alveolar macrophage harvest following the administration of exactly the similar kind and regimen of steroids to the same species of animals (221). However, the difference in the number of lavaged cells can be based on the differences in the techniques used to obtain the pulmonary alveolar macrophages.

Another study by Domby and Whitcomb (124) demonstrated that in contrast to the marked deerease in the percentage of T lymphocytes in the peripheral blood of steroid treated animals, there

was no significant decrease in the percentage of T lymphocytes in the broncho-alveolar cell population. However, the absolute number of T lymphocytes in the broncho-alveolar cell population was depressed, indicating that the effect of corticosteroids on T lymphocytes may be the same throughout the body. This study also demonstrated that neither the pulmonary alveolar macrophage number nor the Fc receptor activity of these cells were affected by the corticosteroid administration in the guinea pigs. Because Pennington (367) had previously demonstrated that the administration of corticosteroids and cyclophosphamide had no effect on the non-Fc receptor mediated phagocytic activity by the alveolar macrophages, Domby and Whitcomb concluded that corticosteroids apparently had no effect on the alveolar macrophage phagocytic function.

Interestingly, that while Hunninghake and Fauci observed that neither in vitro nor in vivo hydrocortisone (100 mg/kg for 7 days) had an effect on PHA induced or antibody-dependent cellular cytotoxicity of the alveolar macrophages, cortisone acetate, the depo-preparation which gives sustained elevations of plasma cortisol levels similar to those found for a brief period after the intravenous injection of hydrocortisone, caused a marked decrease in the cytotoxic effector function of the alveolar macrophage (227). This observation indicates a difference in the host cellular defense response to different types of corticosteroids and raises the question whether the intravenously used types of corticosteroids (e.g., methylprednisolone and hydrocortisone), which are the ones commonly used in emergency settings rather than the cortisone acetate which was used by Gudewicz, would have a similar depressive effect on the alveolar macrophage phagocytic function.

In another experiment, the suppressed killer cell function of the alveolar macrophages from steroid treated animals was found to be related to an intrinsic defect in killing of bound target cells since the defect was overcome by increasing the density of antibodies and PHA on the target cells (227).

Voisin et al examined the effect of suspending alveolar macrophages in a medium containing different concentrations of beclomethazone dipropionate, a corticosteroid used topically in asthmatics. At 24 hours, they demonstrated a depression of the bactericidal activity against Staphylococcus aureus, only at a concentration above 50 mg per 100 million cells. However, the fungicidal activity against Candida albicans did not change at similar concentrations. This led to the conclusion that patients using corticosteroids in an inhalation form may become more susceptible to lung bacterial infections (492) and presents another example of diversity in the response of the alveolar macrophage to different antigenic stimulants.

Methotrexate

Sieger looked at the effect of prolonged methotrexate treatment on the number and functions of rabbit alveolar macrophages. Giving his animals a dose of 2.3 mg/kg methotrexate twice weekly for a period of 1 to 9 months, he noted a decrease in the numbers of alveolar macrophages per gram wet lung weight which was extremely marked at the end of the 9 months. The fact that neither leukopenia nor monocytopenia had occurred suggested that the defect was not that of a toxic bone marrow. When the bactericidal activity of the alveolar macrophages from the methotrexate treated animals was assessed in vitro against Staphylococcus aureus and Pseudomonas aeruginosa, it showed a depression 6 months after the initiation of treatment (446).

BURNS

Sepsis continues to be a major life threatening complication in patients with extensive thermal injury (9). While bacteremia is encountered in 15% of all nosocomial infections, its incidence may rise to greater than 50% in patients with severe burn injury (5). Almost all the expressions of the immune responses that have been studied show impairment, cell mediated immunity being most consistently and profoundly suppressed among these responses (5,336). Perhaps the most interesting development in the understanding of

the mechanism of such immunosuppression is the reporting of the emergence of a suppressor T cell and perhaps a suppressor monocyte population in the post-traumatic period and after major thermal injuries (260). Keeling et al studying the restoration of normal immune responses in the burn-trauma patient and recognizing the emergence of suppressor T cell system following major burns, cautioned against the use of agents which stimulate immunologically competent cells without further recognizing its modulatory effect. He described an in vitro method for assessing the effect of modulators on peripheral lymphocyte response to antigen stimulation (260).

Added to the various cellular and humoral immune derangements, there is substantial evidence that vascular leukostasis occurs in major burn patients in a manner similar to the one previously described in the post-traumatic ARDS. Erikson et al observed the changes in the mesenteric micro-vasculature before and after a distant full thickness cutaneous burn in rats. He calculated the number of WBCs sticking, then emigrating from the vascular compartment during a 6 hour period as many as 0.51×10^9/kg. He concluded that this mechanism of consumption of granulocytes can deplete the body stores and may explain the decrease in body granulocytes seen following major thermal trauma (143).

ANESTHESIA

Post-operative pulmonary infections are known to occur more commonly after the use of inhalation anesthesia when compared to spinal anesthesia (359). This has been attributed generally to aspiration, retention of broncho-pulmonary secretions and occasionally to contaminated equipment. The age of the patient, the type of surgery performed and the duration of anesthesia have been found to be contributory factors (325).

Manawadu and LaForce studied the effect of halothane anesthesia on lung clearance of aerosolized Staphylococcus aureus in mice and the in vitro ciliary motility of tracheal epithelium. They found a significantly greater percentage of viable bacteria in

the lungs of the mice that had been anesthetized (41.8-43.1%) when compared to controls (29.3%). They, however, found no or minimal effect on the ciliary activity when up to 3% halothane anesthesia was used, and only at 4 to 5% halothane anesthesia for a long period were they able to notice a dramatic effect. Since in clinical practice, 4.0% halothane concentrations are rarely briefly used for induction, they concluded that at the usual concentrations of halothane, minimal depression of ciliary activity is expected and is reversible after termination of the exposure. They also concluded from an ultrastructural point of view based on the observation of distorted micro-tubules of the halothane exposed alveolar macrophages and from the substantial evidence of the diminished bacterial clearance detected in the lungs of the halothane exposed mice that the alveolar macrophage phagocytic and bactericidal activity were depressed. They theorized that the mechanism behind this was the depressive effect of halothane on the oxidative phosphorylation of the aerobe-dependent alveolar macrophage (307).

However, a study by Goldstein et al failed to conclude a depressive effect of halothane on the alveolar macrophage, in contrary to that observed when methoxyflurane and cyclopropane were used (175).

Studying the effect of local anesthetics used in bronchoscopy on the human alveolar macrophage, Hoidel et al sprayed a maximum of 7 ml of 4% lidocaine in the upper airways and poured up to 30 ml of 1% lidocaine through the inner channel of the bronchoscope resulting in a measurable level of lidocaine uniformly present in the broncho-alveolar lavage returns. The mean concentration of lidocaine detected in the fluid recovered from the initial 60 ml injected into 8 subjects was 5.5 mM. In this study, they found that lidocaine in a concentration of 16 mM, or tetracaine in a concentration of 2 mM decreased both oxygen consumption and superoxide anion release by the alveolar macrophage by 67%. This effect was found to be reversible since washing the cells in Hank buffered saline solution (HBSS) was followed by recovery of their normal meta-

bolic functions. The site of effect of cationic anesthetics on the alveolar macrophage, described in their experiment, was thought to be in part the cell membrane, a conclusion based on the striking morphological changes in the surface membrane of alveolar macrophages incubated with tetracaine when compared to control (212).

OXYGEN TOXICITY

Normobaric Oxygen

Although oxygen therapy has been of clear benefit in many clinical settings, it also carries a risk of tissue damage. All the tissues of the body can be injured by sufficiently high oxygen concentrations, but the lung is exposed directly to the highest partial pressure of inspired oxygen. The precise concentration of oxygen that would be toxic is determined by many factors in the exposed person, including his age, nutrition, endocrine status and previous exposure to oxygen or other oxidants (91,340).

Singer et al showed that 21 to 44 hours of 100% oxygen at sea level supplied following open heart surgery had no detectable physiologic alteration (452). On the other hand, Barber et al (26) found increases in wasted ventilation and in arterio-venous shunting in patients with irreversible brain damage after 40 hours of 100% oxygen for 25 hours at sea level, and Camroe et al (96) found a decreased vital capacity in persons exposed to 100% oxygen for 25 hours at sea level.

Sackner et al (425) found tracheitis and decreased velocity of tracheal clearance of mucus in normal men exposed to 90 to 95% oxygen for 6 hours; and Nash et al (348) found that pathologic changes in patients treated with ventilation for several days correlated directly with the concentration of oxygen administration.

Although many of these studies appear controversial to each other, a possible conclusion is that although an early tracheitis probably develops in human beings, they can tolerate 100% oxygen

at sea level for 24 to 48 hours with little if any serious tissue injury and that pulmonary damage occurs from longer periods of exposure. The safe level of inspired oxygen is not known and may vary individually, but we know that less than 50% oxygen can be tolerated for a longer period of time without deleterious effects (91).

The pathology of oxygen toxicity is non-specific and consists of atelectasis, edema, alveolar hemorrhage, inflammation, fibrin deposition and thickening and hyalinization of alveolar membranes (91,371). Capillary membrane leakage edema occurs in experimental animals (246) and the pulmonary surfactant is altered increasing the chances for atelectasis. Type I alveolar lining cells are also injured early, and bronchiolar and tracheal ciliated cells can be damaged by 80 to 100% oxygen (373).

Where the alveolar macrophage stands from all this, and what its contribution may be in the pathological process is not clear. Oxygen in vivo appears to increase the number of macrophages in sections of rat lung, but may not increase the number of cells obtainable from a standard lung lavage procedure (151,340). Exposure of animals to 100% oxygen significantly decreases bacterial clearance from their lungs (221). However, the in vitro phagocytic activity of alveolar macrophages from oxygen exposed animals did not differ from normal (337), which suggests a rather different mechanism for the observed diminished lung clearance of bacteria.

Of interest in this area is the suggestions that the oxygen superoxide radicles produced by the actively phagocytizing alveolar macrophage may contribute to the pathology of lung damage in hyperoxia (340,426). However, this requires to be further examined. The toxic effect of oxygen on some of the lung cells is believed to be counteracted by the production of superoxide dismutase enzyme (448). Species and age differences seem to effect the cellular response to excessive oxygen. For example, while neonatal rat macrophages produce superoxide dismutase in response to hyperoxia in vivo and in

vitro, adult rats do not (116). On the other hand, mature mice show an increase in the superoxide dismutase in response to high oxygen levels in vitro (387).

Deneke and Fanburg (117) concluded that compounds that increase cellular respiration or the production of oxygen free radicals are likely to potentiate oxygen toxicity through an unknown mechanism. Dismutase (antabuse) was found to increase oxygen toxicity in rats at levels similar to those considered therapeutic in man. In probably a similar manner, hyperthyroidism and temperature elevation may put a patient receiving oxygen at similar risks.

Certainly, a logic approach to the problem of oxygen cytotoxicity would seem to be through administrating an antioxident, though alpha-tochopherol and ascorbate did not offer protection in non-deficient animals (117).

VENTILATION AND PERFUSION

Pulmonary embolism, cardio-pulmonary bypass and various low flow states may interfere with lung alveolar perfusion. In the absence of adequate ventilation, diminished alveolar perfusion may result in rapid biochemical and structural deterioration of the lung (327). Modry and Chiu demonstrated that the lung can maintain its bio-energetic levels for 5 hours in the lack of pulmonary artery perfusion (327). However, they indicated that with longer periods of perfusion interruption, the lung is not adequately protected by ventilation (284,326). The deterioration observed in the capillary endothelial integrity, together with the upset of ATP/ADP ratio within lung parenchyma and the marked mitochondrial de-arrangement noticed when the bronchus was occluded, may have its severe impact on the alveolar macrophage which is highly dependent on the process of oxidative phosphorylation. However, this remains a speculation which requires examination.

NUTRITIONAL DISORDERS

Law et al demonstrated that the in vivo and in vitro immune

functions mediated by T lymphocytes were impaired in protein-calorie malnutrition and that nutritional repletion of these patients using total parentral nutrition produced significant improvement in their T lymphocyte function (284,285). However, since malnutrition is frequently associated with other pathological states such as auto-immune and malignant diseases, it remains difficult to establish a cause effect relationship between nutritional disorders and immunoparesis.

Haffejee et al evaluated the nutritional status of 15 negro patients suffering from unresectable carcinoma of the mid thoracic esophagus before and after palliative pulsion intubation. All the patients were shown to be in negative nitrogen balance and to have a compromised non-specific cellular immune response. When correction of protein-calorie malnutrition and achievement of positive nitrogen balance were obtained, there was an associated increase in the absolute lymphocyte count and T lymphocyte response to PHA. They concluded that because an improvement in immunological reactivity had occurred without an attempt at therapeutic reduction in the tumor bulk, the observed diminished cellular response was due to the impaired nutritional status (177).

Protein-calorie malnutrition continues to be frequently encountered in our surgical patients. Many studies hav shown that infectious diseases contribute to the nutritional deterioration in protein-calorie malnutrition and that malnutrition, in turn, alters host defense mechanisms against infectious agents (440). Scrimshaw described this cyclic inter-action between infectious diseases and malnutrition as being "synergistic" (439). The major impact of protein-calorie malnutrition has been identified to affect the T lymphocytes, the complement system, and very modestly, the intra-cellular bactericidal activity of polymorphonuclear leukocytes and the B cells function (479).

Douglas and Schopfer demonstrated normal phagocytosis of latex and antibody coated erythrocytes by monocytes derived from children with acute Kwashiork in the Ivory Coast (126).

Bhaskaram looked at the morphology and the bactericidal capacity of peripheral blood monocytes from 11 children with protein-calorie malnutrition. He reported no effect of such disease on the monocytes (37).

Keusch et al who developed a rat model of protein-calorie malnutrition studied both the cellular and humoral immune functions in these animals. They found no defects in the chemotactic or bactericidal activity of peritoneal macrophages from protein deficient animals, although phagocytosis associated oxygen consumption and hexose monophosphate shunt activity were depressed (264). However, the authors noted a marked impairment of humoral chemotactic factors generated in the peritoneal cavity by the injection of glycogen as well as an alteration of the heat labile serum opsonins required for proper phagocytosis of Staphylococcus aureus, Escherichia coli, Salmonella typhimurium and Salmonella enteritidis.

ALCOHOL ABUSE

Today alcoholism afflicts approximately 9 million citizens in the United States with almost similar prevalent figures throughout the western world (363). With a higher incidence of morbidity in these patients, a higher number is expected to present to the surgeon. Perhaps the most alarming factor that increases the morbidity and mortality rates of alcoholism is their susceptibility to infection. The etiology of such susceptibility seems complex. Certainly their life style, exposure to bacteria, nutritional disturbance and trauma attribute to this. In addition, the direct toxic consequences of alcohol excess on the cellular and humoral host immune defenses have been repeatedly reported (363).

One of the first observations on the adverse association between alcohol abuse and infection is that by Benjamin Rush in 1785 who noted a susceptibility to tuberculosis in alcohol abusers. Osler in 1905 reported that alcoholism was the most potent predisposing factor to lobar pneumonia. Many reports show that bacterial pneumonias occur with an increased morbidity in alcoholics (89,

293,330,375,430). However, in spite of the higher incidence of major complications to such infections in these patients (19,235,375), alcoholism does not appear to increase the mortality rates significantly (330,363).

Undoubtedly, the primary event in alcohol abuse related pulmonary infections is more frequent aspiration, with or without vomiting. This increase in the inoculum of bacteria is not associated with a similar increase in the clearance rates (363). Cough reflexes, mucociliary (281) and cellular (160) clearance mechanisms were all reported to be depressed.

DeMeo and Anderson noted a defect in the leukocyte chemotaxis in cirrhotic alcoholics (115). A similar depression in the chemotactic ability of alveolar macrophages was reported by Guarneri and Laurenzi (190). Green and Green suggested that alcohol ingestion selectively inhibits the alveolar macrophage killing ability allowing an enhanced growth of a single pathogenic bacterial species in the alveolar compartment (184).

PNEUMOCYSTIS CARINII PNEUMONIA

P. carinii pneumonitis occurs almost exclusively in the compromised host, being an infant, child or adult. The diagnosis of P. carinii pneumonitis is an indication of the presence of an immune deficiency state. Malignancy, immuno-deficiency, nutritional disorders and others all predispose to pulmonary infection (407). Surgeons may be required to face such a problem in two ways; the first is that without the awareness of the possible occurrence of P. carinii pneumonitis in their immunosuppressed patients, a significant mortality and morbidity may result (223,230,500). Second is the fact that in spite of the development of many serological tests for the detection of such illness, definite diagnosis requires the demonstration of the causative organism in the diseased lung, a procedure which may require an open lung biopsy (13,86,360,407,531).

SPLENECTOMY

The spleen considered by the Greeks an impediment to the performance of athletes, was often subjected to splenectomy by hot irons in an effort to improve performance. Galen thought the spleen a source of black bile and melancholy. Its functions have remained poorly understood. Its clearance ability towards particulate antigens as well as injured or old cells is now fairly understood. It comprises about 25% of the total body lymphoid mass and is an immuno-competent organ.

Likhite reviewed in detail the immunological functions of the spleen and the effect of its removal. It has been found that removal of the spleen has little effect on the net functional potential of antibody production, although it has been established to be a major source of antibody production. Humoral immune responses following subcutaneous administration of soluble antigen in splenectomized individuals were found to be normal. However, primary intravenous administration of particulate antigen (i.e., Rh-positive RBCs to a Rh-negative recipient) evoked little, if any, humoral immune response (294).

Two decades elapsed before the question of whether splenectomy increases susceptibility to infection was answered. Evidence came in a gush of articles and case reports, and immediately interest grew to explain the mechanism of immune dysfunction which may be associated with removal of the spleen.

Although most infections are actually handled normally in splenectomized or congenitally asplenic patients, these persons are prone to overwhelming and often fatal septicemia (121). Streptococcus pneumoniae were found to be responsible for approximately 50% of the infections. Hemophilus influenzae, Staphylococcus aureus, group A Streptococcus and Neisseria meningitides were involved in the remaining 50% (121,125,141). It is interesting to read that the majority (75%) of these infections (total incidence, 5% to 8%) take place within two years after splenectomy (383). Although it is repeatedly reported that fulminating sepsis more frequently occurs in

children splenectomized for such diseases as Thalassemia or idiopathic thrombocytopenic purpura, or in congenitally asplenic or sickle cell autosplenectomized children (294), splenectomized adults are increasingly being reported to develop such infections (389). Eraklis et al noted the importance of the presence of a primary defective reticuloendothelial system in the splenectomized individuals. Of 467 cases reviewed, those with a primary disease involving their reticuloendothelial system (Gaucher's disease, portal hypertension, Thalassemia and Wiskott-Aldrich Syndrome) were the most susceptible to bacterial infection following splenectomy (141).

Radiotherapy to the spleen seems to have an effect similar to that of splenectomy. Ginsburg et al demonstrated that resistance to infection can be significantly impaired after irradiation to the left upper abdomen such as is frequently used for the treatment of malignancies (Wilms tumor, neuroblastoma and Hodgkin's disease) (165). Irradiation to the spleen in rats was found to result in higher mortality when type 3 pneumococci were inoculated intra-peritoneally, when compared to the mortality in similarly inoculated non-irradiated controls (165).

Malangoni et al demonstrated that ligation of the splenic artery did not increase mortality in rats challenged with type 2 Streptococcus pneumoniae, while total de-arterialization of the spleen resulted in an increased mortality from pneumococcal challenge that is similar to that seen after splenectomy (306). This suggested that an arterial communication of the spleen is required for its proper immune response, and that its ability to filtrate circulating particles may be the major role in its ability to control infection.

Studies in experimental animals have verified the primary importance of the spleen in clearing and trapping cellular antigens. Splenectomized animals and congenitally asplenic mice were found to relfect defective production of immunoglobulins such as IgM and IgG_2 that may parallel the inefficient thymus-bone marrow synergism observed in these animals (28).

The intra-vascular clearance of sensitized, autologous erythrocytes in guinea pigs has shown that after splenectomy there is an important defect in the clearance of IgG-coated erythrocytes, a defect that can be corrected by increasing the amount of IgG used to sensitize the erythrocytes (152).

Bogart et al noted an impairment of intra-vascular clearance of pneumococcus type 3 following splenectomy (57). The intravenous administration of pneumococci in splenectomized animals resulted in deficient intra-vascular clearance and increased mortality, as compared with clearance and mortality in controls undergoing a sham operation (57,216,514).

Hosea et al studied the opsonic requirements for the intravascular clearance of pneumococci in guinea pigs and of sensitized erythrocytes in human beings after splenectomy. They were able to correct the post-splenectomy impairment in clearance of injected pneumococci by immunization of the animals. This improvement was thought to be due to the noticed increase in hepatic sequestration of organisms. A similar finding was noted with the IgG sensitized erythrocytes, which required a four-fold increase in sensitizing antibody for a significant improvement in clearance to occur. This also was related to an increased hepatic sequestration in the splenectomized patients (216).

Brown et al studied the rates of removal of pneumococci from the blood stream in normal and splenectomized guinea pigs and the extent of its hepatic and splenic sequestration. A relationship was observed between the virulence of pneumococcus and the extent of its clearance by the spleen. In general, it was found that while the liver is able to clear less virulent pneumococci, its inability to clear virulent types was compensated by the sequestration of those virulent types in the spleen. Removal of the spleen hence induces a defect in the clearance of virulent penumococci from the blood (74).

Biggar et al demonstrated that the opsonic activity of sera from young rats obtained 5 days after splenectomy did not change as

evident by a normal in vitro phagocytosis of pneumococci type 3 by polymorphonuclear leukocytes. However, when the serum opsonic activity was tested 3 months following splenectomy, a deficiency in supporting phagocytosis in vitro was noted (41). This deficiency was attributed to a defect of serum and not of neutrophils since the addition of normal serum instead of splenectomized rat-serum to the bacteria-phagocyte suspension had resulted in a normal phagocytic activity of the splenectomized rat polymorphonuclear leukocytes.

Detection of such serum deficiency stimulated the study of both specific and non-specific serum immunoglobulins in the deficient splenectomized. In addition to the observation that the level of IgM (which is thought to be more superior in its bactericidal and opsonic properties than IgG) was low in young children (416), it was found that splenectomized children had even significantly lower levels of serum IgM compared to normal children. The asplenic children were also found to have a deficiency in serum pneumococcal antibodies (41).

Splenectomized patients were also found to exhibit delayed macrophage mobilization as well as sub-optimal levels of opsonins and cytophilic antibodies (294).

The tetrapeptide (1-threonyl-L-lysyl-L-prolyl-L-arginine) tuftsin, the functional unit of the carrier molecule leukokinin, was found to simulate all the known biological activities of granulocytes and mononuclear phagocytes, the phagocytic, pinocytic as well as the motility and bactericidal activity.

Leukokinin, a leukophilic γ-globulin fraction which binds specifically to blood neutrophils resulting in the stimulation of their phagocytic activity, requires that tuftsin be cleared from it to be utilized by the cell, if phagocytic stimulation is expected (482). The leukokinin then becomes inactive, is shed and replaced by fresh tuftsin carrying leukokinin which

bind to the free cell receptor to make available further quantities of tuftsin and effect further stimulation of phagocytosis. In such a manner, the phagocytes maintain in a state of continuous stimulation (342). The tetrapeptide tuftsin is present in the heavy chain of leukokinin as part of the Fc portion. For the complete release of tuftsin from leukokinin, it is first cleaved at the arginylglutamyl bond by a splenic enzyme, tuftsin endocarboxy peptidase, to produce leukokinin-S with a free carboxyl end of tuftsin. The second cleavage takes place after leukokinin-S binds to the phagocytic cells and is induced through the effect of the membrane enzyme leukokininase (342). In the absence of a functional spleen, tuftsin cannot be cleaved at its carboxy terminal arginine. Consequently, leukokininase is incapable of releasing a free tetrapeptide even if it could cleave tuftsin at the second site of cleavage, the threonine amino-peptide bond (341). Thus, while a normal tuftsin exists in the leukokinin of such cases, it cannot be released, a necessary step in order to exercise its full function.

Deficiency in leukokinin was observed in dogs (345) and rats (295) following experimental splenectomy. Patients with sickle cell anemia or mylogenous leukemia in whom splenic function has been curtained and exhibit a high rate of severe infections were found to show an absence of tuftsin activity (97,346). Assay of tuftsin level in post-elective-splenectomy patients revealed virtual absence of any activity (97,343). Spirer et al used the more accurate radio-immunoassay to confirm this fact (465). The serum levels of tuftsin in 20 cases of elective splenectomy (118 ng/ml^{-1}) were significantly lower than in 35 normal subjects (255.71 ng/ml^{-1}).

Long after splenectomy, no deficiency in any of the γ-globulin phosphocellulose fractions (I-IV) (343), complement components 3 and 5 as well as various immunological components of γ-g and γ-m (344) were found. Only leukokinin-S seemed to be defective and consequently, it was not surprising that normal and splenectomized subjects present similar tetrapeptide yield values in

their γ-globulin analysis (342). In another study, subjects who had experienced rupture of the spleen which was treated by splenectomy appeared to have leukokinin-S with normal tuftsin levels (97). This was thought to be the result of implantation and growth of various proportions of spilled splenic tissue in the peritoneal cavity.

Downey et al examined the immune functions of 22 splenectomized patients of an age range 12 to 63 years old, 4 years after the removal of their spleen. A significant increase in the number of peripheral blood lymphocytes and a decrease in the level of IgM were noticed. The lymphocyte response to phytohemoagglutinin in splenectomized patients was also found to be depressed (128). These observations suggest that aberrations in cellular and humoral immunity are persistent and involve adults as well as young children. However, Baker et al demonstrated an early macrophage dependent hypo-responsiveness to phytohemoagglutinin in traumatized asplenic patients which returned to 94% of normal in 25 days (128), suggesting that in vitro responsiveness of lymphocytes and macrophages may not necessarily recover similarly.

Chandry et al demonstrated an increased pulmonary retention of particulate matter after splenectomy (87). Kovacs et al further studied pulmonary sequestration of circulating ^{131}I-lipid emulsion following splenectomy. A defect in the pulmonary macrophage function was arbitrarily held responsible for this defect. The increase in the pulmonary retention of ^{131}I-lipid emulsion observed in the splenectomized rats may have been due to many factors other than a pulmonary macrophage defect; the fact that removal of the spleen decreases the circulation potential for such particulates may, in itself, be responsible for this defect. On the other hand, the increased pulmonary retention may indicate an increase rather than a decrease in pulmonary macrophage phagocytic uptake.

Skamene et al examined the mechanism behind the increased resistance of splenectomized mice to Listeria monocytogenes infection. They noted that although phagocytosis by reticulo-

endothelial cells of test particles (^{51}Cr-labeled sheep erythrocytes) is the same in splenectomized and control mice, a 24 hour exposure to Listeria which failed to influence phagocytic activity of normal mice, greatly enhanced the blood clearance and liver uptake of the test particles in splenectomized mice (454). The increased anti-listerial resistance in splenectomized mice was noticed to decrease with time following splenectomy and to be no longer demonstrable 10-12 weeks post-splenectomy (453). With these observations, it was possible to suggest the existance of a cell pool in the spleen responsible for the control of macrophage activity, which disappears with splenectomy to be slowly replaced from another source. A hypothesis that was meant to suggest the presence of a cell population, if analogous with other similar models of suppressor cells, would likely originate in the thymus, and if operative in the previously noted experiment would represent the first known instance of a suppressive influence of lymphocytes on mononuclear phagocyte production or activation (454).

It seemed logical with the immunological deficits observed in the post-splenectomy patients to consider splenic preservation in the management of traumatic injury to the spleen. Moreover, an interest grew in boosting up of systemic immune responses in splenectomized individuals by specific immunization or by performing autologous splenic implantation. However, reports from the literature are most confusing in this matter.

Weiss et al studied the blood bacterial clearance rates in splenectomized, sham operated and subcutaneously splenic auto-implanted rats. Bacterial counts from the blood, liver and lung samples of splenectomized rats as well as those from the splenectomized and auto-transplanted rats were significantly much higher than those of control rats, suggesting that the presence of a small piece of auto-transplanted spleen in the splenectomized rats had no protective effect in clearing pneumococcal bacteremia, and in fact, may have resulted in impeded bacterial clearance, since the mean counts in this group were markedly higher than the splenectomized group (506).

This finding of a lack of a protective effect of auto-transplantation in rats was also demonstrated by Schwartz (437) and by Whiteside et al (518), who demonstrated that 4 weeks following splenectomy and auto-transplantation, the rats had a survival rate comparable to that of the splenectomized rats when both groups were challenged with an intra-peritoneal injection of type 2 Streptococcus pneumoniae.

Tesluk and Thomas suggested that the dose of the bacterial inoculum may be a critical factor in the variation of immune response obtained in different experimental models. Contrary to the lack of protection of auto-transplants observed as previously stated, they were able to detect an improvement in the survival rates following intravenous inoculation of type 2 Streptococcus pneumoniae in auto-implanted splenectomized rats (481). However, this improvement was only partial and did not compare to the complete protection observed in another report (296) when a smaller number of bacterial inoculum was used.

Dickerman et al studied the effect of exposure to an aerosolized suspension of type 3 Streptococcus pneumoniae on splenectomized mice who had either all or half of their splenic tissue cut up and re-implanted into the abdominal cavity 8 weeks prior to bacterial challenge. They determined that both groups had a mortality rate similar to that from a sham control group and significantly lower than that of a splenectomized control group (122). This study showed a protective effect of splenic auto-implants when bacteria are presented through the respiratory system and may, indeed, suggest that the failure of most investigators to demonstrate that splenic implants in animals can protect against pneumococcal infection might be related to the intravenous route of bacterial challenge used in these studies.

Since pneumococcal infections in humans are acquired via the respiratory tract (75), attempt to study the problem of pneumococcal infections in splenectomized individuals must consider the role of the lung host defenses in the clearance of

such organisms, and whether splenectomy may affect the local cellular and humoral immune responses in the lung.

Stein-Streilein et al demonstrated that the presence of the spleen was not necessary for a specific antibody forming cell response in the pulmonary draining lymph nodes. However, an enhancement of the local response was noticed to occur if the animals were systemically immunized and, therefore, demonstrated active participation of the spleen in the specific immune response. A finding which supports the hypothesis that, although a local response may occur in the pulmonary draining lymph nodes in the absence of a spleen or asplenic response, the presence of a systemic or splenic response appears to be important for the enhancement of local specific IgM forming cell response. This observation may also suggest that the immune defenses involved in the lower respiratory tract may differ from those in the upper respiratory tract and other mucosally lined organs in that the response of the spleen to antigen affects the local response to that antigen (471).

CHAPTER II

PULMONARY HOST DEFENSES

THE ALVEOLAR MACROPHAGE

PULMONARY HOST DEFENSES

The lung immune response is critical to its defense against potentially harmful antigenic invaders. The amount and kind of antigen presented to the lung is usually determined by the extent of escape of such antigen from the respiratory tract or systemic defenses.

To understand the immunological derangements pertinent to the pathogenesis of lung diseases, thorough examination of both humoral and cellular immune functions is essential. However, the clearance of inhaled particulate materials from the respiratory tract is not entirely dependent on such responses.

Aerodynamics

When considering the flow of air through the respiratory system, it has been found that filtration commences at the vibrissae within the nares, the air stream after which changes direction so sharply at the nasopharynx impacting inhaled particles on the posterior wall of the pharynx (354). The air then enters the trachea containing only few particles larger than 10 to 15 µm which will impact mainly at the carina or within one or two bronchial divisions (333). For particles in the size range of 5.0 to 0.2 µm, sedimentation as a result of gravitational forces in lung regions of low flow is probably the most important determinant of deposition from approximately the fifth bronchial divisions to the terminal lung units (433).

The sudden opening of the glottis which occurs during coughing results in a drop of tracheal and major bronchial pressures with an increase in the more distal transbronchial pressure and a marked augmentation of flow rates throughout the bronchial tree. Ross et al noted that the linear velocity of air flow during coughing may reach values which approach 85% of the speed of sound (410). This high velocity of air flow associated with narrowing of lumen propels the mucus plug containing the leaking particulates toward the mouth.

Mucociliary Mechanisms

Particles deposited between the posterior two-thirds of the nasal cavity and the nasopharynx and from the larynx to the terminal bronchioles, land on airways lined by mucus-covered ciliated epithelium, and are removed by means of the mucociliary transport mechanisms (354).

Mucus glands and goblet cells abound in the major airways. However, at the level of the terminal bronchioles, very few goblet cells are seen in normal man (354). Goblet cell and mucosal-gland secretions join the moving layer of mucus while still attached to the mouths of their secretor cells, tethering the mucus blankets but easily overcomed by the ciliary action under normal circumstances. The mucus blanket, ever moving upwards at a rate of about 2 cm per minute (499), was found to consist of two functional layers, the superficial gel layer of which provides an important barrier to biologic agents because of the presence of immunoglobulins, such as secretory IgA, and lysozyme that provide a high degree of bactericidal activity.

In the ciliated mucosa of the respiratory tract, each cell accommodates approximately 200 cilia, 5 µm long and 0.3 µm in diameter. Each cilium has an ultrastructure consisting of two central microtubules, the function of which is thought to be co-ordinative, and nine paired peripheral microtubules which are "contractile" deriving their energy from adenosine triphosphate (499). The complex ciliary beat is observed to result from the sliding of the paired peripheral filaments across one another, first on one side of the cilium and then on the other.

Bronchial Secretions

Relatively little is known about the synthesis and secretion of immunoglobulins in the lower respiratory tract, a mechanism which might contribute to the regional anti-bacterial activity. Leehand and Cantey studied immunoglobulin synthesis and secretion in the rabbit's lower respiratory tract, both in the normal and after infection with Diplococcus pneumoniae or Listeria monocytogenes.

They found that normal and infected lower respiratory tract secretions contained IgA and IgG and that the ratio of the first to the second differed with the type of organism used. However, the normal respiratory tract produced small quantities of immunoglobulin most of which was IgG (195). This came to be contradictory to expectations since IgA was thought to be the major immunoglobulin in rabbit lower respiratory tract, and the predominant immunoglobulin in several external secretions of many mammalian species. However, there is evidence that IgA-containing cells in human bronchial mucosa are equal to or exceed other Ig-containing cells (310, 482).

There is support to believe that secretory IgA is preferentially secreted onto mucous membranes. Although more IgG than IgA was synthesized in the rabbit's lower respiratory tract and serum IgG levels were 50 times those of IgA, the concentration of IgA was greater than that of IgG in lower respiratory secretions (195).

Whichever the immunoglobulin component present dominantly in the lower respiratory tract, it is their anti-bacterial role either by lysing the micro-organism secondary to complement fixation or by functioning as an opsonin that determines their immune activity and not its agglutination (244). Reynolds and Thompson studied the opsonic properties of immune rabbit bronchial secretions. IgA and IgG antibodies separated from them were assessed in vitro for their ability to enhance phagocytosis and intra-cellular killing of Pseudomonas aeruginosa by alveolar macrophages. They found that IgG was more superior as an opsonin than IgA; and that IgG whether isolated from bronchial fluid or serum, performed similarly regarding their opsonic function (396). However, the opsonization of Pseudomonas aeruginosa and its phagocytosis by alveolar macrophages in the presence of immune IgG was found to be more superior than that in the presence of non-immune IgG. These findings suggest that IgG may be the principle opsonin in the normal respiratory secretions since complements and IgM are present in only minute amounts (396).

Warr et al compared selected proteins recovered from 36 healthy

non-smokers and 19 healthy cigarette smokers. They noted no quantitative differences between the two groups in immunoglobulin A, α_1 antitrypsin, α_2 globulin, transferrin or albumin content. However, the IgG content of bronchial fluids from smokers was found to be twice as high as that from non-smokers, indicating either the selective stimulation of local bronchial immunoglobulin production or the selective exudation of plasma immunoglobulin into alveolar spaces in response to inhaled cigarette smoke (504).

Finally, in the matter of bronchial secretions, an assortment of bio-regulatory molecules have been identified in tumor tissue extracts and in lung washouts. These have the capability of regulating immune responses which may allow tumors or pathogenic organisms to escape normal immunologic control (329). Mohagheghpour et al demonstrated that non-cytotoxic aqueous extracts of esophageal tumors inhibited the spontaneous uptake of ^{3}H-thymidine by human peripheral blood mononuclear cells. It also inhibited mitogen- and antigen-induced mononuclear cell blastogenesis, mitogenesis and labelled thymidine uptake (329). One cannot avoid thinking of the possibility of a similar mechanism functioning in the lungs in relation to the susceptibility to infection in patients with bronchogenic carcinoma.

Role of Complements

In a recent investigation of the immunological alterations associated with pneumococcal pneumoniae, a depression of complement components was found in all patients dying of their disease (114). No casual relation between complement depression and infection could be made, since complement levels before the occurrence of infection were not available. C_3 deficiency had been reported to have an increased incidence of bacterial respiratory infection, however, because the number of reported cases was small, such cause and effect relation remained unconfirmed (9,25).

Diseases associated with depressed complement factors, such as systemic lupus erythematosis and sickle-cell disease have a high incidence of bacterial infections, suggesting the importance

of the complement system in the defense against bacteria (241,).

Gross et al studied the effect of complement depletion by cobra venom factor on the mice lung clearance of four different bacteria species. Four hours after aerosolization with Streptococcus pneumoniae, complement-depleted animals had cleared only 75% of the initial number of organisms, whereas controls cleared 91%. Aerosolization with Pseudomonas aeruginosa resulted in a two-fold greater growth of organisms in the complement-depleted animals (446% of original deposition) as compared to the saline-treated controls (211% of original deposition). Clearance of Klebsiella pneumoniae and Staphylococcus aureus were similar in complement-depleted animals and saline-treated controls (189). Indeed, these experiments suggest that hypocomplementenemia predisposes to bacterial pneumonia and may explain the increased susceptibility to pulmonary infections in patients having impaired complement activity.

Niehaus et al demonstrated that opsonic fibronectin deficiency can exaggerate the increase in lung vascular permeability induced during Pseudomonas aeruginosa bacteremia (356).

Bronchus-Associated Lymphoid Tissue (BALT)

Anatomically organized lymphoid tissue in direct contact with the respiratory mucosa of bronchi was found to consist of follicles containing small and medium sized lymphocytes, but lacking capsules, germinal centers and plasma cells typical of lymph nodes (39). These lymphoid nodules are scattered along the mucosal surface of large and medium sized bronchi and more concentrated at points of bifurcation. BALT bears a resemblance to that of Peyer's patches and other gut-associated lymphoid tissue (GALT). It is made of plasma cells and lymphocytes of which 20% bear a surface marker for T-cells. Although specific antibody-forming cells do not seem to appear after local immunization, suggesting that BALT lacks B-cells that are sufficiently differentiated to synthesize immunoglobulin (40), it has been

demonstrated that BALT cells may repopulate the lamina propria of both gut and lung mucosa with IgA, producing cells after lethal irradiation of recipient animals (39), indicating that BALT is composed of B-cell precursors of IgA-producing cells and raises the possibility that BALT serves as a cellular reservoir for immunocytes contributing to the mucosal clearing system in the respiratory tract (245).

Local Cellular Immune Defense

In the lung, the alveolar macrophage is considered the most important cellular defense element; its functions seem to adapt to varying degrees of slowly occurring changes in the alveolar micro-environment. However, sudden alterations in such an environment may have detrimental effects on alveolar macrophage functions (361). Alveolar macrophages seem to play an important role in both induction and abortion of disease in the lung. The next part of this thesis is devoted to the basic study of the alveolar macrophage.

THE ALVEOLAR MACROPHAGE (AM)

Origin and Kinetics

It is agreed that alveolar macrophages like other tissue macrophages, arise from bone marrow derived blood monocytes (487). This theory was based on studies of monocyte influx during an inflammatory response (493), and whether a similar population origin would be obtained under resting conditions remains controversial (60).

Bowden et al proposed a multicompartment model for the kinetics of the alveolar macrophages, where the direct precursor for these cells is the macrophage of the lung interstitial compartment which is derived from the peripheral blood monocyte (61, 62).

The alveolar macrophages face a constant threat of sudden ex-

cessive particulate exposure which requires a rapid and massive cellular response. This was thought to require a lung cell population with a potential of reproduction. The in vitro and in vivo proliferative capability of murine pulmonary macrophages, both the alveolar and interstitial, is well established ([145, 349,462]). The need of the peripheral monocytes to adapt to its new environment, its enzymatic and metabolic characteristics which are different than those of interstitial macrophages ([60]) and the immediate availability of the later cells render them a favorable source for effective rapid replenishment of the alveolar macrophage population.

In support of the rescue attitude by interstitial macrophages when free alveolar macrophages are required in demand conditions was the observation by Bowden and Adamson ([60]) that in some viral pneumonias proliferation of interstitial cells is a dominant feature, and that in granulomatous conditions such as sarcoidosis, tuberculosis and silicosis as well as in experimentally induced granulomas induced by the injection of freund adjuvent proliferation of interstitial cells is accompanied by an efflux of free alveolar macrophages. Also of interest in this area is the observation that leukemia patients with no circulating monocytes are capable of maintaining a normal population of alveolar macrophages (772).

In the steady state, AMs are developed from 15% of the blood monocytes, their direct precursors. These cells leave the circulation to settle in the lung ([54]). The mean turnover time for AM in mice and rats was calculated to be 27 days ([54,167,441]).

In humans, evidence of bone marrow origin of AM was demonstrated by the fluorescent Y body staining of alveolar macrophages in bone marrow transplanted recipients. In transplants from female donors to male recipients, there was a disappearance of macrophages containing the Y body ([534]). In these experiments under the conditions of bone marrow transplantation, the host macrophages were replaced in less than 100 days.

Hocking and Golde were able to demonstrate that human alveolar macrophages are capable of scheduled DNA synthesis; the tritiated thymidine labelling index of normal human AM ranged from 0.35 to 1.25%, suggesting that only a small fraction of the cell population was replicating (169,172,209).

Macrophages may carry genetic defect markers when such markers are present in the bone marrow precursor cells. Indeed, alveolar macrophages may be a product of a neoplastic clone. Chronic myelogenous leukemia patients with positive philadelphia chromosomes had the marker chromosome on their macrophages (168). Female patients heterozygous for glucose-6-phosphate dehydrogenase enzyme possessed macrophages with only a single enzyme type and therefore, clonely derived from the neoplastic progenator (148).

Development in the Lungs

In the rats which have a gestation period of 22 days, no macrophages can be detected in the developing airways before day 20. At this stage, interstitial cells show predominant synthesis of DNA, a mitotic activity which coincides with the initial appearance of macrophages in the primitive alveoli. These fetal macrophages are not fully mature, with few pseudopodia and sparse cytoplasmic lysozymes. They also show poor phagocytic potential towards available osmiophilic lamellar bodies released by Type 2 cells in the fluid filled alveolar sacs (60). In humans, functional immaturity of newborn mononuclear phagocytes is evident in a lower chemotactic response of the peripheral monocytes (513).

In the immediate post-natal period, a dramatic increase in the number of alveolar macrophages appears to be associated with high mitotic rate of interstitial cells when new alveoli develop (122,123).

Morphologic and functional maturation gradually increases with age in the alveolar macrophages. In rabbits, the bactericidal activity of an adult level is reached by the fourth week (353).

The findings of a poorly developed, gradually maturing AM may explain the higher susceptibility to pulmonary infections in newborns. It was suggested by Bowden and Adamson that the functional maturation and the increase in number of alveolar macrophages after birth are innate adaptive responses to the sudden change from a sterile intra-uterine environment to the external milieu of ambient air (60).

Harvest

Many investigators noted that microscopic examination of AMs in lung sections provide a restricted view and only a fragmentary insight of their morphology (60). Functional studies were yet even more difficult.

Myrvik et al in 1961 described a technique to procure AMs from rabbits by washing the lungs (341). Since then, broncho-pulmonary lavage as a method of obtaining free AMs for the study of morphology, metabolism and function became widely used. Brain et al demonstrated the superiority of using pulmonary lavage for the study of AMs by noting that a yield of 3-15 x 10^6 cells/gm of lung may be obtained by this method in eight mammalian species (65).

In humans, AMs are harvested by performing subsegmental, broncho-fiberscopic, saline, pulmonary lavage (95,111,170, 395). Such a procedure would require the patients to give an informed consent. Prospectively, in patient suffering particularly from recurrent chest infections, bronchial carcinoma and pulmonary fibrosis, it presents a method of collecting lung cell samples for assessing the local cellular response. The information obtained would be potentially valuable in managing the patients on individual bases. In the patients with recurrent lung infections and in the absence of a systemic immune defect, evaluation of the lung local immune functions would be of most importance in the management of these patients. In patients with bronchial carcinoma, bronchoscopy is frequently performed. Obtaining broncho-

alveolar cells for the study of the local natural killing ability of the alveolar macrophages, may enable us to explain individual variations in their responses to both the tumor and its therapy. Such studies would ultimately help to identify patients whose host response can be stimulated, and would allow standard treatment the greatest chance of success (95).

Technique of Broncho-alveolar Lavage

Many authors described the use of the fiberoptic bronchoscope transnasally to perform broncho-pulmonary lavage. After the respiratory tract is anesthetized with a local anesthetic, the tip of the bronchoscope is passed into a dependent bronchus and the lavage performed with the patient in the upright position (70,381,504).

In the method described by Cole et al, bronchoscopy was performed under local anesthesia, the patients being premedicated with omnopan 10-15 mgm and atropine 0.6 mgm and given oxygen by a nasal catheter throughout the bronchoscopy. Following inspection, the bronchoscope was impacted usually in a segment or a more peripheral bronchus of the lower lobe segments. It is then held in place by the assistant. Using a hand held syringe, 60 ml of sterile 0.9% saline corrected to pH 7.0 is injected while the patient inhales very slowly to total lung capacity and then quietly exhales to functional residual capacity. A total of 120-180 ml is injected. The tip of the bronchoscope is then withdrawn 1-2 cm and aspiration commenced while the patient breaths quietly within normal tidal range. In order to obtain a lavage sample of 100-150 ml, a total of 300 to 500 ml of saline was injected (95). However, unless the sample retrieved is to be used for an extensive cellular and biochemical analyses, it is conceivable to obtain a sufficient cellular yield for functional studies from a much smaller lavage sample. The highest cell yield obtained in their series (1.4×10^8) is that from a healthy cigarette smoker, the lowest yield was 2×10^6 obtained from a non-smoker (95, 381,504).

Morbidity of Broncho-alveolar Lavage

Out of 120 patients that had pulmonary lavage, 3 developed acute respiratory distress which lasted for one minute after immediate termination of the lavage procedure and removal of the fiberoptic bronchoscope. Three more patients developed syncope during or immediately following the procedure and recovered rapidly after removal of the bronchoscope and lying flat, except one patient who required 2.4 mgm atropine I.V. Eight of the 42 patients developed fever and 6 of these had radiologic shadowing in their lungs. However, 5 of the fevers and 4 of the shadowings were in patients who previously had recurrent pulmonary infections and responded rapidly to antibiotics and physiotherapy (95). In another series 11 of 100 normal volunteers who were subjected to broncho-pulmonary lavage, no difficulties or complications were encountered.

It appears that this procedure is tolerated well by most patients and that the potential risk included acute respiratory distress, vasovagal syncope, fever, pulmonary infiltration, and vocal cord damage. It is associated with a mean fall of Pa 0_2 of 3.0 KPa (22.7 mm Hg) which remains reduced for 120 minutes, and which makes routine oxygen supplement during the procedure recommended (95). Bronchoscopic lavage for the previous reason was contra-indicated in the respiratory or cardiac risk patients.

Cell Separation

The mammalian lung contains over 40 different types of cells (19). Only with the progress in the techniques of isolation and characterization of each cell type will pneumology advance. Although many of the pulmonary functions are thought to be understood, the use of isolated perfused lung, lung slices and subcellular fractions in the presence of diverse heterogenous cell populations limits the interpretation of many studies (284).

Although difficulty is usually encountered in cell isolation techniques (311), it presents the advantage of working in a

controlled and chemically defined medium in the absence of other cell types. Cell isolation can be obtained by tissue dissociation requiring incubation of the tissues in a medium, its enzymatic digestion and the use of mechanical shear to clean tight junctions (496). It is now evident that these techniques could not pass without frequently affecting cellular function. Another manner for cell isolation is using different cell separation procedures. In summary, this includes the use of physical methods, the most common of which are the gradient centrifugation, unit gravity and centrifugal elutriation, all of which sediment cells depending upon its size and density (76,110,313,324,496). The use of electrophoresis, flow microfluorimetry, affinity columns, selective destructions and primary cultures is dependent upon the characters of cells to be separated and the type of study to be performed. The equation for velocity sedimentation of an unchanged spherical particle is presented below -

Unit Gravity

$$V = \frac{d\,x}{dt} = \frac{2\,gr^2}{\eta}\,(P\ cell - P\ med.)$$

where:
- v = velocity of sedimentation (cm/sec),
- x = vertical position (cm),
- t = time (sec),
- r = spherical radius (cm),
- P cell = density of cell (g/cm^3),
- P med = density of medium (g/cm^3),
- η = fluid viscosity (gm/cm/sec).

Structure

The predominant morphological feature of lavaged AMs is their heterogeneity (50). Their size varies from 15 to 50 µm in diameter (209) with a variable nucleus to cytoplasm ratio. This, however, is commonly about 1:3.

In the Giemsa-stained preparation, the cytoplasm appears gray, and planished with dark blue granules and cytoplasmic vacuoules. As any other mononuclear phagocyte, it contains abundant amounts of non-specific esterase and is positively stained with the periodic acid-Schiff technic. The variations in structure and size are largely dependent upon the age of the cell, its activity, and phagocytic content (60). While immature non-working cells have smooth endoplasmic reticulum, those involved in phagocytic activity show an increase in rough endoplasmic reticulum.

Alveolar macrophages viewed under the scanning electron microscope (SEM) show typical ruffled membranes and pleomorphic cell shapes. Of these, in a monolayer two distinct types of cells were detected, flat and round cells. Both cells maintained adherency to cover slips, but the flat cells were spread with thin extended periphery. In the non-smoker, 55% of AMs are flat and 45% are rounded (111). When both types of cells (flat and rounded) were allowed to phagocytize iron particles which could be seen within cells using a back-scatter mode of the SEM, the flat cells were 4 times more efficient as phagocytes compared to the round cells (111).

The heterogeneity of the AM population is thus an expression of its activity. Following phagocytosis, the intra-cellular acid phosphatase activity moves from its exclusively perinuclear location to a more general distribution throughout the cytoplasm (60). Alveolar macrophages may occasionally be multi-nucleated, and frequently show a prominent nucleus. Free ribosomes, well developed golgi apparatus, mitochondria and glycogen are easily detected (203,308). Occasionally, cells may be highly vaculated with a foamy cytoplasm. However, lipids are normally scarce and can be detected by oil red 0 stain (209).

Since AMs are normally aerobic, Voisin et al attempted to study the AM in an environment similar to that in the lung. He maintained the cells on a filter which would allow the macrophage to obtain nutrients by capillary action while their exposed surface was facing air with an optimal 0_2 content (491). These macrophages were noted to be rather rounded in difference to

those put on glass (280) In a different fixation procedure the surface of AMs facing the air was found to be smooth, while that in contact with the alveolar surface had spread pseudopodia (273).

Effects of O_2 Change

In the lungs, local environmental changes 182 and disease (183,282) may alter its ability to clear bacteria

Acute hypoxia has been shown to decrease net bacterial lung clearance of Staphylococcus aureus and Staph epidermidis but not of proteus mirabilis (183 This decrease in net bacterial lung clearance in the presence of acute hypoxia was attributed to alteration of in situ killing a function attributed to the highly O_2 dependent alveolar macrophage

In another experiment by Harris et al the clearance of Staph aureus, Klebsiella pneumonia and E coli was found to be impaired in the presence of hypoxia, while streptococcus pneumonia was cleared normally under the same hypoxic condition They postulated that in contrary to the previously used bacteria, the clearance of streptococcus pneumonia was oxygen independent (201

Goldstein et al studied the effect of exposure to 2-5 ppm of ozone for 5 hours on the lysosomal enzymes and intra-cellular killing of AMs engaged in phagocytosis of Staph. aureus (173) He found a diminished rate of bacterial clearance demonstrated by decreased ingestion, increased intra and extra-cellular micro-colonization by staphylococci and an absence of enzyme activity in the macrophages containing bacterial micro-colonies He observed that the absence of enzyme activity occurred only in macrophages subjected to the dual insult of ozone exposure and ingested bacteria, but did not occur in macrophages without ingested bacteria which suggest a relationship between impairment in the bactericidal capacity and the cellular activities of the lysosomal enzymes.

Bio-energetic Differences

While phagocytosis by the mononuclear phagocytes and neutrophils is accompanied by an increased respiratory activity and hexose monophosphate shunt activity, alveolar macrophages fail to show a similar increase in their oxygen consumption (21,253,254,413).

In contrast to other tissue macrophages, the alveolar macrophages operate in an aerobic atmosphere (PO_2 ~ 100 torr), whereas peritoneal macrophages develop and function in a relatively anaerobic environment (PO_2 ~ 5 torr) (449). Under normal resting conditions, AMs have a relatively high O_2 consumption as compared to peritoneal macrophages (362,449) Histochemically, the AMs were shown to have higher succinic dehydrogenase and cytochrome oxidase activities (105) and their mitochondria were found to be greater in both number and size with more dense cristae than peritoneal macrophages (255), which emphasizes the dependence of the AM on aerobic metabolism.

Simon et al demonstrated that changes in the ambient oxygen tension alter enzyme activity in the cultivated macrophage. He suggested that a high O_2 tension in the lung may act on precursor cells to produce high activities of oxidative phosphorylation enzymes and low activities of glycolytic enzymes, and that opposite effects may occur in the other tissue macrophages (449). He then went forward to hypothesize some pathophysiologic implications of his observations Acute hypoxia will depress O_2 dependent energy provision in the AM and significantly decrease energy dependent AM function (e g , phagocytosis and pinocytosis). With more chronic hypoxia, decreased molecular O_2 would alter AM energy metabolism enzymes resulting in a more favorable pattern for energy provision in the diseased lung (449).

Functions

(1) Phagocytosis

"The First Description of Pinocytosis"

"I first observed this phenomenon in May, 1859, in a specimen of thetis fimbria, which I had injected with an aqueous suspension

of fine indigo particles in connection with a study of the vascular system. When I put the fine vessels running in the transparent subcutaneous tissues of a loose flap on the animal's back under the microscope, I was more than a little surprised, a few hours later to find the colorless blood cells filled with fine indigo particles. These indigo particles had penetrated the blood cells, sometimes only a few, but sometimes in large numbers, and had formed aggregates, mainly around the slightly oval nucleus" (193).

Haeckel (1862)

in his book "Die Radiolaren"

"The Starfish Experiment"

A century ago, Metchnikoff was the first to describe the macrophage and the microphage. He recognized that phagocytes not only serve as scavengers, but also play an important role in the host defense against micro-organisms and championed the cause of phagocytosis as the basis of survival against pyogenic infection

One day in Messina, Metchnikoff was quietly alone at home at his microscope, recovering from traumatic political experiences that had led to his decision to leave his post at the University of Odessa in Russia. He was working at his research "with passion" in his new and beautiful environment. His family had gone to the circus to see some performing apes. He sat watching the mobile wandering cells in a starfish larva, which was beautifully transparent and had long been a favorite experimental object in his studies. The idea struck him, that mobile cells, which were able to ingest solid particles, could serve to defend the organism against "noxious intruders". In great excitement, he developed his simple experiment: to place a thorn under the skin of the larva to see whether the wandering cells would mobilize around its tip at the point of injury. After a restless night, his expectations were fully realized. "This experience served as the basis of the phagocytic theory, to the development of which I consecrated the next 25 years of my life".

Metchnikoff (32)

a) Recognition

Having arrived at the inflamed or invaded site, the macrophage must recognize what to attack. This remarkable selectivity is believed to be determined by the surface characters of the object to be ingested. Although the stimulus on the surfaces of certain particles that trigger engulfment is still enigmatic with subtle chemical features, the field has been extensively explored and the recognition of the role of antibodies and opsonins are clear to an extent.

b) Opsonic Requirements

The realization that humoral factors and phagocytes co-operate is a concept which was popularized by George Bernard Shaw in the Doctor's Dilemma "The phagocytes won't eat the microbes unless the microbes are nicely buttered for them. Well, the patient manufactures the butter for himself all right; but my discovery is that the manufacture of that butter, which I call opsonin, goes on in the system by ups and downs ..... There is at bottom only one genuinely scientific treatment for all diseases, and that is to stimulate the phagocytes".

The only antibodies to be found opsonically active are those of the class IgG (474), and of these the subclass IgG_1 and IgG_3 are the species that actually participate in the process of opsonization (222). The expression of IgG opsonic activity requires that both its Fc and Fab portions be intact. The small amount of this type of opsonin present in normal serum can be augmented by the subjection of the organism to hyperimmunization (382,529).

While opsonins of the IgG group are heat stable, another group of heat labile opsonins is entirely attributed to complement proteins. A quantity of IgG which may be insufficient as an opsonin may react with bacteria, resulting in the activation of the hemolytic complement protein C_1, C_4 and C_3. Bacteria on their own or in the presence of IgG can also activate the properdin system. The latter system will then promote the

deposition of an opsonically active fragment of C_3 on the surface of the microbe (163,240,460,476).

C_5 which is present in very minute amounts in the serum, and is structurally similar to C_3, may contribute to the opsonic power of fresh serum (462). However, IgG and activated C_3 are the most effective opsonins of serum (474).

Following opsonization, the ingestion of IgG or complement coated particles by macrophages proceeds by first the attachment of these particles to specific receptors on the phagocytic membrane followed by its interiorization, a process which is regulated not only by the distribution of IgG or complement on the particle's surface, but also by the availability of membrane receptors for these ligands on the surface of the phagocytic cells (322).

Measuring the ingestion rates of particles by quantitative methods have yielded many findings in relation to the receptor-particle binding. Antibodies directed against the plasma membrane of phagocytes have altered the ingestion rates of opsonized and unopsonized particles (213). In vitro, macrophages continue to respond to antibody coated particles, while C_3 opsonized particles may cease to provoke a similar response ( ). And while C_3 activates the maximal ingestion rates without increasing the affinity of particles for the receptor (475), IgG opsonized particles increase both the maximal ingestion rate and the affinity of the particles for the receptors (473).

Macrophages and neutrophils show differences in their behavior towards C_3 and IgG coated particles. While macrophages respond to more lightly opsonized particles, neutrophils require the presence of a larger amount of opsonins (474).

In addition, there are qualitative and quantitative differences in the requirements for optimal ingestion of particles which relate not only to the type of the organism, but also to the type of the phagocyte. Hof et al compared the opsonic requirements for uptake of radio-labelled Staphylococcus aureus or

Streptococcus pneumoniae Type 7 by human alveolar macrophages or neutrophils. Alveolar macrophages readily phagocytized S. aureus organisms pre-opsonized with 1% albumin, serum, or heated complement deplete serum; whereas optimal uptake of S. aureus by neutrophils occurred only with unheated complement replete serum of 2.5% concentration. In contrast, the uptake of S. pneumoniae by alveolar macrophages or neutrophils required high concentrations (more than 40%) of unheated serum (210).

Murphey et al demonstrated that normal rabbit serum was a satisfactory opsonin for the phagocytosis of Staphylococcus aureus, but not for Pseudomonas aeruginosa by rabbit alveolar macrophages. They also noted that an intact alternative complement pathway enhanced phagocytosis when the concentration of staphylococcal immune serum was lowered to 0.3%, while at a concentration of 10%, an intact alternative complement pathway was not required. On the other hand, phagocytosis of Pseudomonas aeruginosa was enhanced significantly by either the classic or the alternative complement pathway when the concentration of pseudomonas immune serum was lowered to 1% (338). This study implicates the importance of complement in enhancing phagocytosis by the alveolar macrophages when antibody concentrations are low, a situation which may be the case in vivo.

An observation by Bloom dating back to 1927 (48) that antibody coated avian erythrocytes clustered about macrophages like the petals of a daisy, is now used to identify IgG and complement receptors on the surface of macrophages. The characteristic formation of rosettes by erythrocytes coated with IgG molecules or to which opsonic fragment of C_3 has been affixed, may parallel those of antibody and complement activated antibodies (474).

Harmsen and Jeska studied the presence of IgM, IgG and complement receptors on porcine alveolar macrophages using the previously mentioned principle. Approximately 90% of the

alveolar macrophages formed rosettes with IgG coated sheep erythrocytes and 23% formed rosettes with complement coated sheep erythrocytes. In addition, while IgG was capable of mediating both attachment of and endocytosis of the target cells, complement in the absence of IgG mediated attachment only (200).

c) Events in Particle Ingestion

When the macrophage encounters a tasty particle, it extends pseudopodia to surround and engulf it. These pseudopodia characteristically have no phase-dense organelles and lack definable structure. In electron micrographs, they are frequently composed of overlapping 5-7 nm (actin) filament (206). The filaments in the advancing pseudopod first appear randomly oriented. With further advancement, they become parallel to the long axis of the pseudopod and more densely packed.

Next, the granules move to fuse with the formed phagosome at the plasma membrane, releasing their enzymatic contents into what is now called the phagolysosome and degranulation of contents is initiated.

There is no question that contractile proteins control the macrophage movements in phagocytosis. Hartwig et al purified actin, myosin, actin binding protein (ABP) and a calcium-dependent actin regulator protein (CDAR) from rabbit lung macrophages. Using the contractile behavior of crude extract as a model, it was possible to make those purified proteins behave similarly. It was also found that all contractile processes performed by the macrophage are proceeded by the establishment of cytoplasmic rigidity gradients which are possibly generated by the cross linking of actin filament by ABP, and that free calcium controls this rigidity, and finally, that a sliding actin filament mechanism develops the shearing force of movement which in itself is automated by myosin (206).

d) Cyclic Nucleotides and Phagosome-Lysosome Fusion

Following the ingestion of particles by the macrophage, the lysosomes fuse with the phagosomes and a blend of enzymes is set to degrade this particle. However, in vitro studies have shown that several pathogen species are able to survive and multiply inside the macrophage within the phagosomes by either inhibiting phagosome-lysosome fusion or another mechanism. These organisms include Mycobacterium tuberculosis (16), Toxoplasma gondii (302), Microbacterium microti (242) and Chlamydia species (154). This failure of phagosome-lysosome fusion can be reversed by coating the organisms with antibody prior to ingestion (154).

The failure of phagosome-lysosome fusion was believed to be induced by an increase in cyclic adenosine 3'-5' monophosphate (cyclic AMP) in infected macrophages (302), the extent of fusion being dependent on the balance between opposing effects of cyclic AMP - a fusion inhibitor and cyclic GMP - a fusion promoter. However, when exogenous cyclic nucleotides were introduced into phagosomes via positively charged liposomes, no effect was detected on fusion (302).

(2) Chemotaxis

Chemotaxis is defined by Zigmond as a bias in the movement of a cell or organism along the axis of a chemical gradient which results from some parameter of the locomotion, e.g., the speed of movement and/or the frequency, magnitude or direction of turns, altering as a function of the direction of locomotion in the gradient (533). Chemotaxis is of particular interest in the analysis of the mechanisms behind the congregation of phagocytes at the site of an inflammatory response.

The interaction between micro-organisms and host tissue has been known for many years to cause the generation of chemotactic factors either directly or by the activation of complement factors with the subsequent release of protein fragments with

chemotactic activity (465). The generation of complement fragments in the cleavage of C_3 and generation of C_3a by plasmin along the early contact phase of the intrinsic system of blood coagulation is also thought to lead to the production of chemotactic factors (38).

Chemical substances in their environment may influence the locomotion of cells in at least two ways: by determining the speed of movement (chemokinesis) and by determining the direction of movement (chemotaxis) (261).

Chemokinetic reactions may be determined by the nature of the substratum on which cells move. Wilkson and Allan observed that if a surface (micropore filter or glass) is coated with albumin and then washed, chemokinetic locomotion of neutrophils on the albumin coated surface is still observed, even though the fluid phase is albumin free. They concluded that albumin may act as a chemokinetic agent by providing a suitable substratum for cell movement, whereas uncoated filters or glass do not (521). In a later study, the same authors presented evidence of similar chemokinetic effects of albumin and IgG on human blood monocytes (522). Gallin et al monitored the membrane potential changes in cultured guinea pig and human macrophages during exposure to chemotactic factors using direct intracellular recording techniques. Cultured macrophages exhibited spontaneous membrane hyper-polarization associated with an increase in membrane permeability to potassium. Exposure to endotoxin activated serum, partially purified C_5a, and chemotactically active synthetic N-Formylmethionyl peptides resulted in a prolonged membrane hyper-polarization sometimes preceded by a smaller depolarization. This chemoattractant-obtained hyperpolarization was similarly produced when the calcium ionophore A23187 was used (158). From this study, it was concluded that changes in membrane potential are early events in macrophage activation by chemotactic factors.

Invading micro-organisms may lead to the generation of factors which are either primarily chemotactic or by activating serum

complements. This complement activation can occur by a specific reaction in which bacterial antigen-antibody complex activates the hemolytic complement components C_1, C_4 and resulting in the consequent activation of C_3 and C_5 both with chemotactic activities (463). The generation of C_3a and C_5a in the absence of specific antibodies or in response to antibodies of certain subclass specificities can occur by activating the incompletely defined alternate complement pathway or properdin system (428). Ward et al noted that non-specific proteases released from bacteria or damaged tissues can attack C_3 and C_5 directly to yield C_3a and C_5a (503).

A protein released by aggregating platelets was observed to generate a chemotactic factor from C_5a (507). Kallikrein and Plasminogen activators were also found to contain chemotactic powers (248), indicating that intrinsic reactions in serum have the capacity to produce chemoattractants. In addition, macrophages, lymphocytes and neutrophils in the absence of serum were found to be able to release their own chemotactic factors (10,108).

Massive complement activation occurs intra-vascularly in several situations, such as in gram-negative septic shock, pneumococcal bacteremias, acute pancreatitis and SLE (232). Bower et al hypothesized that granulocytes which are uniformly filled with activated complement in such situations would not be able to perceive any further activated complement messages in their environment and would be, thereby, essentially "paralysed". The in vitro exposure of granulocytes to activated complement, indeed, resulted in their failure to respond to chemoattractants in chambers (93).

Ward observed that alveolar macrophages respond poorly to migrational stimuli in in-vitro chemotactic assay systems (502). This observation was later supported by Dauber and Daniele who stressed that the poor response to certain serum derived chemotaxins was not sufficient evidence to suggest

that the alveolar macrophage lacked the potential to respond chemotactically (106). They also hypothesized that the variation in the response of guinea pig alveolar macrophages may be attributed to metabolic alterations. In fact, they showed that with optimal Po_2 concentrations, alveolar macrophages responded to chemotactic formylmethionyl peptides.

Shwartz et al using different protein concentrations and separate molecular weight fractions of adult rhesus monkey lung lining lavage presented evidence of the latter having a chemotactic effect on alveolar macrophages. This chemotactic effect was directly related to the concentration of proteins in the lavage fluid and was maximal in the molecular weight fraction below 5,000 daltons (445).

Kemp et al (263) demonstrated that human IgA, but not IgG precipitate, inhibited both chemokinesis and chemotaxis of human neutrophils and monocytes and since IgA does not activate complement by the classical pathway (521), it does not produce chemotactic factors from serum (230) and does not opsonize bacteria or cells (525,534), which suggests that IgA may dampen down potentially damaging inflammatory responses at mucosal surfaces (219).

Pierce et al, using histological techniques, quantified the amount of polymorphonuclear leukocyte infiltration into the bronchi and alveoli 4 hours after an aerosol inoculation of mice with bacteria. They demonstrated that Klebsiella pneumonia or Escherichia coli aerosol inoculation significantly increased the polymorphonuclear leukocyte infiltration, while Staphylococcus aureus did not, indicating that the cellular response varies according to the organism used (263). Rylander and associates supported this interpretation by noting that the number of polymorphonuclear leukocytes, but not macrophages among free lung cells, obtained by lavage of guinea pigs, increased after aerosol exposure of E. coli or K. pneumoniae, but not after exposure to Bacillus subtilis (419).

Hunninghake et al demonstrated that guinea pig alveolar macrophages

involved in phagocytizing heat killed Staphylococcus aureus generated a chemotactic factor which attracts monocytes, but more preferentially neutrophils (229). Dauber and Daniele recently detected a chemotactic factor in the medium of guinea pig lung macrophage cultures. This factor manifested maximum chemotactic effect on neutrophils and least effect on lung macrophages. Lymphocytes also responded to this factor which suggests that alveolar macrophages secrete chemoattractants with abilities to initiate both acute and chronic inflammatory reactions by directing the influx of neutrophils, macrophages and lymphocytes (107).

(3) Intra-cellular Bactericidal Function

In the mononuclear phagocytes, anti-microbial events are either oxygen dependent or independent. Oxygen, is partially reduced in the cells to yield intermediates, namely, the superoxide anion, hydrogen peroxide and hydroxy radicals, and the interaction between those species may lead to the production of excided oxygen (singlet oxygen). Oxygen independent mechanisms are attributed to the fall in intra-phagosomal pH and the release of lysozyme, lactoferrin, and cationic proteins into the phagolysosomes (267).

a) Myeloperoxidase

The myeloperoxidase-mediated system contributes significantly to the anti-microbial activity of polymorphonuclear leukocytes, peripheral blood monocytes and recently recruited exudate macrophages. Following phagocytosis, myeloperoxidase is discharged into the phagosome where it interacts with H_2O_2 produced by either the phagocyte or as part of the microbial metabolism in what is considered a suicidal attempt. The phagocytosis induced respiratory burst does not necessarily reflect an increased intra-cellular killing activity by the phagocytes and it is of interest that myeloperoxidase deficient polymorphonuclear leukocytes have a markedly impaired microbicidal activity even in the presence of an increased respiratory burst (267).

b) Superoxide Anion

The morphological, functional and metabolic manifestations of activation in elicited or infection-activated macrophages are accompanied by an enhanced superoxide anion release when phagocytosis or membrane perturbation occurs. Johnston et al demonstrated an enhanced superoxide anion response when macrophages were exposed in vitro to proteolysis or subjected to certain components of micro-organisms. They concluded that whether induced in vivo by lymphokines, enzymes from other phagocytic cells, or microbial products, such priming of macrophages could accentuate their oxidative response to invading micro-organisms or malignant cells (239).

c) Hydrogen Peroxide

Our understanding of the mechanisms of hydrogen peroxide release by mononuclear phagocytes is still fragmentary.

Studies with alveolar macrophage have given results in variance with those of blood monocytes and peritoneal macrophages. While the latter produce considerable amounts of hydrogen peroxide, extremely low levels of hydrogen peroxide are released during phagocytosis by the alveolar macrophages in rats and rabbits (42,498). Simon et al postulated that these cells which are acclimated to an oxygen-rich environment may have peroxide-detoxifying mechanisms which would allow minimal H_2O_2 escape from the phagocytic vacuoles into the extra-cellular milieu (449).

The process of phagocytosis by macrophages is associated with dramatic changes of oxidative metabolism. Widely, it is accepted that the biological significance of the respiratory burst is that of providing a mixture of reactive compounds which can kill the micro-organism whether it is intra-cellular or extra-cellular. Rossi et al studied the respiratory burst of rabbit alveolar macrophage. In normal cells, this was associated with the formation of superoxide anion and hydrogen peroxide. However, these inter-

mediates were not released owing to the high efficiency of an intra-cellular mechanism for their transformation and degradation. When alveolar macrophages were activated by BCG, a greater respiratory burst was accompanied by a change in the equilibrium between synthesis and degradation of the intermediates, resulting in the extra-cellular release of high amounts of such intermediates when phagocytosis was initiated (412).

d) Molecular Oxygen

Oren et al reported that alveolar macrophages exhibit substantially higher oxygen consumption than peritoneal macrophages, and while phagocytosis by peritoneal macrophages, which is provided by glycolysis, is not impaired by anaerobiosis or by cyanide treatment, alveolar macrophage phagocytic activity is dependend on oxidative energy metabolism and is decreased by anaerobiosis and cyanide treatment (361). These differences in metabolic responses are only a part of the end of line differentiation in the functions of mononuclear phagocytes as they settle in their new environments whether it is peritoneal, alveolar or other.

Axline et al studied the effect of acute and chronic in vitro hypoxia on alveolar macrophage phagocytic functions. When the phagocytes were maintained under chronic anaerobiosis (96 hours), their phagocytic function only reached approximately half the maximal value achieved by cells cultivated aerobically. They also provided evidence supportingthe role of molecular oxygen as a metabolic regulator when the glycolytic capacity was found to exhibit marked reciprocal changes in response to changes in the oxygen concentration of the medium (20).

e) Controversy in Methods of Studying Intra-cellular Killing

Study of intra-cellular killing of micro-organisms by macrophages can be approached in two main ways. The first is microbiological where killing by phagocytic cells is estimated from the decrease in the number of viable, cell associated, bacteria during in-

cubation of macrophages together with bacteria (384). This method carries the disadvantage that the rate of bacterial killing is dependent on the continuous ingestion of new bacteria. A decrease in phagocytic activity may not be discriminated from a decrease in the intracellular killing ability in the studied cells. The second approach is a chemical where conversion of oxygen into bactericidal agents superoxide anion and hydrogen peroxide during the metabolic burst associated with phagocytosis is used as an indirect measure of the bactericidal capacity of the phagocytic cells (267). However, intracellular killing itself is not elucidated because the metabolic burst is measured during the continuous ingestion of the micro-organism.

Leijh et al modified the usual microbiological method by allowing pre-opsonized micro-organisms to be ingested for a very short time, then separating the uningested bacteria from the phagocytes with their contained ingested bacteria by differential centrifugation, followed by performing viable counts on the cultured phagocytic cells. The decrease in the number of viable intra-cellular bacteria determined in relation to time represents the intra-cellular killing (289). However, this method carried the disadvantage that, if viable counts obtained are low, it is not possible to know whether this would be a reflection of low phagocytic activity or a high intra-cellular killing ability.

f) Extra-cellular Requirements for Intra-cellular Killing

It was found that all catalase-positive micro-organisms studied (Staphylococcus aureus, Staphylococcus epidermidis and Escherichia coli) required extra-cellular serum to be effectively killed intra-cellularly by human monocytes, and almost no killing was observed in the absence of serum. On the other hand, the killing of catalase-negative micro-organisms (Streptococcus pneumoniae, Streptococcus pyogenes and Streptococcus faecalis) did not necessitate the presence of serum, and the stimulation by serum proved to be additional to the killing obtained in its absence (289).

[illegible] the same between the intra-cellular killing requirements for catalase-negative and catalase positive micro-organisms were found in phagocytes of patients suffering from chronic granulomatous disease (249)

The intra-cellular killing of Staphylococcus aureus by human monocytes was shown to be suboptimal in the absence of IgG, the killing being stimulated by an interaction of the Fc part of IgG with the Fc receptor in the monocyte membrane. Complement components generated through the alternative pathway were shown also to stimulate the intra-cellular killing of Staphylococcus aureus. A decrease in the number of C3b receptors after treatment of the monocytes with pronase or anti-monocyte serum correlated with a decrease in the intra-cellular killing of Staphylococcus aureus, indicating that C3b receptor interaction stimulates intra-cellular killing of these micro-organisms (290).

4. Secretory Functions

a) Enzyme Release and Superoxide Anion Production

Only recently has the secretory functions of the pulmonary macrophages received attention. It has been suggested that proteases secreted by macrophages may play a role in the lysis of collagen and the remodelling of scars (464). This idea is based upon the knowledge that whereas the unstimulated or quiescent macrophage is concerned primarily with phagocytosis and endogenous digestion, the activated macrophage, identified morphologically as an epithelioid cell, is capable of exogenous secretion. Proof of the postulate is not at hand however, and the question is further complicated by the functional duality of the fibroblast as secretor and degrader of collagen and by the lack of suitable markers which will distinguish fibroblasts and macrophages within the general population of pulmonary interstitial cells (60).

The pulmonary macrophage has been implicated in the pathogenesis of emphysema by its ability to secrete proteolytic enzymes. Enzymatic secretion by the macrophage usually follows some form of extrinsic stimulus and since cigarette smoking is a dominant

factor in the etiology of emphysema, the response of the macrophage to tobacco smoke has attracted considerable interest. Although significant morphological changes have been observed in the alveolar macrophages of smokers, its function significance is not known (71).

The macrophages which are adapted to cigarette smoking may indeed be involved in the destruction of terminal airways and air sacs with their secretory potential. Proteases with elastic-like activity have been demonstrated in macrophages from various sites.

White et al reported that an unstimulated alveolar macrophage produces more elastase than an activated peritoneal cell and that in smokers, this enzyme activity is greatly enhanced (517).

On the other hand, the enthusiasm for a macrophage role in destructive diseases of the lung did not obscure the protective role of this cell. The macrophages are known to bind and incorporate neutrophil derived elastase (80), phagocytosis of elastase rich neutrophils and the neutralization of their enzymes in the lungs of smokers may, in fact, be more important than the postulated role of the macrophage as a digester of elastic fibers (47).

Recently, this concept was confirmed by the work of White et al who demonstrated that Alpha-2-macroglobulin, one of the major protease inhibitors which insignificantly crosses endothelial barriers is secreted locally in the alveoli of smokers by the alveolar macrophage (515). In rats, it was shown that alveolar macrophages were able to secrete Alpha-1-proteinase inhibitor, which suggests that the accumulations of these cells at sites of damage in the smoker's lung represent an effort to protect the alveoli against proteolytic attack (516).

Alpha anti-trypsin (93) was also identified in human pulmonary alveolar macrophage. Its activity was found to be elevated in pulmonary alveolar macrophages obtained from smokers, and in one patient with severe homozygous α-anti-trypsin deficiency,

macrophages were found to have reduced α-anti-trypsin activity (360).

Selective release of lysosomal enzymes by alveolar macrophages was noticed to occur during the process of phagocytizing zymosan particles and while smokers' alveolar macrophages were shown to secrete elastase in vitro, non-smokers' cells released no detectable elastolytic secretions in response to a phagocytic stimulus (404). Thus, it seems that the ingestion of particulates in tobacco smoke by the alveolar macrophage may lead to the release of proteolytic enzymes into the bronchio-alveolar spaces.

Many other enzymes have been detected in alveolar macrophage secretions, platelet activating factor, B-glucuronidase, neutral proteases and possibly slow reacting substance of anaphylaxis (SRS-A) (22, 33, 243). Superoxide anion production by alveolar macrophages have been previously mentioned and its role in the etiology of many lung diseases is currently the subject of interest of many researchers.

During the last few years, immunoglobulins of the E class have been shown to bind to rat, baboon and human mononuclear phagocytes, resulting in their activation and conversion into cytotoxic effector cells (243). Rat monoclonal IgE protein was able to bind to the macrophage membrane within 15 minutes (120) and to induce selective release of lysosomal enzymes after subsequent addition of anti-IgE antibody (119).

The role of immunoglobulin E in the activation of human alveolar macrophages and the induction of their secretion have been further endorsed by the finding that the specific release of lysosomal B-glucuronidase and neutral proteases was successively obtained when alveolar macrophages were incubated with IgE and then with anti-IgE. When IgE opsonized zymosan was added to IgE incubated cells, superoxide anion O_2 generation was obtained. Finally, when alveolar macrophages were cultured with serum from patients allergic to house dust or to grass pollen, and then with the specific allergen, the cells were found to release B-glucuronidase (243).

An interesting analogy can be drawn by the system previously described and the possibility of inducing mast cell degranulation in the lung by the same stimulating agents, i.e., anti-IgE or specific allergens (365). Alveolar macrophages may be considered as a second cell population involved in immediate hyper-sensitivity together with mast cells (243).

(b) Secretion of Chemotactants

Complement components have been reported to be synthesized by macrophages and their monocyte precursors by several investigators. Peritoneal macrophages synthesize the third and fourth components of complement (470). Human monocytes in a longterm primary culture synthesize and secrete the second component of complement (134).

Kazmierowski et al observed that following broncho-alveolar lavage of a primate lung, the population of polymorphonuclear leukocytes increased from an initial 0% at the onset of lavage to form 45 to 90% of the cellular contents in the lavage fluid 4 hours later. To explain the ingress of the polymorphonuclear leukocytes, the lavage fluid was tested for the presence of chemotactant factors. Two components were identified, a larger component with an estimated molecular of 15,000 daltons was chemotactic for both polymorphonuclear leukocytes and mononuclear phagocytes. Because this component was inactivated with anti-serum against the fifth component of complement, resistant to heat and unaffected by anti-serum against C_3, this factor was considered analogous to the cleavage product of the fifth component of complement, C_5a. The other component identified was chemotactic to polymorphonuclear leukocytes only with a smaller molecular weight of 5,000 daltons, a heat labile character. These chemotactic factors were also found to be secreted by alveolar macrophages in vitro when stimulated by phagocytosis of opsonized Staphylococcus albus or by attachment to a glass surface (256).

A study taken by Hunninghake et al delineated further the role of the alveolar macrophage as a secretor of chemotaxin in the

modulation of acute pulmonary inflammation when they demonstrated that the generation of a chemotactic factor with preferential activity for neutrophils by guinea pig alveolar macrophages followed the intra-tracheal injection of heat killed Staphylococcus aureus in vivo or the phagocytosis of the same bacteria in vitro (228).

Recently, Dauber and Daniele demonstrated that chemotaxins secreted by guinea pig lung macrophages attracts neutrophils, as well as peripheral blood monocytes, peritoneal macrophages and lymphocytes derived from tracho-bronchial lymph nodes (107).

c) Chemical Modulators and Other Active Substances

In cultures, many kinds of substances have been identified as products of macrophages. However, it is not known whether alveolar macrophages produce several or all substances at the same time. Transferrin (469) and transcobalamine II (386) were shown to be produced in macrophages. After endotoxin and phagocytic stimulation, all kinds of mononuclear phagocytes release endogenous pyrogens (18, 56). However, this response is only detected in vivo. Angiotensin converting enzyme has been recently reported to be secreted by mononuclear phagocytes in culture, specially after exposure to dexamethasone (153). The clinical implication of angiotensin converting enzyme in the lungs is still uncertain. The prospect that hypertension of "renin excess type" might respond to inhibition of the converting enzyme has stimulated a search for an immunologic approach for treating high blood pressure.

Colony stimulating factor (CSF), a glycoprotein with a molecular weight of 70,000 daltons, which acts on the mononuclear phagocyte and granulocyte precursors, was found to be produced by human alveolar macrophages in vitro (171). This may suggest that alveolar macrophages have the potential to release factors which can stimulate the production of phagocytic cells by the bone marrow, which may then migrate to the lungs as rapid deployment forces.

Another substance which is probably released by macrophages at the site of inflammation is the factor increasing monocytopoiesis (FIM). This factor can enhance the proliferation of promonocytes and monoblasts. However, the possibility of its production by cells other than macrophages at the site of inflammation has not been completely ruled out (488).

Prostaglandins are produced by mouse and human mononuclear phagocytes (275). Endotoxins and phagocytosis enhances its secretion in vitro. Further, prostaglandins were found to inhibit proliferation of precursor cells of the granulocyte and the mononuclear phagocyte series (276).

Arginase was found to be released from activated macrophages both in vitro and in vivo. This deprives the medium from arginine, thus interfering with the growth of other cells, e.g., tumor cells (102,276).

Interferon has been shown to be synthesized and released by non-infected macrophages. When macrophages are virus infected, its production is increased (461). Fibroblasts as well as endothelial cell proliferation has been induced by factors secreted by stimulated macrophages (288,380).

Thus, it seems obvious that the alveolar macrophage can be a potent secretor and that this function can affect many systems in the body. However, most of the interest in its secretory function was directed towards examining the role which alveolar macrophages have in interaction with lymphocytes.

(5) Removal of Obsolete Surfactant

The alveolar macrophages reside in the alveoli within a lining layer of surfactant which fills the irregularities of the alveolar surface and lowers the surface tension between the air and the tissues. Surfactant, primarily a phospholipid in composition, is produced by Type II epithelial cells of the alveoli in intracellular organelles called lamellar bodies (164). However, only recently was the mechanism of its clearance from the lungs under-

stood. Naimark (347) administered labelled palmitate, a precursor of dipalmitoyl lecithin, one of the predominant components of surfactant, intravenously into rats and analyzed its uptake and subsequent fate in the lung. He found that the labelled precursor is first taken by the lung tissue, presumably by the Type II alveolar (epithelial) cells during the synthesis of surfactant. It is then released into the alveoli and appears in the surface lining layer, and at a significantly later time the label appears in the alveolar macrophages.

Using different methods, Geiger and colleagues studied the clearance of aerosolized dipalmitoyl lecithin (DPL) from the lung. The cellular distribution of tritium-labelled DPL was followed with frozen section autoradiography at intervals after administration of the aerosol. The label was immediately taken up by the lung tissues, but was lost progressively from the Type I alveolar (epithelial) cells, to appear in the liver, spleen, kidney, blood and urine. However, it was retained by the Type II alveolar epithelial cells, and increased in amount in macrophages at the same time. The authors concluded that DPL is rapidly absorbed by the Type I cells and transferred to the blood and lymph (161). Some technical problems arose with his method, in that only 3% of the labelled DPL reached the terminal air space. Furthermore, the half-life of radioactivity was approximately 3 hours, so that synthetic surfactant was cleared from the alveoli significantly more rapidly than normal surfactant in vivo. The impact of these factors on the results is difficult to assess.

Sheishi et al presented evidence of a different sort concerning the turnover of surfactant. Using antibodies prepared against a specific protein of surface active material lavaged from normal rabbit lungs and conjugating fragments of the antibody to horseradish peroxidase, the authors were able to localize a protein component of surfactant in the lung by means of the Graham and Karnovsky cytochemical test for peroxidase. They found localization in the alveolar lining material, in the cough endoplasmic

reticulum, golgi complex and multi-vesicular and lamellar bodies of the Type II alveolar (epithelial) cells. They also detected localization in surface pinocytic vesicles of Type I alveolar epithelial cells. Finally, they found dense accumulations of the protein in the secondary lysosomes of alveolar macrophages, indicating its apparent relation to the clearance mechanism of that surfactant component (478).

Macrophage Lymphocyte Interaction

The macrophage has an important role in both the afferent and efferent limbs of the immune response. The afferent functions were found to be dependent on the close anatomical proximity which occurs between macrophages and lymphocytes in the lymphoreticular tissue (113). Sell (443) summarized the possible mechanisms of functional cellular interaction between B-cell, T-cell and macrophages. The antigen is bound to or near the surface of the macrophage or is ingested by the macrophage, then degraded mostly and a small part containing antigen determinants is then released. The antigen may be modified by the macrophage to become more immunogenic, complexed to low molecular weight RNA or it may induce formation of a specific messenger RNA. The term "antigen focusing" has now become popular in describing the role of the macrophage in relation to the lymphocyte. Walker noted that macrophages may present functional heterogeneity and that different groups may have considerably different functions (498).

The modification of the antigen presented to the T and B cells by the macrophages allows a suitable response, by possibly altering its concentration, presenting a multi-valent form, or merely by providing a rigid substrate (82).

Pierce and Kapp demonstrated that under certain circumstances macrophages may eliminate tolerogenic antigen, preventing the occurrence of immunological tolerance (376).

On the efferent side of the immune response, the interaction between antigen and sensitized T-cells at the site of an

inflammation results in the release of lymphokines which attract monocytes, immobilize them at the site of inflammation and induce their maturation. These mature activated macrophages are non-specifically more active in the phagocytosis and killing of bacteria (131).

Macrophages also perform tumoricidal functions, however, how this is done is not clear yet. Mature, stimulated or activated macrophages can kill tumor cells non-specifically, and macrophages armed with specific cytocidal factors produced by T-lymphocytes can kill tumor cells specifically. Soluble mediators, possibly lysosomal in origin may be involved (82). The observed phagocytosis of tumor cells by macrophages is not known whether to occur before or after tumor cells are killed (7). In addition, macrophages are present in considerable numbers in many tumors and the progression of such tumors is thought to be inversely related to their macrophage content (82).

Macrophages take up, process and present the antigen to pre-committed but inactive T-lymphocytes, which are there by triggered to proliferation (410). However, Rosenthal and Shevach demonstrated that not all macrophages are capable of stimulating antigen specific T-lymphocytes (409). It is hypothesized that T-lymphocytes, in order to become stimulated, must recognize not only a fragment of the protein antigen, but in addition, must recognize major histocompatibility complex determinants of the macrophage that presents the antigen (443).

It has always been considered that the T-lymphocyte must get into close physical contact with macrophages for the activation to take place. Nonetheless, supernate factors from cultures of antigen-pulsed macrophages were demonstrated to be of importance in macrophage-dependent T-lymphocyte activation. Farr et al noted that the interaction of immune T-cells and macrophages require direct physical contact and that separation of these cells by a membrane impermeable to cells prevented the production of these mediators (146).

Genetic analysis revealed that the lymphocytes and macrophages

must share regions of genetic homology for interaction to occur, a sub-region of the major histocompatibility complex, the I region, being the one responsible for such restriction (113). The I region has been known for quite a while to determine the ability of different mouse strains to respond to various antigens (268).

Rosenwasser et al noted that in contrary to the guinea pig macrophages which are genetically restricted in their interaction with lymphocytes, mouse was found to have both a genetically restricted and a non-genetically restricted macrophage function. The former appears to require direct physical contact, the latter to be available via a soluble supernatant factor (410).

In a reciprocal fashion, a number of macrophage functions may be modified by interaction with immune T-cells and the appropriate antigen. Following such interaction in vitro, macrophages were shown to increase their metabolic activity (473), bacteriostatic and tumoricidal activity (45,300). An increase in macrophage secretory activity was also noticeable following its interaction with T-lymphocytes and an appropriate antigen. The secretion of lysozyme, plasminogen activator, elastase and collagenase were all found to be significantly increased (486).

Beller et al noted that macrophages release a number of lympho-regulatory molecules in cultures containing immune T-cells and normal macrophages (29).

Rosenwasser et al confirmed that macrophages are required for antigen-specific T-cell activation. In a macrophage-dependent, antigen-specific, in vitro, murine, T-lymphocyte-proliferation assay, they were able to show that T-lymphocytes depleted from adherent cells do not respond when challenged in vitro with the immunizing antigen, and that this phenomenon can be reversed by adding macrophage-rich peritoneal exudate (410).

Enhancement of microbicidal and tumoricidal properties of mono-

nuclear phagocytes can be induced in vitro by exposing immunologically activated or inflammatory macrophages to either viable sensitized lymphocytes and antigen or the supernatant fluid from this interaction. In both cases, soluble lymphocyte products (lymphokines) require the presence of T-cells for their generation (339)

In some systems, unelicited macrophages from normal mice can be induced after 48-72 hours of lymphokine treatment to display in vitro some of the properties of in vivo activated cells. A constant stimulus such as daily exposure to fresh lymphokine seems to be necessary in these instances to maintain the cell activation process. Withdrawal of such stimulus would result in decline of the macrophage level of activation over 24-48 hours (339).

Hormone Receptors on Alveolar Macrophages

The first step in the biological activity of hormones is the interaction with their specific receptors in virtually every target tissue. Hormones bind to receptors of macrophages in a specific interaction, the affinity of which correlates with their biological effects. In vivo, evaluation of hormone receptors is influenced by many factors of which the rate of hormone metabolism and the plasma protein transport mechanism are of most importance.

The currently held concept of glucocorticoid mechanism of action on macrophages is that it permeates the cells by free diffusion and then combines with high affinity protein receptors in the cytoplasm. A temperature dependent activation of this hormone receptor complex takes place. Finally, the later complex enters the nucleus where it binds to chromatin acceptor sites (511,512). The glucocorticoid receptor which is normally taken up into the nucleus, in the presence of glucocorticoids, remains in the nucleus. Once steroids are removed from the medium, dissociation of the glucocorticoid receptor complex occurs and the original receptors recycle into the cytoplasm (510). Rousseau et al noted that this

recycling does not require RNA or protein synthesis (415).

Schwartz et al observed that monocytes rather than lymphocytes were the insulin-binding cells of the peripheral blood mononuclear cell preparations (438). In this matter, insulin binding to monocytes seems to show negative co-operativity where the affinity of the receptors is high when unoccupied, but decreases sharply when occupancy increases, owing to site-site interactions that accelerate the rate of dissociation of insulin from its receptors or to the interaction of two classes of receptors with different affinity.

In an excellent review by Werb, she suggested that it is possible that the function of this negative co-operativity is to provide a homeostatic mechanism buffering acute elevations in hormone concentrations. She observed that in contrast to insulin receptors, glucocorticoid receptors behave as a single class of molecules obeying the law of mass action and showing no negative co-operativity in binding hormone, and that cortisol and progesterone compete for receptor binding which suggests that their relative concentrations in biological fluids may determine their eventual effect on macrophages (510). The fact that progesterone and cortexolone, substances that sometimes act as anti-glucocorticoids compete for receptors (510), may theoretically present a possible method for blocking untoward effects of corticosteroid treatment.

Mononuclear phagocytes from human, rabbit and mouse were found to contain 4,000 to 9,000 glucocorticoid binding sites per cell, with dissociation constants of 1-8 nmol/L (511,512). Although monocytes and resident macrophages have higher concentrations (sites/mg protein) of receptors than other cells of smaller size (510), differences in the number of glucocorticoid receptors do not necessarily correlate with differences in response to steroids (458).

CHAPTER III

THE EXPERIMENTS

EXPERIMENTAL

Aim

The aim of this study was to examine the lung cellular immune responses in some surgical conditions which are thought to be associated with a high lung susceptibility to infection. An emphasis was put on the role of the alveolar macrophage since it is considered the most important element in the host lung defenses. The following conditions were induced experimentally in animals:

1. Septic shock lung
2. Starvation
3. Lung atelectasis
4. Splenectomy
5. Use of pharmacological doses of corticosteroids

On examining the previous models, several questions were raised. How does the alveolar macrophage function under such surgically relevant conditions? Could there be an alteration in its antibacterial responses which may result in lung susceptibility to infection? On the other hand, could there be a change in alveolar macrophage function which may be etiologic in the pathogenesis of respiratory dysfunction? These questions were put prior to commencing our study. A few more were raised in due of findings obtained further in the progress of work. Its place seem to fit on description of each experiment in the next part.

Technically, we had to start by choosing the optimal methods of detecting phase related defects in the mechanism of bacterial clearance. Chemotaxis, attachment, phagocytosis, and intracellular bacterial killing were to be examined each separately. The value of singling phase related defects is of obvious importance in understanding the cause and effect relation of the studied diseases.

Furthermore, we modified elements of the methods used to create resemblance to what may occur in clinical states. For example, the target cells used in our phagocytic assays were changed from

Pseudomonas aeruginosa in the lung atelectasis and septic shock lung experiments to streptococcus pneumonia in the splenectomy study.

Early Observations

Initially, attempts were made to assess the different techniques for study of the chemotactic and phagocytic functions of the harvested alveolar macrophages.

Serum opsonic requirements were quantitated for rat and pig alveolar macrophage phagocytic ability, against Pseudomonas aeruginosa and streptococcus pneumonia. We found that the maximal opsonic effect of both pig and rat serum were exerted in serum concentrations between 10-25%. We also found that in general, alveolar macrophages phagocytize Pseudomonas aeruginosa more efficiently than they do pneumococci.

Autologous fresh serum obtained from either pigs or rats was pooled and frozen at -35°C to be used within a week. During this period, no change in its opsonic activity could be detected.

Our experience with the chemotactic assays was rather unsuccessful. When we used the Boyden chambers, we had either obtained a remarkable chemotactic response or only a minimal one to the same chemo-attractants under a similar experimental setting. We attributed this to a discrepancy in the thickness and the size of the pores. We had used endotoxin activated serum and N-Formylmethionyl peptides as chemo-attractants for our assays.

In the agarose wells, the problem was that of reading and interpreting our findings. While the size of the wells was standarized, that of the interface between the agarose and the plate was not. The implications of differences in the size of this space on cell migration is obvious. To decide upon which was the leading cell when measuring the distance of migration was another problem.

In the experiments thus presented in the following pages, we will be looking primarily at the morphological characters as well as the viability, phagocytic and bactericidal functions of the alveolar macrophages.

A total of 28 Yorkshire swines and 616 Sprague Dawley rats of either sex were used. The methods and materials as well as the results and conclusions will be described and discussed separately in each of the studies.

PULMONARY BACTERIAL CLEARANCE AND ALVEOLAR MACROPHAGE FUNCTION IN SEPTIC SHOCK LUNG

ABSTRACT

The association between the pulmonary bacterial clearance and the development of septic shock lung had been demonstrated in porcine and canine experimental models. In order to elucidate the role of pulmonary reticuloendothelial system in bacterial clearance, the functions of alveolar macrophage (AM) obtained by bronchopulmonary lavage were studied. Five piglets were infused i.v. with Pseudomonal aeruginosa labelled with methyl $-^3H$ thymidine at $3\text{-}6 \times 10^8$ CFU/kg/min. Septic shock and ARDS developed within one hour and pigs died in 2-3 hours. Pulmonary bacterial clearance was 93% initially, and progressively decreased to 29%, as PaO_2 decreased and lung water increased. The number of bacteria in the serial lung biopsy specimens increased steadily although the distribution was not homogenous. Differential centrifugations, repeated washings and scintillation countings of the lavage fluid showed that in vivo AM phagocytosis was nil in spite of the abundant bacteria found in the lavage fluid. However, when these AMs were tested in vitro in the presence of optimal concentrations of opsonin and oxygen, their phagocytic capability was well preserved and not significantly different from the controls. It is concluded that the lungs clear bacteria not primarily by AM uptake, but rather by mechanical leakage into the pulmonary space. The apparent dysfunction in vivo is not intrinsic, and likely to be caused by the micro-environmental factors.

INTRODUCTION

Clinical studies have repeatedly shown that bacterial sepsis is the most consistent factor associated with adult respiratory disease syndrome (157,250,490). Crocker et al (100,101) recently demonstrated that continuous intravenous infusion of Pseudomonas aeruginosa in pigs produced dose-dependent pulmonary failure.

While the mechanism by which sepsis, with primary focus in the lungs or elsewhere, may produce pulmonary failure remains unclear, there is evidence of impaired systemic host immune defenses in septicemia (11,12,250,421,422,423) which may suggest a failure in the local lung immune responses as well.

Using a porcine experimental model which has some similarity to man, the development of ARDS was found to be associated with high lung clearance rates of circulating bacteria (101). On the other hand, in the dogs, the lungs neither cleared circulating bacteria efficiently nor developed respiratory failure (100).

We studied the role the pulmonary reticuloendothelial system may play in the clearance of circulating bacteria in a pig model. The effects of the continuous infusion of an ARDS-inducing dose of Ps. aeruginosa on the alveolar macrophage phagocytic activity in vivo was examined. The effects of altering the opsonic and aerobic environments in a controlled in vitro state on the AM phagocytic function was also assessed.

METHODS

Five conditioned piglets of weights between 12-16 kg were studied. Each animal was lightly anesthetized with intravenous pentobarbital 30 mg/kg. An endotracheal tube was introduced and the animal kept breathing on room air using an Ohio Harvard Volume Respirator at a tidal volume of 15 ml/kg and respiratory rate of 14/min. An arterial line was inserted through the femoral artery and a Swan-Ganz catheter placed via the femoral vein.

Bacterial Preparation

Pseudomonas aeruginosa (N.C.T.C. 10662-England) were innoculated to grow overnight in trypticase soy broth containing 0.08 mci/ml (methyl-^{3}H) labelled thymidine (specific activity 78.1-80.3 cm/mmol - NEN, Boston, Mass.) at 37°C. Labelled bacteria were then centrifiged at 2000 g for 15 minutes at 4°C, washed and

resuspended in normal saline to yield a final bacterial concentration of 1-3.5x10^8 colony forming units (CFU)/ml by plate colony counts.

Administration of Bacteria

After two hours of baseline measurements in the lightly anesthetized pigs, the bacteria which were reconstructed in 500 ml of saline were infused intravenously at a rate of 3-6x10^8 CFU/kg/min until death of the animals (2 1/2-3 hours).

Parameters Monitored

Serial measurements were done of arterial blood pressure, pulmonary artery pressure, pulmonary wedge pressure, pulmonary vascular resistance, cardiac output (using the thermal dilution technique), arterial blood gases and arterial blood pH. Differential white cell counts, hemoglobin, hematocrit and platelet counts were also taken serially.

Lung Clearance of Circulating Bacteria & Lung Bacterial Contents

The amount of bacteria cleared in the lungs from each pulmonary circulation at different intervals was examined by obtaining serial blood samples simultaneously drawn from the pulmonary artery (PA) and the aorta (A). Lung bacterial clearance was calculated as $\{100\ (DPM_{PA}-DPM_A)/DPM_{PA}\}$ where DPM_{PA} and DPM_A represent disintegrations per minute obtained by counting 0.1 ml of treated (12) blood samples from PA and A respectively in a scintillation system.

At 1/2 hour intervals, one hemi-thorax was opened through the fourth intercostal space and lung biopsies were taken from the upper, middle and lower lobes, as well as from anterior and posterior segments. The content of viable bacteria in the lungs (including its blood) was assessed by culturing lung samples on blood agar plates for 24 hours.

Pathological Examination

Lung water contents were estimated from biopsied lung tissues using the method of Pearce, Yamashita and Beazell (366). The results were expressed as percent lung weight of water and as a ratio of extravascular lung water to dry weight of bloodless lung (QWL/qdL) (70). Measuring the hemoglobin concentration in the blood and lung samples and assuming the hemoglobin concentration and water content of blood at the time of biopsy to be equal to that of residual lungblood (70), the wet weight of bloodless lung was obtained by subtracting the residual blood weight from the total wet lung weight, and the dry lung weight equalled the wet weight of bloodless lung minus extravascular lung water.

The lungs were examined grossly at 1/2 hour intervals and the tissue biopsies examined under light microscopy.

Harvest of Alveolar Macrophages

At 1/2 hour intervals, bronchoalveolar lavage was performed using a size 14 polyethylene tube introduced with the aid of a rigid Jackson bronchoscope into the lower lobes. The tube was wedged distally and aliquots of 10 cc PBS were injected and suctioned using a triple-way stopcock to a total of 50 ml. The returning lavage fluid ranged between 40-44 ml.

Handling of Lavaged Cells

The bronchoalveolar lavage fluid was collected in polypropylene tubes (Corning, 50 ml centrifuge tubes), which were kept on ice. The sample was then strained through gauze to remove mucus. Immediately, total cell counts were done using turk blood diluting fluid (Anachemia Chemicals Lts., Montreal, P.Q.) in an improved Neubauer hemocytometer, and differential cell counts were performed using a Diff Quik stain set (Harleco Diff Quik, Dade Diagnostics, Aguada, Puerto Rico, U.S.A.). Alpha-naphthyl acetate esterase stain (Sigma, St. Louis, Mo.) was also used to confirm the identity of the alveolar macrophages. Trypan blue exclusion test (Grand Island Biological Co., Grand Island, N.Y.) was carried

out to assess the viability of the alveolar macrophages in the sample. The total number of viable alveolar macrophages in the retrieved bronchoalveolar lavage sample was calculated and the macrophages were then centrifuged for 15 minutes at a speed of 400 g at 4°C, and the sedimented cells were resuspended in Newman and Tytell serumless medium (Gibco Laboratories, Grand Island, N.Y.) to yield a cell distribution of $2x10^6$/ml.

In Vivo Adherence and Phagocytosis

0.1 of the AM suspension containing $2x10^5$ cells was added to 10 ml of aqueous counting scintillant (ACS, Amersham, Arlington Hts., Ill.) in a triplicate of plastic scintillation vials (Fisher) and counted for 10 minutes in a liquid scintillation counter (ISOCAP/300, Searle Analytic, Des Plaines, Ill.). Counts per minute (CPM) from the liquid scintillation were converted to deteriorations per minute (DPM) using a recently corrected efficiency curve. The counts would represent the total number of AM associated bacteria, both adherent and phagocytized.

The cells were then washed using PBS and centrifuged thrice at 160 g for 5 minutes to detach all adherent bacteria as confirmed by microscopy, and the counts were repeated in the same manner. The latter would reflect the actual in vivo phagocytosis.

Controlled In Vitro Phagocytosis

Tritium labelled bacteria were first opsonized for 1/2 hour with 10% fresh normal pig serum in a shaking water bath at 37°C. Using a modification of the technique described by Verhoef et al (489), from each alveolar macrophage sample to be tested, 0.1 ml containing $2x10^5$ AM cells was added to 1 ml of opsonized labelled bacterial suspension containing approximately $2x10^7$ bacteria in two identical triplicate polypropylene tubes (12x75 mm, Falcon, Oxnard, Calif.) The tubes were then incubated in a shaking water bath in 21% oxygen environment at 37°C for 60 minutes.

Following this, the first triplicate of tubes (A) was centrifuged at 160 g for 5 minutes at 4°C, bringing down only the alveolar macrophages together with the phagocytized bacteria within these cells. The cells were then washed in PBS and centrifuged again, at the same speed, two additional times to remove any adherent non-phagocytized bacteria. The second identical triplicate (B) was centrifuged once at a high speed of 2000 g for 15 minutes to bring down all the bacteria (intra and extracellular). The pellets in both triplicates were then resuspended in 1 ml of PBS and shaken vigorously (Deluxe Mixer, S8220, Scientific Products, Evanston, Ill.), 0.9 ml of the suspension in each tube of both triplicates was then solubilized in 10 ml of aqueous scintillant and counted in a Beta counter, as previously described. An average DPM value was obtained from the first and second triplicates and the phagocytic index was calculated as follows:

$$\text{Phagocytic Index} = \frac{\text{DPM from first triplicate (A)}}{\text{DPM from second triplicate (B)}} \times 100$$

$$= \frac{\text{Counts from Cell Associated Bacterial Pellets}}{\text{Counts from Total Bacterial Pellets}} \times 100$$

Statistics

The results are presented as mean±SEM and compared to those of the baseline control using student's-t-test, or paired t-tests within the group.

RESULTS

Gas Exchange and Hemodynamic Changes

I.V. infusion over 2 hours of live bacteria resulted in the development of hypodynamic septic shock. All the pigs died between 2 1/2 and 3 hours post-infusion. The cardiac output dropped from a mean baseline reading of 175.6±4.4 to 68±7.3 ml/min/kg in 2 1/2 hours ($P<0.001$) (Figure 1). The pulmonary artery pressure rose significantly from a mean of 11.6±.2 mmHg

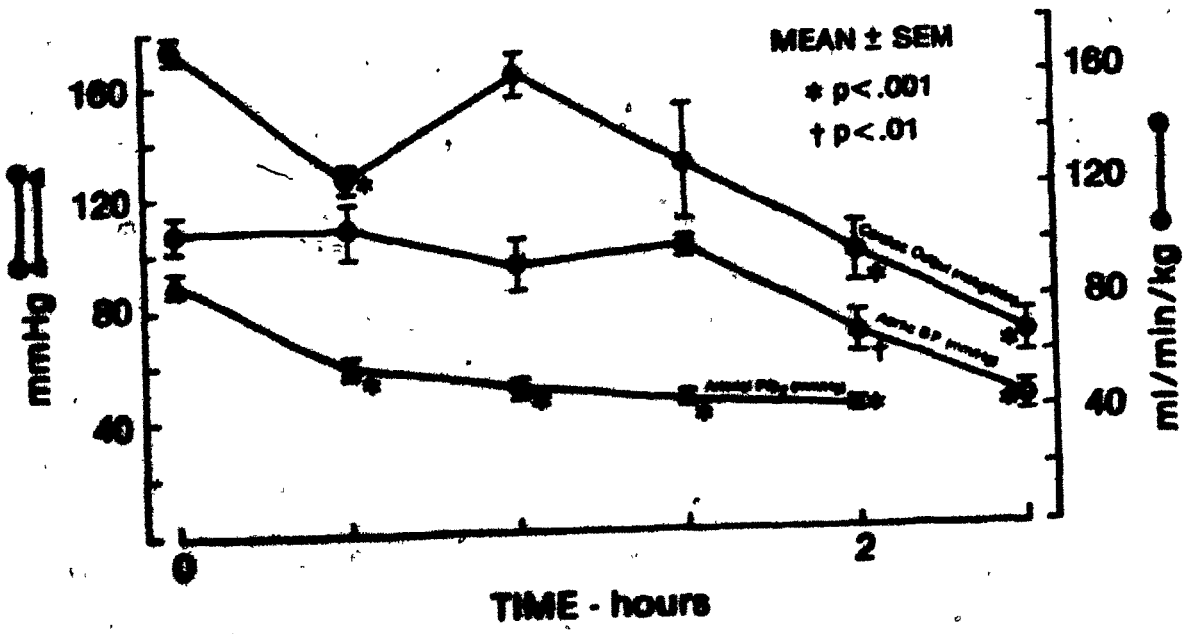


Fig. 1: Effect of administering Pseudomonas aeruginosa intravenously on aortic blood pressure, cardiac output and arterial oxygen pressure.

baseline to 35.6±4.5 mmHg in 1/2 hour and remained high through the rest of the experiment (Figure 2). This was associated with an increase in the pulmonary vascular resistance from 3.4±.2 woods units (mmHg/L) baseline value to 15.3±3.4 woods units in 1/2 hour ($P<0.02$) (Figure 2), an increase of 447% of control measurement. The aortic arterial pressure, however, remained insignificantly changed until the second hour after initiation of bacterial infusion when it dropped from a baseline value of 108.8±6.1 mmHg to 69.4±6.3 mmHg at 2 hours ($P<0.01$) (Figure 1).

The arterial Po_2 dropped from a mean of 91.6±2 mmHg prior to bacterial infusion to 58.9±2 mmHg in 1/2 hour, and continued to deteriorate, reaching a level of 43.1±.0 in 2 hours post-infusion ($P<0.001$), (Figure 1), indicating the onset and progress of respiratory failure.

Gross and Microscopic Lung Changes

During the first hous of bacterial infusion, the lungs appeared to be expanding proportionally, fully, and maintained a normal pink surface. However, on biopsy, marked froth oozed off the cut sections with evidence of progressive intra-alveolar and interstitial edema. In the following period, petchiaes progressively appeared over the surface of the whole lung with interstitial hemorrhages manifested in the cut sections. These changes, however, were more prominent at the posterior dependent areas. In the final 1/2 to 1 hour of the experiment, the posterior segments showed dark purplish coloration with the development of consolidation and apparent atelectasis.

Microscopic Findings

Congestive atelectasis was invariably present in the posterior segments of the lungs. Marked thickening of the alveolar walls with infiltration of both bacteria and leukocytes in the interstitium consistent with the typical picture of septic ARDS was evident. Bacteria and leukocytes were also prominently present in the lung vascular compartment.

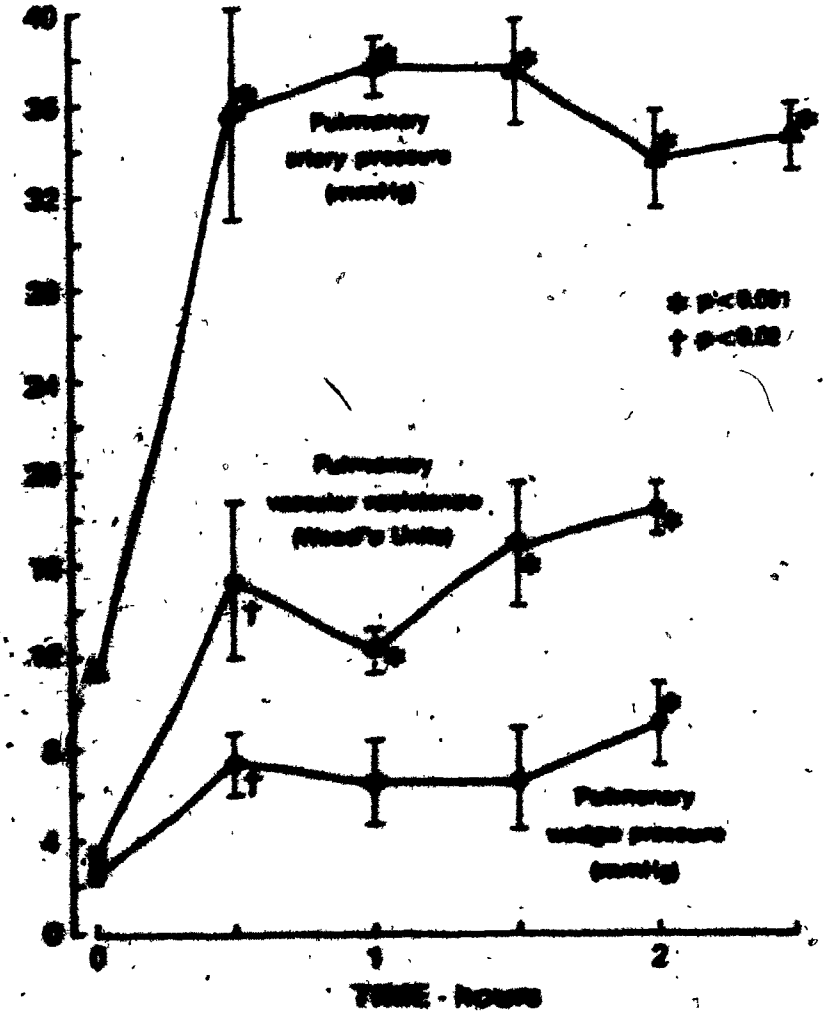


Fig. 2: Pulmonary hemodynamic changes with intravenous infusion of Pseudomonas aeruginosa (Woods unit: mmHg/L/min).

Lung Water

During the first 1/2 hour of bacterial infusion, the lung water content was noticed to have significantly increased from 72.1±2.6% of wet lung weight at 0 hours to 79.9±1.6% at 1/2 hour post-infusion ($P<0.01$). In 2 hours, the lung water had increased to 82.6±.7% of wet lung weight ($P<0.001$) with significantly higher water content in the posterior lung segments compared to the anterior segments (85.4±1% wet lung weight in posterior segment; 81.3±.9% wet lung weight in anterior segment) ($P<0.02$) (Figure 4). The QWL/qdL increased significantly to 160% of control values in one hour and to 200% of control in 2 hours of bacterial infusion ($P<0.001$) (Figure 5).

Lung Bacterial Clearance

At the initiation of bacterial infusion, the lungs cleared 92.5% of the circulating bacteria. With time, the clearance of bacteria by the lungs decreased progressively to reach 28.8% at 2 hours ($P<0.001$) (Figure 6).

Lung Bacterial Content

Over the period of continuous intravenous bacterial infusion, the lungs seemed to accumulate progressive amounts of bacteria ($2.6\pm.9\times10^6$ CFU/gm lung tissue at 1/2 hour post-infusion; $11.8\pm4.1\times10^6$ CFU/gm lung tissue at 2 hours) ($P<0.05$). The posterior lung segments showed a significantly higher bacterial content than the anterior segments when these were examined at 2 hours post-infusion. (Posterior segments; $17\pm4\times10^6$ CFU/gm lung tissue; anterior segments $1.6\pm.5\times10^6$ CFU/gm lung tissue) ($P<0.02$) (Figure 7).

Hemogram

The Hct increased over the first 2hours of the experiment from a mean of 32.2±1.8 at 0 hours to 36.9±2.4 at 2 hours, ($P<0.001$), (Figure 3). The platelet number progressively decreased from a mean of $2.8\pm8\times10^5$ at 0 hours to $1.6\pm.3\times10^5$ at 2 hours (Figure 3). The total white cell counts progressively deteriorated from a

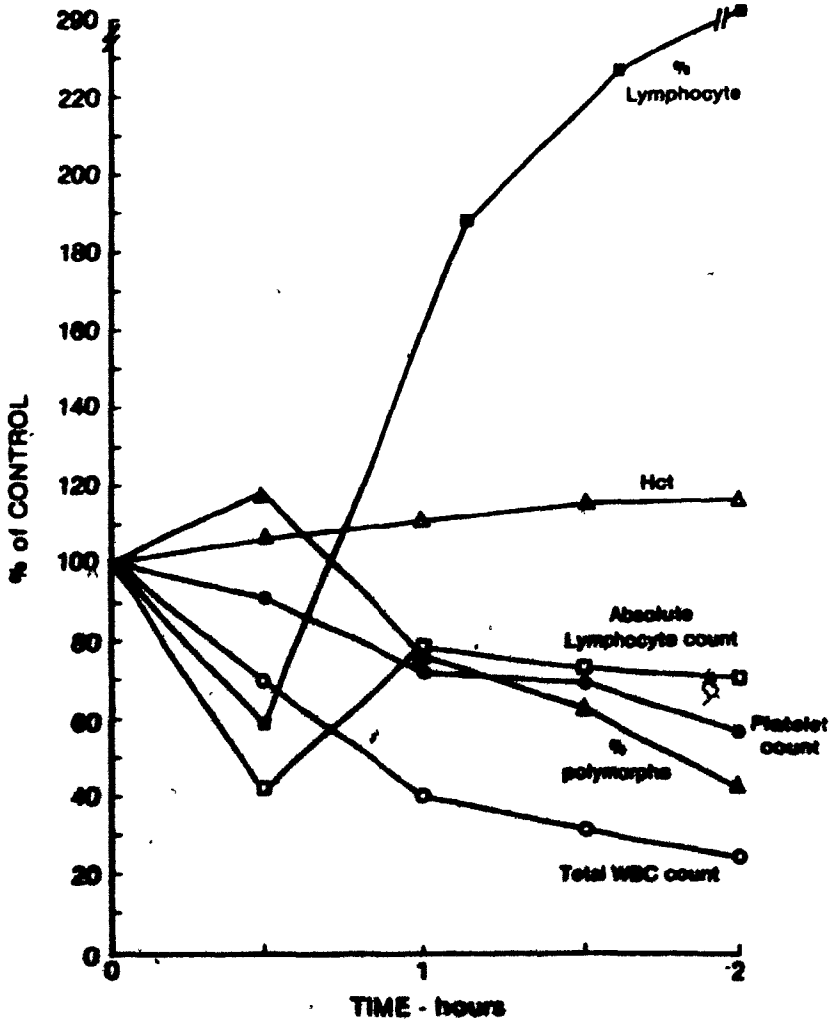


Fig. 3: Hemogram changes with progression of septic shock lung.

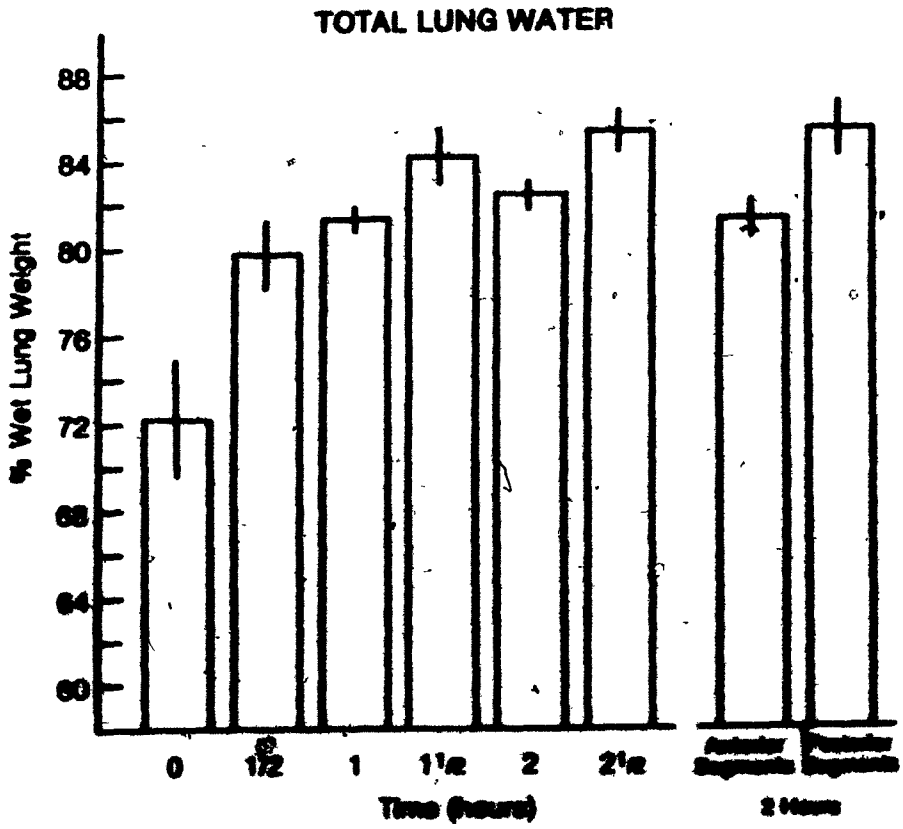


Fig. 4: Change in total lung water content expressed as percentage of wet lung weight.

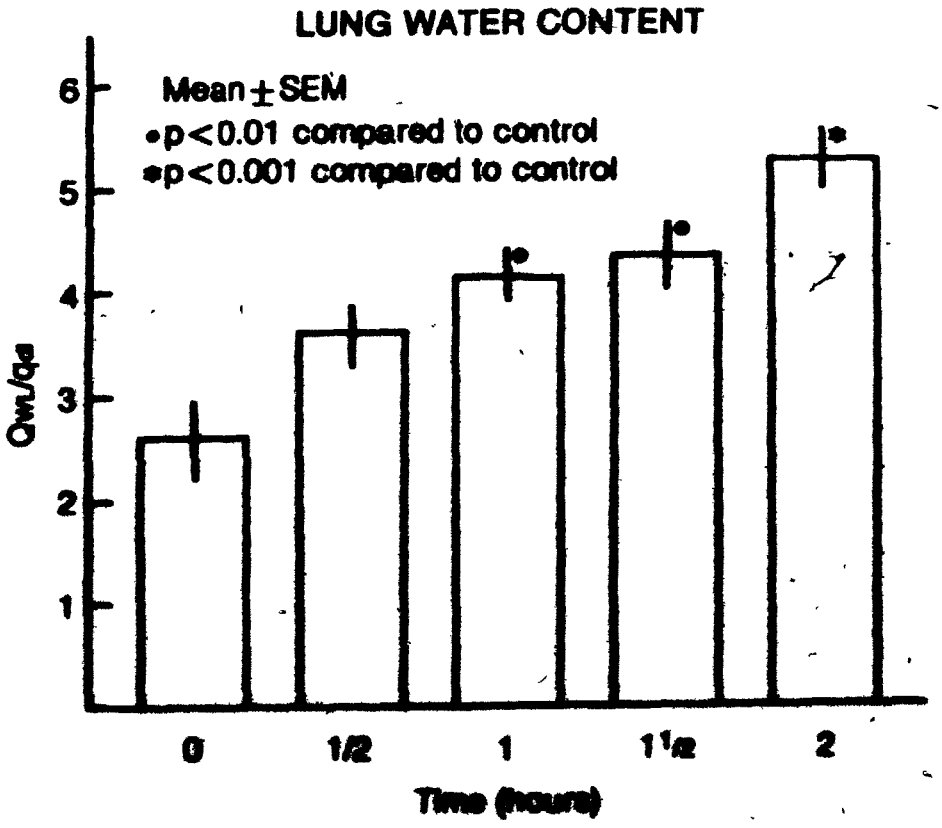


Fig. 5: Lung water changes in septic shock lung (QWL/QBL - Extravascular lung water/ Dry weight of bloodless lung).

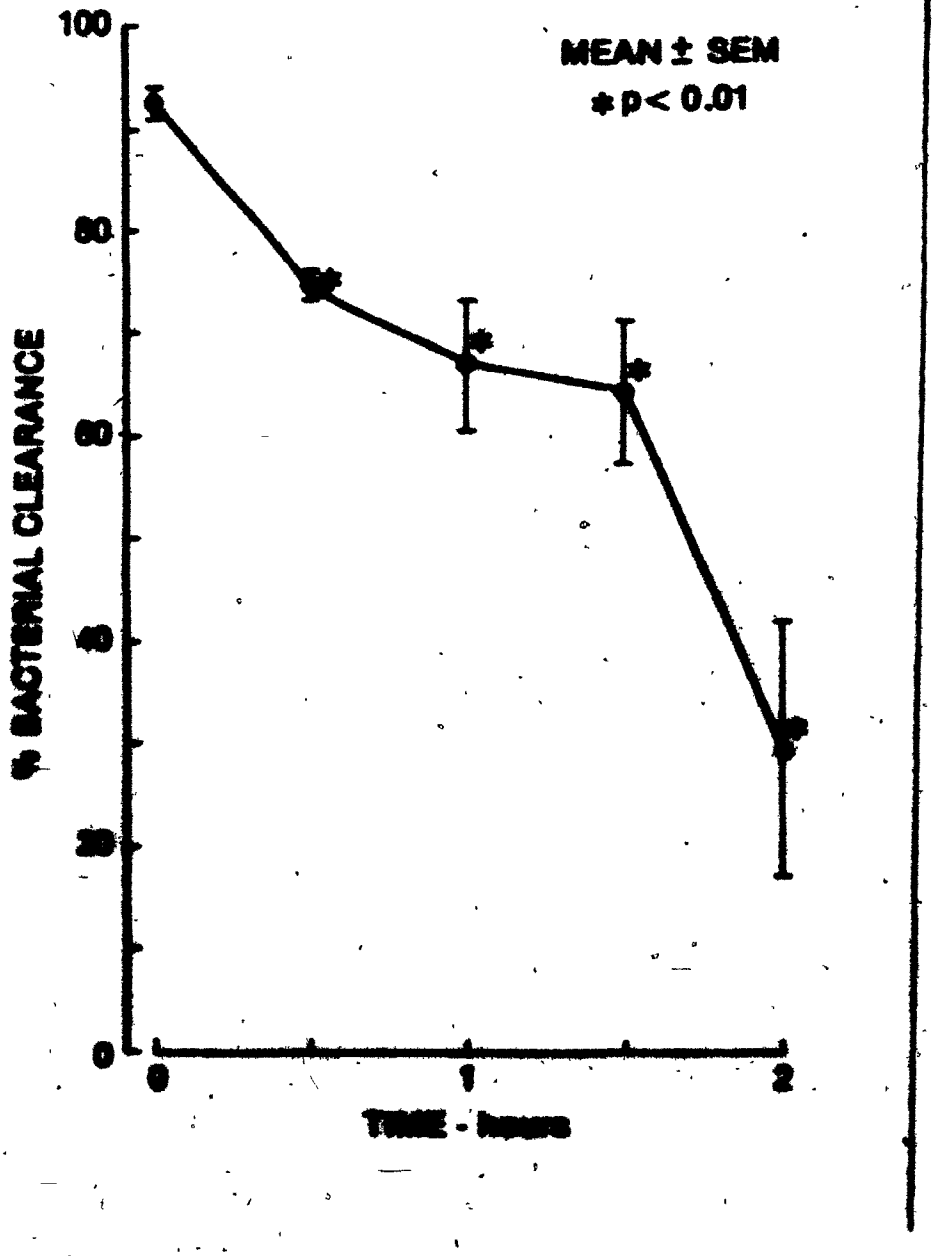


Fig. 6: Pulmonary bacterial clearance of circulating Pseudomonas aeruginosa.

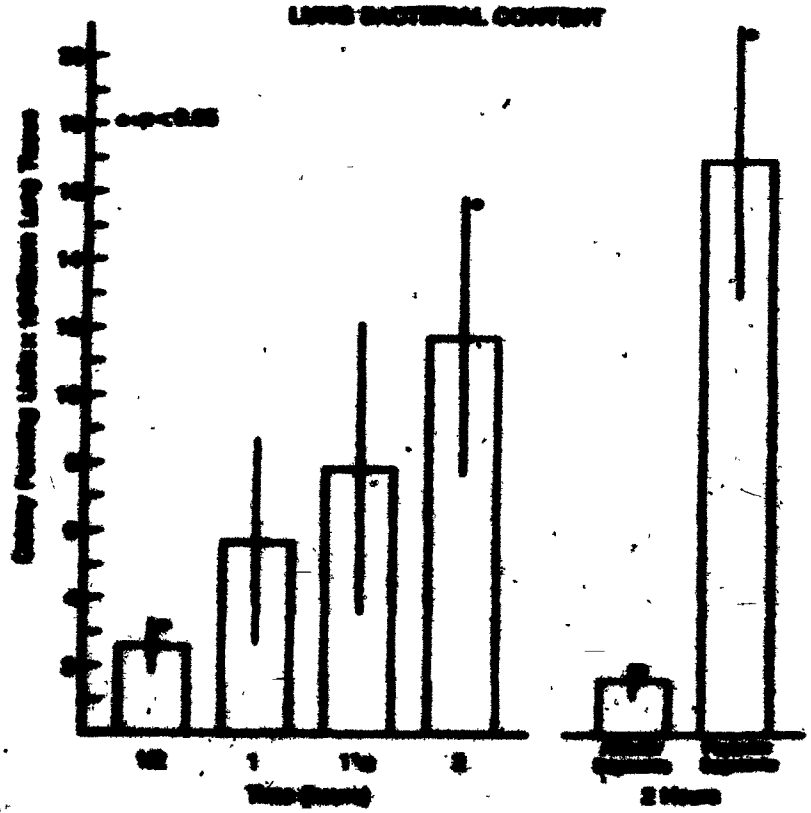


Fig. 7: Lung bacterial content

baseline number of $23\pm4.7\times10^3$ to $5.6\pm2\times10^3$ at 2 hours ($P<0.02$), (Figure 3). However, this decrease affected the polymorphonuclear leukocytes more than it did the lymphocytes, as reflected by the progressive increase in the percentage of lymphocytes in the total white cell count (23.3±4.6% at 0 hours to 67.3±3.8% at 2 hours), (Figure 3). The decline in both the polymorphonuclear leukocyte and platelet numbers may reflect a consumption defect as a result of its extensive deposition in the lung vasculature, as observed microscopically.

Pulmonary Macrophage Functions

No significant change in the total cell yield on bronchoalveolar lavage at different times of the experiment was noticed (0 hours $3\pm1.6\times10^7$; 2 hours $1.2\pm4\times10^7$) (Figure 9). The ratio of alveolar macrophages in the lavage cell population remained unchanged as well (0 hours 83±3%; 2 hours 79±3.5%) (Figure 9). The alveolar macrophages retained their viability as determined by Trypan blue exclusion throughout the period of the experiment (0 hours 81.8±1.8%; 2 hours 80±2.7%), (Figure 9).

In Vivo and In Vitro Phagocytosis

Although an increase in both the number of bacteria contained in the lungs and its adherence to AMs was observed (adherence: 49 ± 14.3 DPM/2×10^5 AM cell at 1/2 hours; 294.3 ± 35 DPM/2×10^5 at 2 1/2 hours), ($P<0.01$), (Figure 8), a lack of actual phagocytosis of the bacteria was noticed, 23.9 ± 5.8 DPM/2×10^5 cells at 0 hours, 22.1 ± 7.8 at 2 1/2 hours), both representing nil bacterial count, as a background count of up to 28 DPM was encountered (Figure 8). However, when the lavage cells were washed and incubated in vitro under an optimal O_2 concentration of 21% and using 10% fresh normal pig serum as an opsonin, the AMs showed a normal phagocytic response (phagocytic index at 0 hours 42.3±7.8%; 2 1/2 hours 31.7±9.3%), (Figure 9).

DISCUSSION

Hypodynamic septic shock and ARDS are reproducible in pigs by the continuous infusion of Pseudomonas aeruginosa. Using such

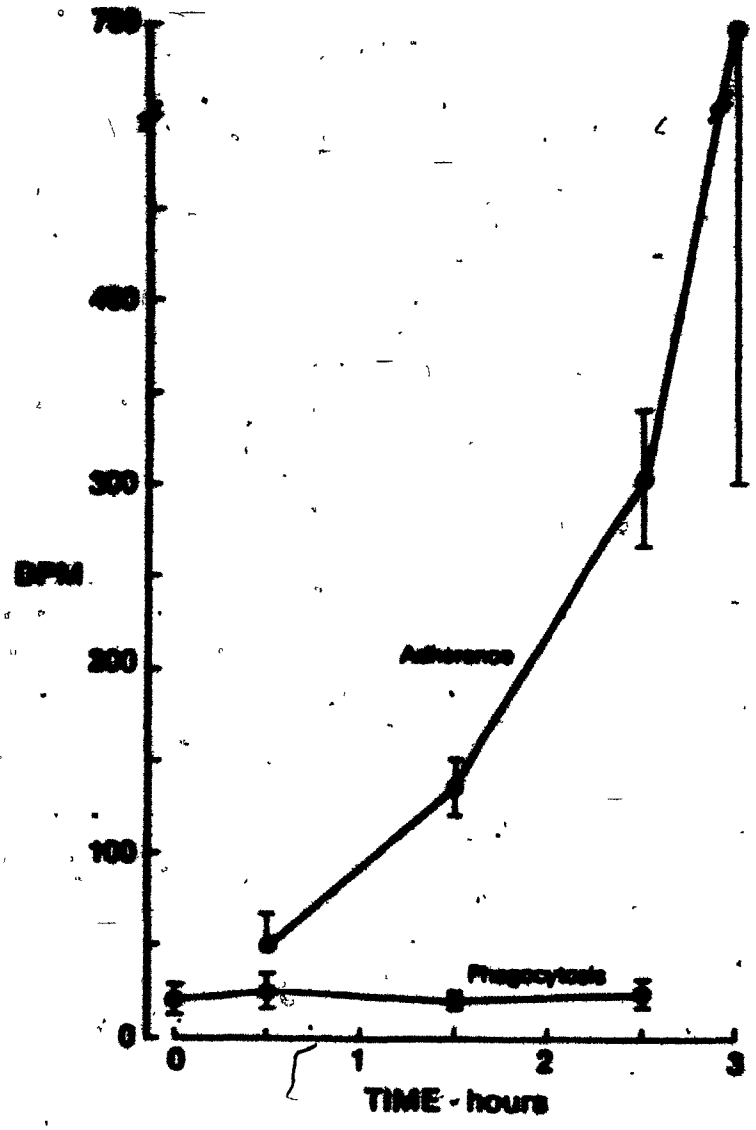


Fig. 8: In vivo response of alveolar macrophages: While the number of bacteria adhering to macrophages progressively increased, the macrophages failed to phagocytize the adherent bacteria.

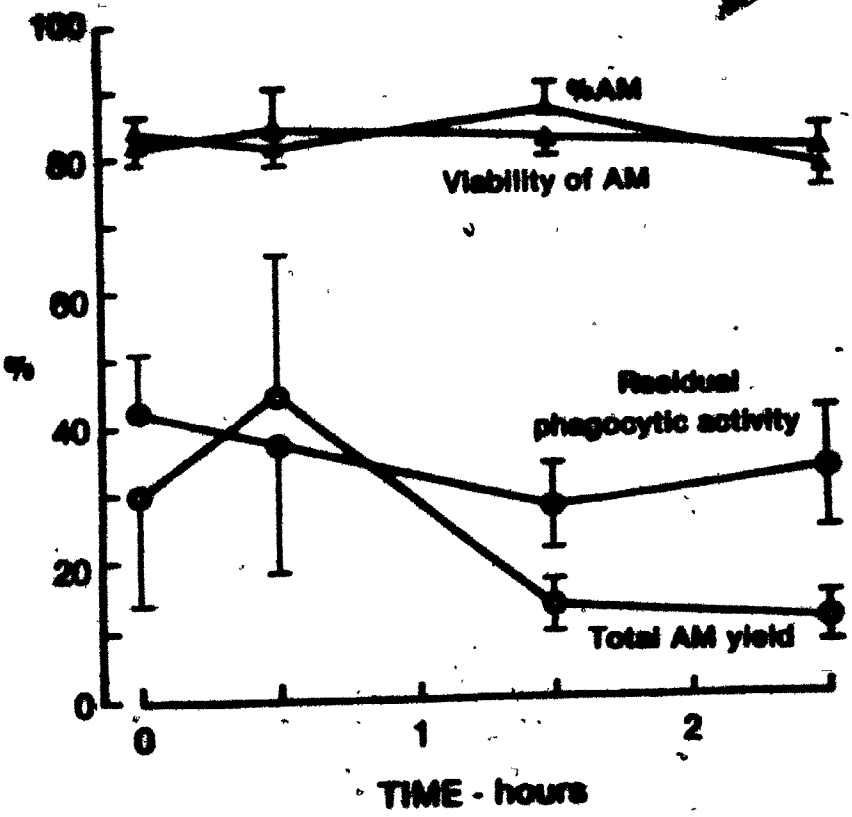


Fig. 9: Assessment of alveolar macrophages in broncho-alveolar lavage from septic shock lungs (residual phagocytic activity indicates ability of macrophages to phagocytize bacteria in vitro after repeated washing and provision of optimal oxygen and opsonin).

a model, we found that the bacterial clearing ability of the lung is not uniform, and differed among various lobes. Although 92-63% of bacterial circulating through lungs were cleared in the first 1 1/2 hours, the pulmonary bacterial clearance decreases to only 28.8% at 2 hours.

The lack of intracellular phagocytized bacteria in the alveolar macrophages harvested from such animals, in spite of the progressive leakage of circulating bacteria into the alveolar spaces and its adherence to the alveolar macrophages, indicates that somehow there was complete paralysis of the in vivo phagocytosis. These findings suggest that the pulmonary clearance of circulating bacteria in this model is not due primarily to an active uptake by the pulmonary reticulo-endothelial system, but rather due to their leakage and entrapment in the potential pulmonary spaces.

In our study, we found a marked granulocyte aggregation in the pulmonary intravascular compartment concomitant with the progressive depletion of polymorphonuclear leukocytes in the peripheral circulation. This, as suggested previously (198,232), in the presence of bacteria, may result in the alteration of membrane permeability, permitting the leakage of circulating bacteria into the pulmonary interstitium and alveolar spaces. The decreased pulmonary bacterial clearance observed later in the course of sepsis could be due to the development of a significant intrapulmonary shunting, or simply a saturation effect.

The failure of in vivo alveolar macrophage phagocytosis may be partially explained on the basis of decreased alveolar oxygen and/or opsonin depletion, secondary to chaotic complement activation in the intravascular compartment, which results in leakage into the alveolar spaces of fluids which are complement depleted. Although we did not quantitate the levels of complements in the bronchoalveolar lavage fluids, the depletion of complements, and therefore, the chemotactic effect may be suggested by the failure of granulocyte migration into the alveolar compartments as noted by the preservation of the same cell population ratios in the bronchoalveolar lavage fluid,

in spite of the progressively increased presence of granulocytes in the lung vasculature.

In the in vitro studies of alveolar macrophage functions, optimal environmental conditions can be provided by adjusting the oxygen and opsonic requirements. As mentioned earlier, the alveolar macrophages retained their viability throughout the period of bacteremia, but were unable to phagocytize in vivo. Washing the alveolar macrophages in vitro with a buffered solution and providing it with an optimal oxygen and opsonic environment resulted in the restoration of their phagocytic capability. This suggests that the alveolar macrophage phagocytic failure in vivo was not due to an intrinsic macrophage dysfunction, but was related to an altered micro-environment under such a pathophysiologic state.

The clearance of circulating bacteria and particles are often throught to be performed by the reticulo-endothelial system. While this may be true in the liver and spleen, this may not always be the case in the lungs.

While some studies have shown that other cells in the lung, such as types 2 and 1, alveolar epithelium are capable of retaining circulating particles, and this contribute to its clearance, others have described many factors that may play a role in the pulmonary clearance (70,100,204,405,420,424,). These include the nature of circulating material, it circulating load, and obviously of importance, the state of the lung itself.

We conclude that the pulmonary clearance of organisms from the circulation in this porcine septic lung model is due to a mechanical failure associated with the membrane defect and leakage, and is not primarily accomplished by an active uptake by the pulmonary reticulo-endothelium. Lack of in vivo phagocytosis by the alveolar macrophage is not due to an intrinsic dysfunction of the AM, but is likely to be related to the impaired micro-environment involving inadequate opsonins and/or oxygen present.

DEPRESSION AND DELAYED RECOVERY OF ALVEOLAR MACROPHAGE FUNCTION ON STARVATION AND ITS REVERSAL

ABSTRACT

The lungs are particularly vulnerable to infection in the starved patients. We studied the hypothesis that starvation may induce alveolar macrophage (AM) dysfunction in rats.

Pulmonary AM phagocytic activity decreased significantly after one week of starvation (49.1% of normal), and remained low after the animals had been refed for one week (47.6%), even though the weight and absolute blood lymphocyte counts had become normal (97.6%). By the third week, alveolar macrophage phagocytic activity returned to normal. In contrast, the intra-cellular killing of phagocytized bacteria remained normal with starvation. Electron microscopic studies revealed poor pseudopodia and less phagolysosome formation in AMs harvested from starved rats and challenged with Ps. aeruginosa, which were consistent with our functional findings. Starvation selectively depresses AM phagocytic activity rendering lungs more susceptible to infection. The delay in recovery may prolong this susceptibility even though other immune functions may be normal.

INTRODUCTION

Information from World Health Organization Nutrition and Immunology Centers indicates that two thirds of the world's population suffers from inadequate nutrition (440). Infection in these groups (442) was found to contribute markedly to their morbidity and mortality (439,440). In addition, many patients develop nutritional deficiencies by nature or consequence of their illness. These patients are frequently recognized to be prone to infection (284,285). Bronchopneumonias are among the most commonly encountered infections in the malnourished (*).

Immune incompetence in the nutritionally deprived was found to affect thymus derived (T) lymphocytes (479), the complement system (**) and the polymorphonuclear leukocytes (**).

---

* Goodhart, R.S., Shils, M.E. (ed.), Modern nutrition on health and disease, pp. 709, (6th edition), Lea Febringer, Philadelphia, 1979.

** Chandra, R., Nutritional assessment-present status, future directions & prospects. Report 2nd Ross Conference on Medical Research, Levenson, S.M. (ed.), pp.111-113, Columbus, Ohio, 1981.

On assessment of the mononuclear phagocyte system, Keusch et al (264) detected no change in the chemotactic or bactericidal functions of peritoneal macrophages from rats subjected to protein calorie malnutrition. Bhaskaram examined peripheral monocytes of children suffering from severe protein-energy malnutrition and found that the monocytes bactericidal capacity in these children was unaltered (37). Douglas and Schopfer demonstrated normal phagocytosis by monocytes obtained from children with acute kwashiorkor (126).

These studies suggest that the mononuclear phagocyte system seems to maintain its normal immune functions in protein calorie malnutrition. However, since different resident tissue macrophages vary in their characterization and metabolic demands from one site to another (449,498), such a conclusion cannot be entertained without further assessment of other macrophages. Although infections in malnourished patients seem frequently to involve the lung (*), no attempts have been made to study the effect of nutritional changes on the alveolar macrophages. In the following experiments, we examined the effects of total starvation on the alveolar macrophages' phagocytic and bactericidal activity.

MATERIAL & METHODS

Six groups of Sprague Dawley rats obtained from Canadian Breeding Farms (Montreal, Canada) were used. The first group was starved for one week allowing only water intake. The second group was starved as previous for one week and then allowed access to ad lib of rat chow for another week. The third group was starved for one week and then refed ad lib of rat chow for three weeks. The other three groups were allowed access to ad lib rat chow throughout the period of the experiment and were used as simultaneous controls to the previous groups. Six animals from each group were used to obtain alveolar macrophages, as described below.

Labelling of bacteria, harvest and handling of the alveolar macrophages and phagocytic and bactericidal assays were done as previously described.

Bactericidal Activity

Bacteria were incubated with alveolar macrophages, as in the phagocytic assay for one hour. Following this, 10 mcg gentamycin sensi-disc (Becton, Dickinson and Co., Mississauga, Ontario) was added to the macrophage bacterial suspensions for 1/2 hour to allow for killing of all extra-cellular bacteria. The tubes were then subjected to repeated centrifugation and washing as in Group (A) in the phagocytic assay. The macrophages were then resuspended in 1 ml of distilled water, after which 3 mm glass beads were added and stirred vigorously for 30 seconds in a vortex mixture to disrupt cell walls and release previously phagocytized bacteria. 100 µl of this solution was then taken to determine the viability by plating on nutrient blood agar. After 18 hours of incubation at 37°C, the CFU were counted. The intra-cellular bactericidal activity was then estimated using the equation below:

$$\% \text{ Intra-cellular Bactericidal Activity} = \frac{\text{NO. TOTAL PHAGOCYTIZED BACTERIA}^{*} - \text{NO. VIABLE PHAGOCYTIZED BACTERIA}^{\dagger}}{\text{NO. TOTAL PHAGOCYTIZED BACTERIA}} \times 10$$

* NO. OF PHAGOCYTIZED BACTERIA AS CONVERTED FROM DPM TO CFU

† NO. OF COLONY FORMING UNITS IN GENTAMYCIN TREATED SUSPENSION

The results are recorded as mean±SEM and were compared to those of simultaneous controls using Student's test. The use of simultaneous controls in such an assay was found to be essential to eliminate possible intra-laboratory differences.

Electron Microscopic Study

Alveolar macrophages were incubated with opsonized bacteria for one hour in the same macrophage/bacteria ratio described in the phagocytic assay. The macrophage bacterial suspensions were mixed 50-50 with 2% buffered glutaraldehyde and centrifuged at 2000 rpm for 15 minutes. The sediment was then post fixed in osmium tetroxide, dehydrated through alcohols and embedded in epon.

Thin sections were stained with uranyl acetate and lead citrate for ultrastructural studies.

RESULTS

Body Weight

One week starvation resulted in a decline in body weight to 81% of pre-starvation weights. This was associated with clinical manifestations of malnutrition such as emaciation, loss of hair, lethargy and decrease in body temperature. Refeeding the starved rats resulted in a progressive improvement of their weights reaching 151% of pre-starvation values within 3 weeks of re-feeding.

WBC Counts

Peripheral blood absolute lymphocyte counts decreased from a mean of 5675±342 control pre-starvation value to 1754±528 ($P<0.001$) after one week of starvation. However, within one week of refeeding, the absolute lymphocyte counts reached a mean of 5727±156 (101% of pre-starvation value) and remained within control values (5624±442 at 3 weeks post-feeding).

Alveolar Macrophage Yield

The total alveolar macrophage yeild from the starved rats was insignificantly higher than that of the control rats ($2.18±4x10^6$ one week starvation; $1.72±.2x10^6$ one week ad lib feed). When refeeding was initiated for one week, the refed, previously starved rats yielded an insignificantly higher alveolar macrophage count in their broncho-alveolar lavage as compared to the ad lib fed simultaneous controls ($4.6±.7x10^6$ refed rats; $3.3±.5x10^6$ control).

AM Phagocytic Activity

One week starvation resulted in a significant decrease in the AM phagocytic activity to 49.1% of the simultaneous control values

(Phagocytic index: 7.85±0.43; starved 3.86±0.16) (P<0.001). When rats starved for one week were fed ad lib rat chow for one week, their AM phagocytic activity remained significantly low (47.6% of simultaneous control) (Phagocytic index: control 23.1±1.66; starved 11±.615) (P<0.001) (Figure 10). When the AMs from rats that were starved forone week and then fed ad lib for three weeks were examined for their phagocytic activity, they showed a phagocytic index which was similar to that of the simultaneous control rats (control 8.2±.39; starved 3 weeks re-fed 8±.57) (Figure 10).

Bactericidal Activity

AM bactericidal activity from starved rats was comparable to that of the simultaneous controls, both being able to kill 100% of the phagocytized bacteria. AMs from rats which were starved for one week and then refed for oneweek were able to kill 80.6±0.74% of the phagocytized bacteria, a figure insignificantly different from that of simultaneous ad lib fed rats (82.2±0.84%). After three weeks of refeeding starved rats, their AMs bactericidal activity was again similar to that of the simultaneous control group, both killing 100% of the phagocytized bacteria (Figure 10).

E.M. Findings

Alveolar macrophages from normal rats showed active pseudopodia formation with different stages of phagosome formation and phagolysosome fusion with marked progression of degradation. Their nuclei appeared actively segmented frequently with multiple nucleoli (Figure 11). On the other hand, alveolar macrophages from starved rats had very minimal pseudopodia with no evident cell membrane activity, a low number of phagolysosomes and mitochondria and inactive nuclei. Multiple dense osmiophilic vacuoles were also noticed (Figure 12).

DISCUSSION

In our experiment, we were able to detect a defect in the alveolar macrophage's ability to phagocytize bacteria when the rats were

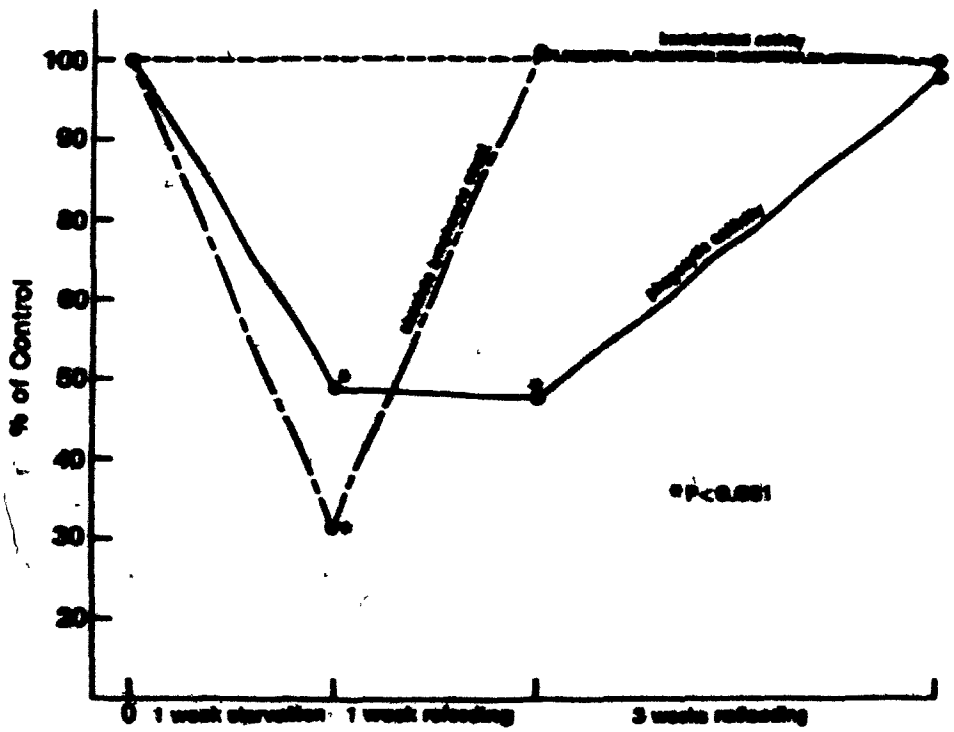


Fig. 10: Effect of starvation and refeeding on alveolar macrophage phagocytic and intra-cellular killing and on peripheral blood lymphocyte count.

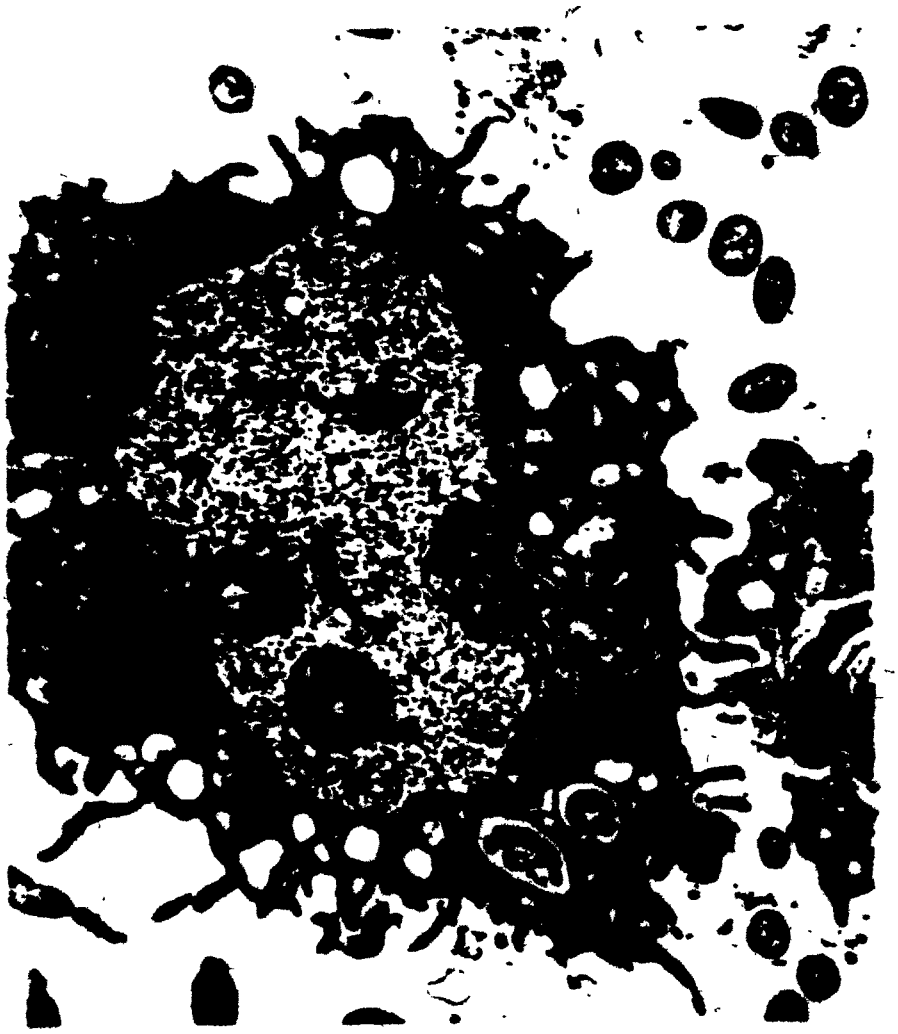


Fig. 11: Normal alveolar macrophage involved in phagocytosis and degradation of Pseudomonas aeruginosa.

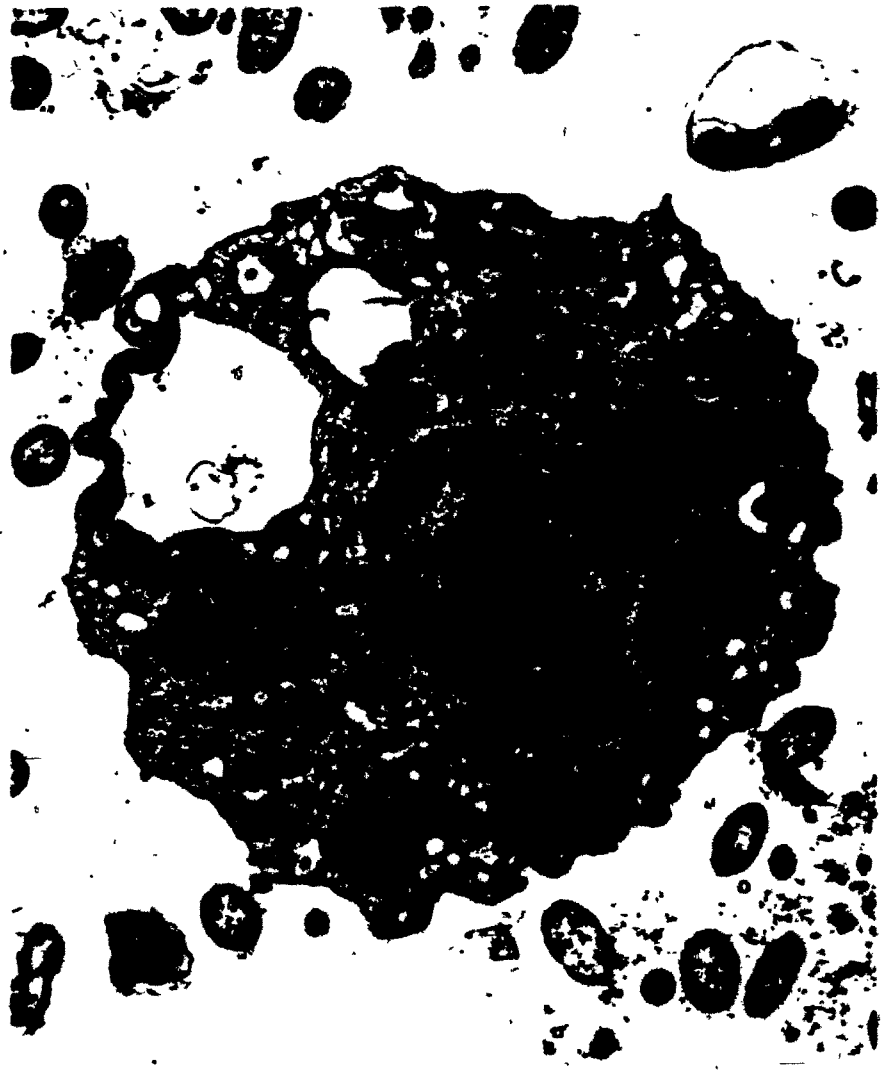


Fig. 12: Alveolar macrophage from starved rats challenged with Pseudomonas aeruginosa.

starved for one week. This depressed phagocytic activity occurred without a concomitant change in the bactericidal activity.

The phagocytic activity having decreased would result in less bacteria to be killed in the phagolysosomes of the phagocytizing macrophages. The decrease in the alveolar macrophages phagocytic activity seems to persist for some time, in spite of the animals having regained a normal total lymphocyte count and an increase in the animals weight to pre-starvation values. This delay in the recovery of the alveolar macrophage phagocytic activity may be of importance if such findings can be extrapolated to patients. While other immunological functions recover early after replenishment of patients, it may require a longer period for the local lung cellular responses to recuperate. The intensity of changes in the phagocytic activity may not be apparent in the peripheral monocytes (126), as it is in the alveolar macrophages which are more efficient scavenger cells.

In rats, the mean turnover rate of alveolar macrophages is about three weeks (441), which seems to suggest that the effect of starvation on the resident alveolar macrophages may be irreversible and that only when the whole macrophage cell population in the lungs have changed would the complete recovery of the phagocytic activity become apparent. However, we have not attempted to study the specific mechanism by which starvation directly affects alveolar macrophage function.

In the nutritional assessment of patients, variable tests have been used to identify deficiencies to the non-specific immunologic defenses. These tests allow us to recognize patients with susceptibility to infection. While lymphocyte counts and cutaneous delayed hypersensitivity to recall antigen may seem simple to perform and are both frequently reduced in under-nourished subjects (**), their alteration and return to normal may not necessarily indicate a parallel improvement of the lung cellular immune responses. Our data reveals the necessity for further assessment of the effects of nutritional changes on pulmonary immune functions.

THE EFFECT OF ATELECTASIS AND RE-EXPANSION OF THE LUNG ON ALVEOLAR MACROPHAGE FUNCTION

INTRODUCTION

Peri-operative and post-traumatic atelectasis are associated with an increased risk of lung infection. The vigorous use of chest physiotherapy has resulted in a decreased morbidity in the atelectic patients. Although the mechanism of such susceptibility and its reversal is still unclear, it is presumed that alteration of the micro-environment in the lung precipitates the occurrence of infection and its prevention. The sterility of the lung alveoli is maintained by the ability of the lung immune system to overcome the pathogenic and proliferative powers of the organisms escaping mechanical clearance. The role of a possible AM dysfunction in the precipitation of infection in the atelectic lung segments was examined. The effect of re-adjusting the alveolar macrophage micro-environment in vivo by re-expanding the atelectic segment with mechanical ventilation and increasing the alveolar oxygen tension by using 100% oxygen was also examined.

MATERIAL & METHODS

Six conditioned male Yorkshire swine (Group 1) weighing 14-15 kg were anesthetized as previously described. Through a midline cervical incision, the trachea was exposed and a small left lateral incision was made in it to allow the insertion of a double lumen balloon catheter into the trachea. The tip of the catheter was then guided using an orally introduced Jackson straight bronchoscope into the right upper lobe bronchus and then further pushed until it wedged (Fig.14). This would allow atelectasis of a small segment of the lung with minimal effect on the rest of the lung parts (Fig.13). The fact that the young swine have a right upper bronchus which branches directly from the trachea immediately prior to its division allows for easy manipulation of the catheter into the desired site. The proximal end of the catheter exited through the neck incision.

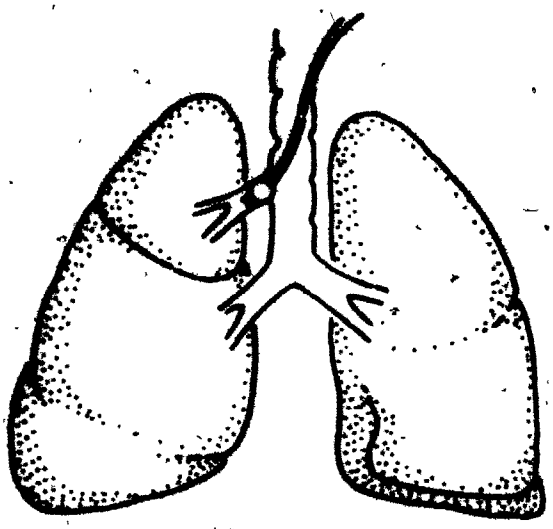


Fig. 13: Balloon catheter inserted in (R) upper lobe bronchus (note opening above carina).

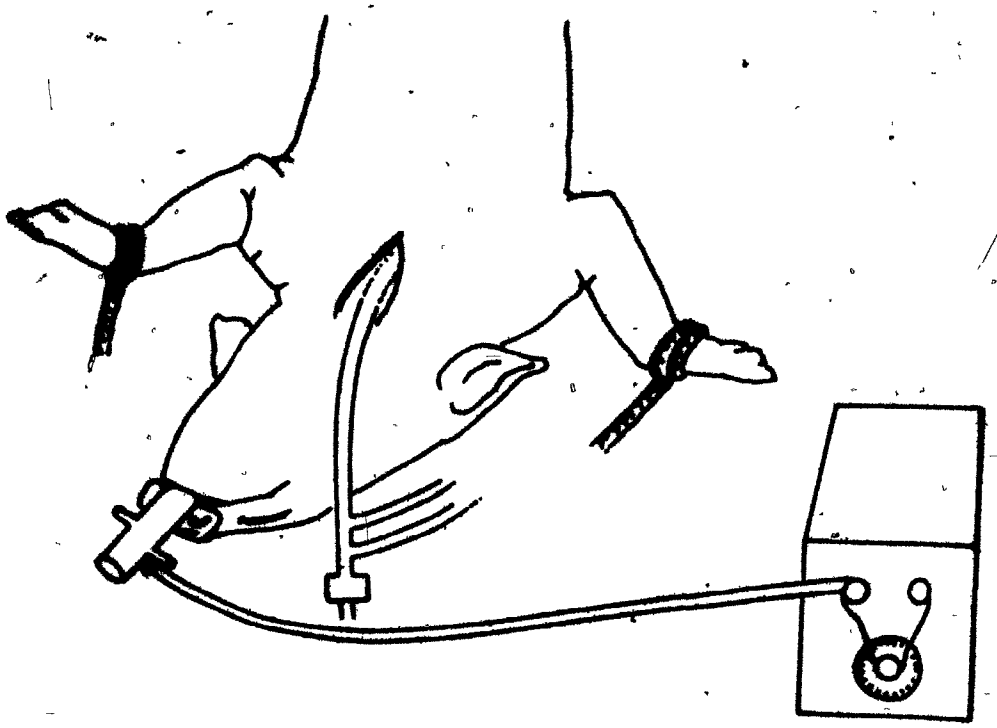


Fig. 14: Introduction of balloon catheter under bronchoscopy.

Broncho-alveolar lavage was performed at 0,2,4,6 and 24 hours. Aliquots of 5 ml phosphate buffer saline were injected and immediately suctioned through a triple way stopcock placed at the proximal end of the occluding catheter. 40 ml were always injected into the collapsed segment with a return of 35-40 ml of the injected solution. Lavage specimens were immediately placed on ice.

In another group of animals (group 2) (n=3), having induced atelectasis as previously described, the RUL was re-expanded by removing the balloon catheter at 6 hours post-occlusion and supplying it with 100% oxygen with the use of intermittent mandatory ventilation at a rate of 16/m, a zero endexpiratory pressure and a tidal volume of 300-350 ml for 4 hours. Broncho-alveolar washouts were done at 0 hours prior to collapse, 6 hours following right upper bronchus occlusion and 4 hours after re-expansion of the RUL in a similar fashion to the first group.

The alveolar lavage cells were then counted. The alveolar macrophage population was identified by its morphology and non-specific-esterase activity and its viability assessed by trypan blue exclusion as described previously.

The alveolar macrophage phagocytic and bactericidal assays were then done in vitro using Pseudomonas aeruginosa as target cells. Results are presented as mean±SEM. Comparison was done using student and paired t-tests.

RESULTS & DISCUSSION

Occlusion of the right upper bronchus with the resultant collapse of the right upper lobe (RUL) resulted in a significant increase in the phagocytic activity of the alveolar phagocytic population to 142.3% of normal (cont. 21.3±3.6/post. collapse 30.3±3.3) ($P<0.01$) (Figure 15) (Table I) in 2 hours. The fact that the percentage of AM in the broncho-alveolar washout had remained the same (95.5±1.1% at OH; 92.3% at 2H) (Table I) (Fig.16) suggests that the observed increase in the phagocytic activity is that of the alveolar macrophage itself and not due to an associated polymorphonuclear leukocyte phagocytosis.

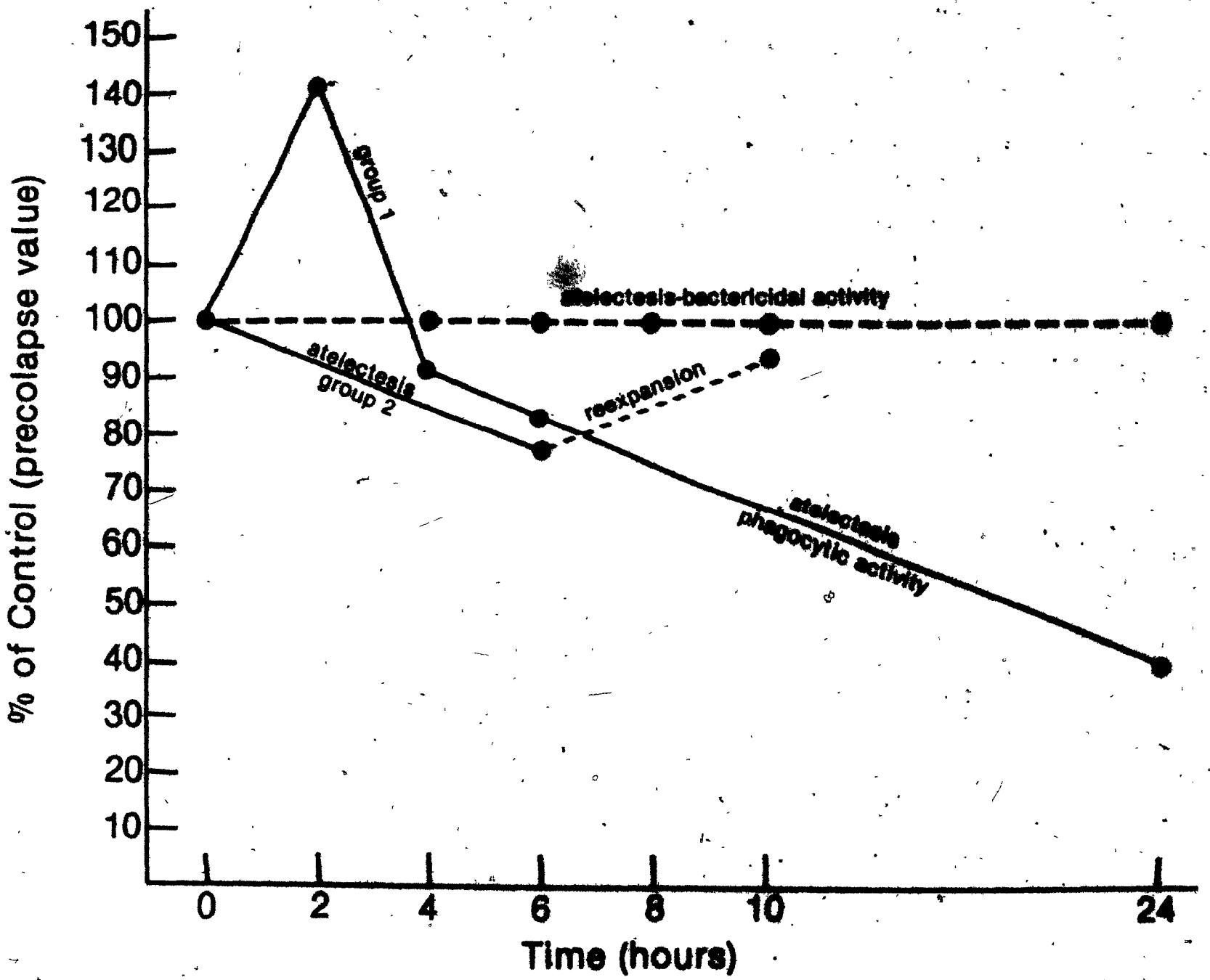


Fig. 15: Effect of atelectasis and re-expansion of the lung on alveolar macrophage function.

In the following hours of atelectasis, the phagocytic activity of the broncho-alveolar washout cell population rapidly and significantly deteriorated to reach 39% of the control pre-collapse values (cont. 21.3±3.6/post. collapse 8.3±1.8) ($P<0.01$) at 24 hours post-collapse (Figure 15) (Table I).

During this period and with the progressive influx of polymorphonuclear leukocytes, the percentage of AMs in the broncho-alveolar washout cell population decreased significantly from a control pre-collapse value of 95.5±1.1% to 37.5±3.9% ($P<0.01$) at 6 hours post-collapse and then increased moderately to only 67±8.2% ($P<0.05$) at 24 hours (Table I) (Fig.16). Since the comparison in phagocytic activity was done using a constant AM number of 2×10^5, the deterioration in the phagocytic activity could not be due to a decrease in the number of AMs in the tested samples. The observed increase in the number of associated polymorphonuclear leukocytes suggests that it may have played a role in such deterioration of AM phagocytic activity. However, the intra-cellular killing of phagocytized bacteria remained almost normal throughout the period of collapse, indicating that the major process affected is the phagocytic uptake of Pseudomonas aeruginosa and not its eventual killing.

In the second part of the experiment, the reversal of atelectasis 6 hours after its initiation by removing the occluding balloon catheter, and using intermittent mandatory ventilation with 100% oxygen for 4 hours resulted in a significant increase in the percentage of AMs in the broncho-alveolar lavage (44.7±2.9% 6 hours after atelectasis/85±2.9% 4 hours following re-expansion. $P<0.01$) (Table I) (Figure 16). This change indicates either the rapid disappearance of the influxed polymorphonuclear leukocytes or the appearance of recently recruited mononuclear phagocytes. This was also associated with the return of AM phagocytic index to almost normal values 94% (cont. 23.8±1.4/reversal of atelectasis 22.4±2.5 - $P<0.05$) (Fig.15) (Table I).

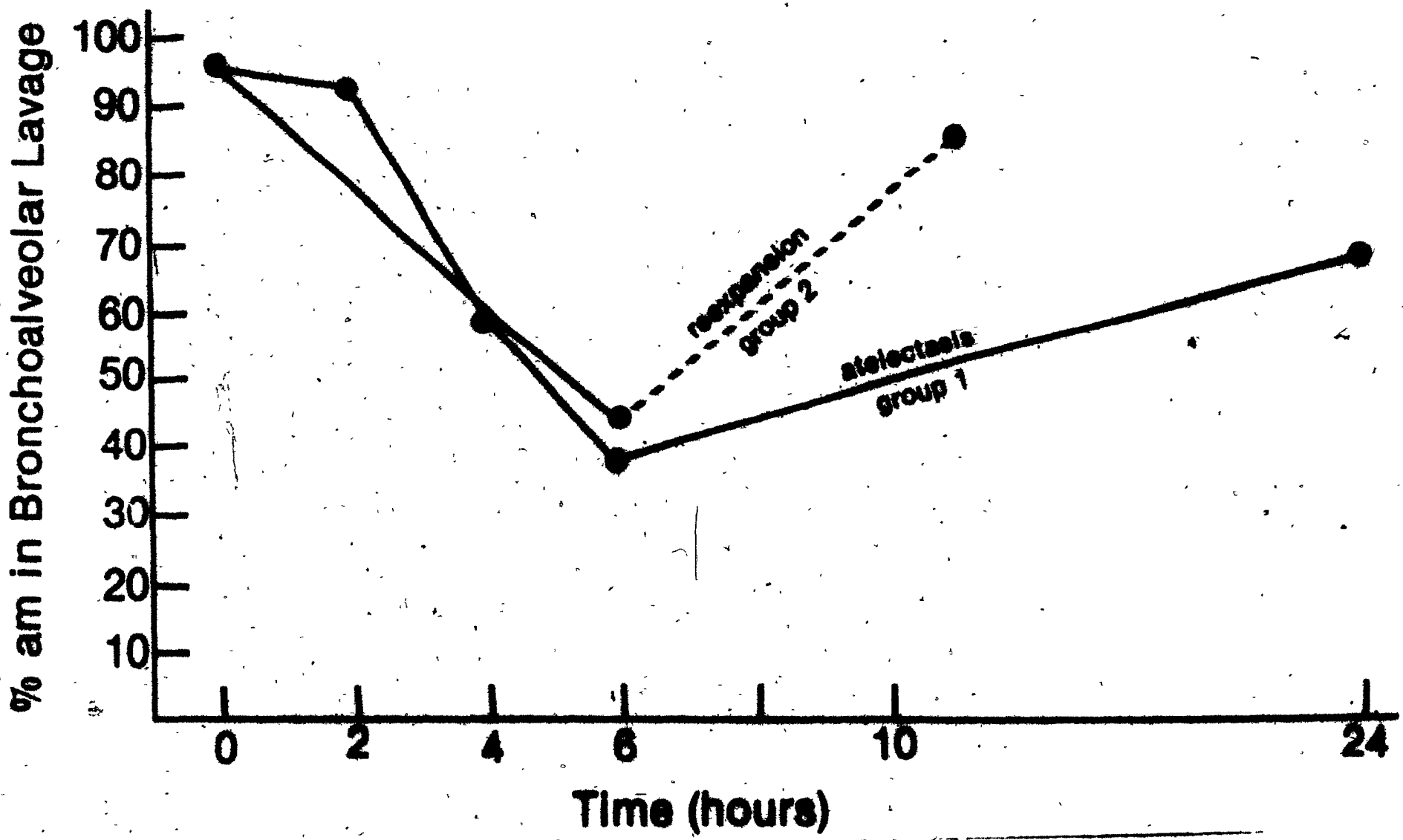


Fig. 16: Effect of atelectasis and re-expansion on broncho-alveolar lavage cell ratios.

TABLE I

GROUP I	PRE-COLLAPSE	POST-COLLAPSE			
		2 hrs	4 hrs	6 hrs	24 hrs
% AM in broncho-alveolar lavage	95.5±1.1	92.3±0.6†*	59.8±16.7	37.5±3.9†	67±8.2†*
Phagocytic index (% of control)	21.3±3.6 (100%)**	30.3±3.3β* (142.3%)	19.6±3.3* (92%)	17.8±2.8 (83.6%)	8.3±1.8β* (39%)
Intracelluiar bactericidal activity (%)	100	100	99.98	99.92	100

GROUP II	PRE-COLLAPSE	6 HRS. POST-COLLAPSE	6 HRS. POST-COLLAPSE x 4 HRS. RE-EXPANSION
Phagocytic uptake (% of control)	23.8±1.4 (100%)	18.3±1.6 (77%)	22.4±2.5* (94.2%)
% AM in broncho-alveolar lavage	96±2.1	44.7±2.9	85±2.9†

COMMENTS

In the lungs, local environmental changes (182,282) and disease (183,281) may alter its ability to clear bacteria.

Acuta hypoxia has been shown to decrease net bacterial lung clearance of Staphylococcus aureus and Staph. epidermidis, but not of Proteus mirabilis (183,184). This observation while indicating a definite effect of oxygen changes on the lung bacterial clearance mechanism, suggests that the lung host defenses vary according to the species of bacteria to which it is exposed to. Such variations were also noticed by Harris et al who showed a decrease in lung clearance of Staph. aureus, Klebsiella pneumonia and E. coli, but not of Streptococcus penumonia when hypoxia is induced (201).

It was suggested that an alteration in the alveolar macrophage phagocytic activity may be responsible for such changes in lung bacterial clearance. This hypothesis relates to the fact that alveolar macrophages, in contrast to other mononuclear phagocytes, operate only under aerobic atmospheres (362,449).

From previous review of the literature, it seems obvious that the surgical patient is subject to a higher risk of infection. Peri-operative and post-traumatic pulmonary atelectasis are thought to predispose to such susceptibility.

In this experiment, we were able to detect a time related alteration in the AM phagocytic function when atelectasis was induced by occluding the RUL bronchus. The initial increase in the AM phagocytic activity at 2 hours followed by a progressive decrease up to 24 hours post-atelectasis reflects the initial potential of the AM to overcome mild environmental changes in the alveoli, which seems then to give way when the deterioration of the alveolar environment status progresses under the effect of atelectasis. These changes were noted to occur even though AMs were put in optimal in vitro phagocytic condition, indicating the development of a cellular defect

secondary to the environmental changes.

When atelectasis was interfered with as early as 6 hours following the occlusion of the airway, by clearing the later and providing assisted mechanical ventilation to assure expansion of the collapsed segments, together with the use of 100% oxygen, the observed AM phagocytic defect was reversed.

Although the environmental changes that occur in the atelectic lung segments are not confined to that of a decreased oxygen tension, the reversal of such a defect with the arrest of the progressive pathological changes is of most importance in the traumatic and post-operative patient who is prone to airway plugging. The supply of oxygen and mechanical respiratory assistance may play a bigger role than just providing optimal blood gas levels.

The observed influx of polymorphonuclear leukocytes in the atelectic segments may play an important role in the development of alveolar macrophage dysfunction. The decrease in the ratio of polymorphonuclear leukocytes in broncho-alveolar lavages on reversal of atelectasis was certainly associated with improvement of alveolar macrophage phagocytic activity.

THE MODULATORY ROLE OF THE SPLEEN ON ALVEOLAR MACROPHAGE FUNCTION

ABSTRACT

The effect of splenectomy on the ability of young and adult rat alveolar macrophages to phagocytize Pneumococci, Types 3 and 14 and Pseudomonas aeruginosa was studied. Young animals show a significant (15%) decrease in the phagocytosis of pneumococci type 14, 4 weeks after splenectomy. This depression increases to 30% in 6 weeks time. Such depression is also noted when young splenectomized rat alveolar macrophages are challenged with Pseudomonas aeruginosa, but not with type 3 pneumococci 6 weeks post-splenectomy. Three months following splenectomy in young animals, the grown rats seem to regain their normal phagocytic activity against pneumococci type 14. Adult rats also show no alteration in their phagocytic activity against type 3 pneumococci. Autotransplantation of the spleen had a protective effect on the phagocytosis of type 14 pneumococci, and an insignificant effect on that of type 3.

This study postulates a modulatory role of the spleen on alveolar macrophage function. Splenectomy may cause the impairment of local lower respiratory immune function, making them vulnerable to specific bacterial invasion. Such splenic modulatory effect on alveolar macrophage phagocytic function seems to be age and antigen specific.

INTRODUCTION

Splenectomized patients are susceptible to overwhelming and fatal bacteremia of which pneumococci are responsible for approximately 50% of cases (451,125,141). The majority of these infections take place within 2 weeks after splenectomy (383,497). Splenectomized individuals, younger than 2 years of age, were found to be at greater risk than those older than 5 years of age (141,140,497). However, such infections may also occur in splenectomized adults (187).

In infants, other than neonates and children, the pneumococcus was found to be a frequent inhabitant of the respiratory tract and the most common bacterial cause of pneumonia and bacteremia (269). The mechanism of its invasion is not fully understood, and the further increase in its invasiveness in splenectomized animals is also not clear.

Studies in experimental animals have verified the primary importance of the spleen in clearing and trapping cellular antigens (216,234,292,434,514). A relationship was observed between the type, virulence of the pneumococcus and the extent of its clearance by the spleen (74). The humoral immune functions of the spleen have also been extensively studied (28,41,294,334). The route, dose and time of antigen administration seem to be important factors in determining the spleen modulated immune responses (122,294).

Although pneumococcal infections are usually acquired via the respiratory tract (75), studies have not considered the role of the lung host defense in the clearance of these organisms in asplenic animals.

The alveolar macrophage, a resident end stage mononuclear phagocyte, is an important component of the lung host defense. Its phagocytic function not only eliminates invading organisms, but also provides antigenic determinants to the lymphocytes which further enhances local cellular and humor immune responses. We have examined the role of a possible alveolar macrophage (AM) dysfunction in the increased susceptibility to infection in splenectomized animals.

MATERIALS AND METHODS

Bacteria and its Labeling

Types 3, 14 pneumococci were obtained from the American type culture collection (Rockville, Maryland) and reconstituted in trypticase soy broth. Every 4 weeks the pneumococci were passaged in rats to maintain its virulence and capsulation. The bacteria were maintained on blood agar plates at 4°C.

Pseudomonas aeruginosa (N.C.T.C. 10662-England) were reconstructed in trypticase soy broth.

On the day prior to phagocytic assay, the bacteria were inoculated to grow overnight (18 hours) in 10 ml of pepton water containing 0.8 mci of (Methyl -^{3}H) labeled thymidine (specific activity 78.1-80.3 ci/mmol - NEN, Boston, Mass.) at 37°C.

Labeled bacteria were then centrifuged at 2000 g for 15 minutes at 4°C, washed and resuspended in phosphate buffered saline PBS to yield a final bacterial concentration of 1-3.5 x 10^8 colony forming units (CFU)/ml as confirmed by plate colony counts. 0.1 ml of the final bacteria suspension was counted in a liquid scintillation system for 10 minutes to obtain a correlation between the counts of bacteria (CFU) obtained by the plates and their radio-active counts.

Splenectomy and Autoimplantation

Sprague-Dawley rats obtained from Canadian Breeding Farms (Montreal, Canada) were used. In the "young animals" group, the animals were recently weaned with weights of 80-95 grams. In the "adult" group, the weights always exceeded 350 grams.

The animals were lightly anesthetized with intra-peritoneal administration of 30 mg/kg sodium pentobarbital. Through a midline incision, the spleen was examined and either removed in the splenectomy group (Splx), reduced back into the abdominal cavity in the sham operated gropu (Cont.), or excised and 1/4 of it wrapped in the omentum with silk sutures in the autoimplanted group (Autoimplant.). The abdominal wall was closed in 2 layers of catgut and silk. In each experiment, a minimum of 6 animals in each group was used. One group of sham operated animals was prepared in each experiment as the simultaneous control. The rats were housed in galvanized steel cages at a constant temperature of 22° to 25°C and were allowed free access to Pruina rat chow and water. Any animal which showed signs of infection or deviation from normal growth was eliminated from the study. The animals were divided into 10 groups (Table 1).

Group I contained young animals subjected to either sham operation, splenectomy or autoimplantation. Its AM phagocytic activity was examined against type 14 pneumococci 4 weeks after the procedure. Groups II and III had either sham operation, splenectomy as in Group I, but were examined 6 weeks after the procedure. Group IV consists of young animals subjected to either sham operation or splenectomy and its AM phagocytic activity was examined against type 3 pneumococci 4 weeks after the procedure. Group V was similar to the previous groups, but contained a sub-group of auto-implanted animals in addition. Group VI was young animals subjected either to splenectomy or sham operation and examined 6 weeks later for the AM phagocytic activity against type 3 pneumococci. Group VII was similar to Group VI, but examined against Pseudomonas aeruginosa.

Group VIII was animals which were subjected to either splenectomy or sham operation in infancy, but were examined for their AM phagocytic activity 3 months later, after they grew into adulthood. Group IX was adult rats which were either splenectomized or sham operated, to be examined for their AM phagocytic activity 6 weeks following the procedure. Finally, Group X was normal rats which were examined for their AM phagocytic activity against type 14 pneumococci susing serum opsonin from either control (sham operated) or splenectomized animals 6 weeks following the surgical intervention. At the end of the experiments, all the rats were subjected to laparotomy and their abdominal cavities were assessed for possible splenosis.

Harvest of Alveolar Macrophages

On the day indicated, the rats were anesthetized as previously. Through a transverse cervical incision, the trachea was exposed and cannulated using a size 14 Medicut (Sherwood Medical Industries, St. Louis, Mo.). The cannula was secured in place with ligatures and a 3-way stop cock was fixed to its end. Broncho-pulmonary lavage was performed by the repeated injection of 5 ml aliquots of PBS and its subsequent withdrawals, to a maximum of 50 ml

total. This procedure resulted usually in the retrieval of 45-49 ml of the original injected fluid. Any sample which showed evidence of contamination with red blood cells was discarded. In a similar manner, those samples which later proved to contain polymorphonuclear leukocytes in an unusual number were excluded from the study.

The retrieved broncho-alveolar lavage fluid was collected in polypropylene tubes (Corning, 50 ml centrifuge tubes) which were kept on ice. The sample was then strained through gauze to remove mucus. Immediately, total cell counts were done using turk blood diluting fluid (Anachemia Chemicals Ltd., Montreal, P.Q.) in an improved Neubauer hemocytometer, and differential cell counts were performed using a Diff' Quik Stain Set (Harleco Diff Quik, Dade Diagnostics, Aguada, Puerto Rico, U.S.A.). α-Naphthyl acetate esterase stain (Sigma, St. Louis, Mo.) was also used to confirm the identity of the alveolar macrophages. Trypan blue exclusion test (Grand Island Biological Co., Grand Island, N.Y.) was carried out to ascertain the viability of the alveolar macrophages in the sample. The total number of viable alveolar macrophages in the retrieved broncho-alveolar lavage sample was calculated and the macrophages were then centrifuged for 15 minutes at a speed of 400 g at 4°C, and the sedimented cells were resuspended in Newman and Tytell serumless medium (Gibco Laboratories, Grand Island, N.Y.) to yield a cell distribution of 2×10^6/ml.

AM Phagocytic Assay

Bacteria were first opsonized for 1/2 hour with 10% fresh normal rat serum in a shaking water bath at 37°C. We used a modification of the technique described by Verhoef et al (489). From each alveolar macrophage sample to be tested, 0.1 ml containing 2×10^5 AM cells was added to 1 ml of the opsonized labeled suspension containing about 2×10^7 bacteria in two identical triplicate polypropylene tubes (12 x 75 mm, Falcon, Oxnard, Calif.). The tubes were then incubated in a shaking water bath at 37°C for 60 minutes. Following this, the first triplicate of tubes (A) was centrifuged at 160 g for 5 minutes at 4°C, bringing down only

the alveolar macrophages with its contained phagocytized bacteria. The cells were then washed in PBS and centrifuged again two additional times to remove any adherent non-phagocytized bacteria. The second identical triplication (B) was centrifuged once at a high speed of 2000 g for 15 minutes to bring down all the bacteria (intra and extra-cellular). The pellets in both triplicates were then resuspended in 1 ml of PBS and shaken vigorously (Deluxe Mixer, S8220, Scientific Products, Evanston, Ill.). 0.9 ml of the suspension in each tube of both triplicates was then solubilized in plastic scintillation vials (Fisher) each containing 10 ml of aqueous counting scintillant (ACS, Amersham, Arlington Hts., Ill.) and counted for 10 minutes in a liquid scintillation counter (ISOCAP/300, Searle Analytic Co., Des Plaines, Ill.). Counts per minute (CPM) from the liquid scintillation counter were converted to deteriorations per minute (DPM) using a recently corrected efficiency curve. An average DPM value was obtained from the first and second triplicates and the phagocytic index was calculated as follows:

$$\text{Phagocytic Index} = \frac{\text{DPM FROM FIRST TRIPLICATE (A)}}{\text{DPM FROM SECOND TRIPLICATE (B)}} \times 100$$

$$= \frac{\text{COUNTS FROM CELL ASSOCIATED BACTERIAL PELLETS}}{\text{COUNT FROM TOTAL BACTERIAL PELLETS}} \times 10$$

The results are recorded in Table 2 as mean±SEM and were compared to those of simultaneous controls using Student's t-test. The use of simultaneous controls in such an assay was found to be essential to eliminate possible intra-laboratory differences.

RESULTS

Alveolar macrophages of splenectomized animals showed a significant 15% decrease in their ability to phagocytize pneumococci type 14 compared to sham controls 4 weeks following surgery. (Group I: Control 11.62±0.71/Splx 9.83±0.19, $P<0.05$) (Figure 17). Six weeks post-splenectomy, a 30% decrease in the phagocytic activity against pneumococci type 14 was observed (Group II: Control 12.1±1.6/ Splx 8.3±0.5, $P<0.05$). (Group III: Control 8.0±0.5/Splx 5.7±0.8,

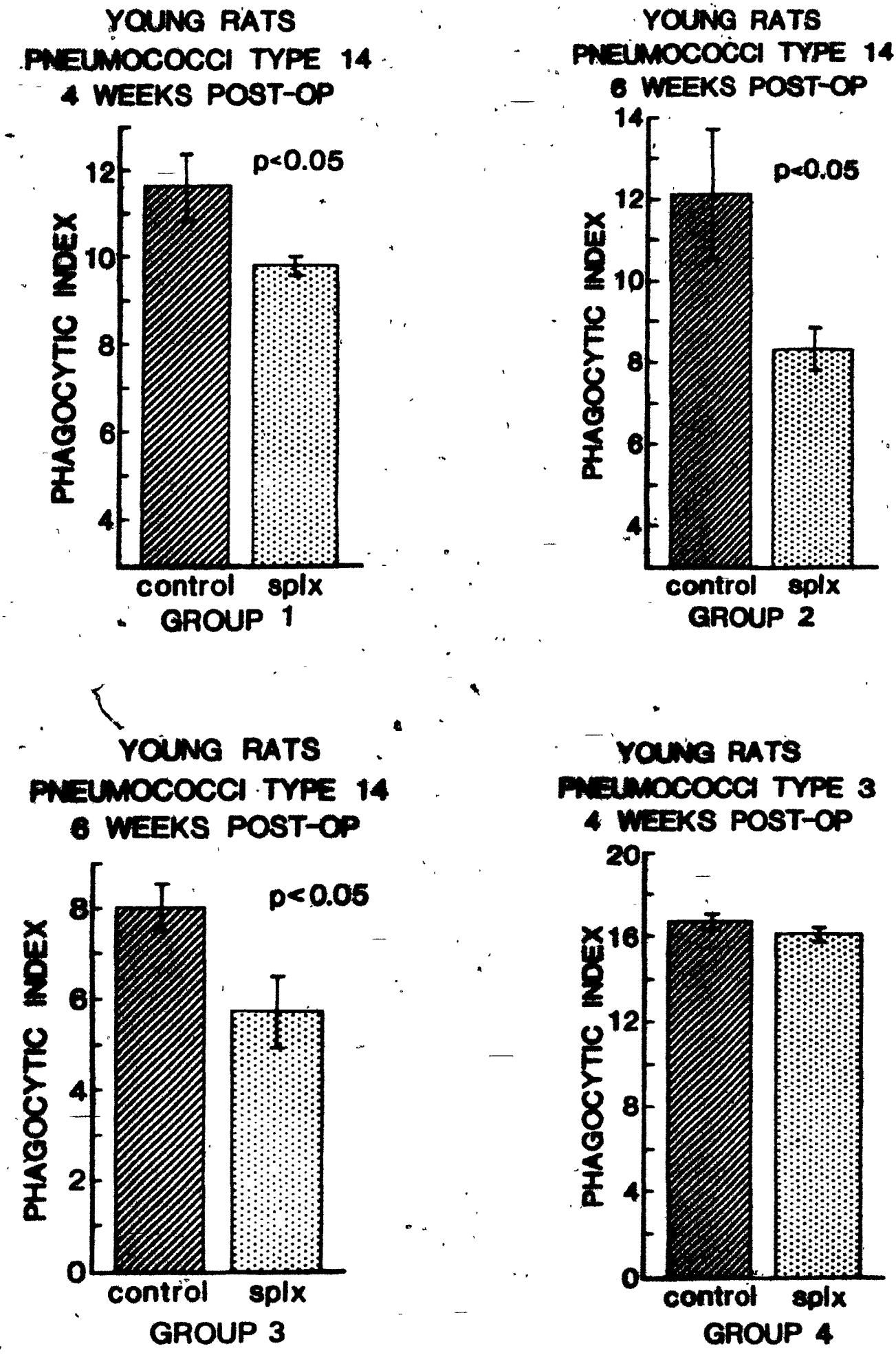


Fig. 17: Phagocytic indices of alveolar macrophages from splenectomized and simultaneously sham operated rats mean±SEM.

$P<0.05$) (Figure 17). Such alteration in the phagocytic index of Splx AM was found to represent a decrease in bacterial uptake of 8×10^5 CFU per 2×10^5 AM (Table 3) when DPM were correlated to CFU.

When young rats' AM phagocytic activity against type 14 pneumococci was compared using as opsonins serum from either splenectomized animals or sham operated animals 6 weeks following surgery (Group X), an insignificant difference was noted (Control 5.7±0.7/Splx 4.7±0.8), (Figure 19), indicating a cellular rather than humoral defect.

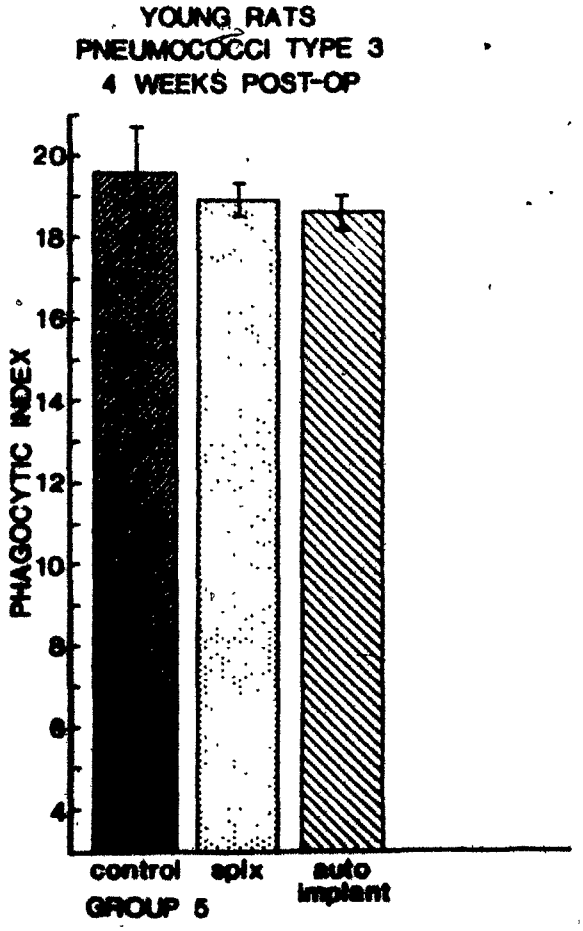
Three months following splenectomy in young animals, and as these rats grew to adult size, their AMs regained their normal ability to phagocytize pneumococci type 14 (Group VIII: Control 6.4±.4/Splx 6.6±.2) (Figure 18).

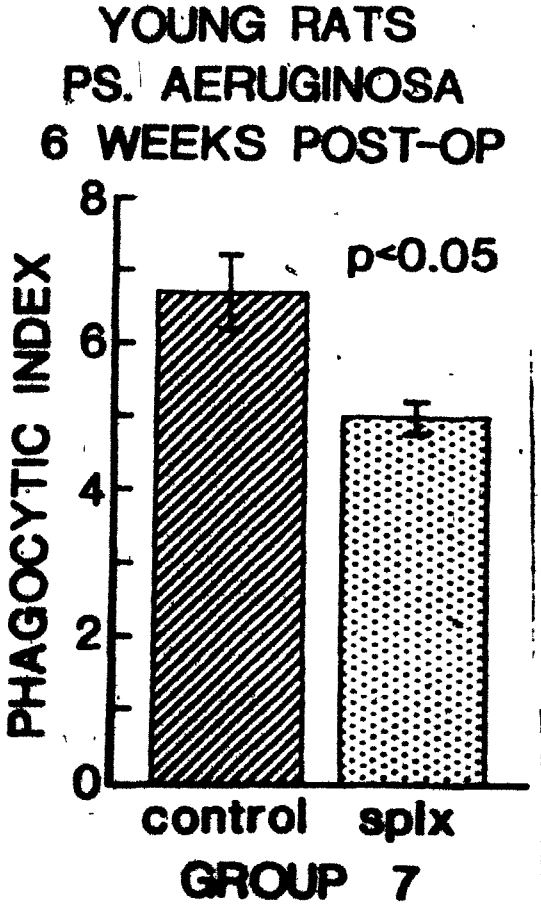
AMs from splenectomized young animals showed no change in their phagocytic activity against type 3 pneumococci at 4 weeks (Group IV: Control 16.7±1.28/Splx 16.1±.33), (Group V: Control 19.56±1.14/ Splx 18.89±.4) (Figures 17 and 18) and 6 weeks (Group VI: Control 19.86±0.85/Splx 19.94±0.36) after splenectomy (Figure 18).

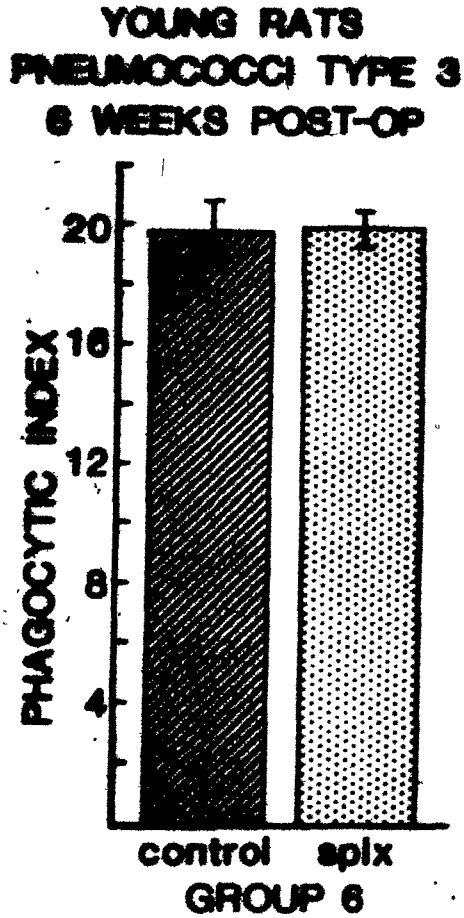
AMs from splenectomized young animals showed a significant 25% decrease in their ability to phagocytize Pseudomonas aeruginosa compared to controls 6 weeks following surgery (Group VII: Control 6.67±0.48/Splx 5.03±.17, $P<0.05$) (Figure 18).

Adult animals showed no change in their AM phagocytic activity when challenged with type 3 pneumococci 6 weeks following surgery (Group IX: Control 10.66±0.58/Splx 10.61±.48) (Figure 19).

Alveolar macrophages obtained from autoimplanted young rats had a significantly higher phagocytic activity against type 14 pneumococci when compared to that of splenectomized rats ($P<0.001$), with the phagocytic index reaching that of control animals 4 weeks after splenectomy and autoimplantation (Group I: Control 11.62±0.71/ Splx 9.83±0.19/Autoimplant 12.27±0.27) Figures 17 and 19). Autoimplantation had an insignificant effect on the phagocytosis of







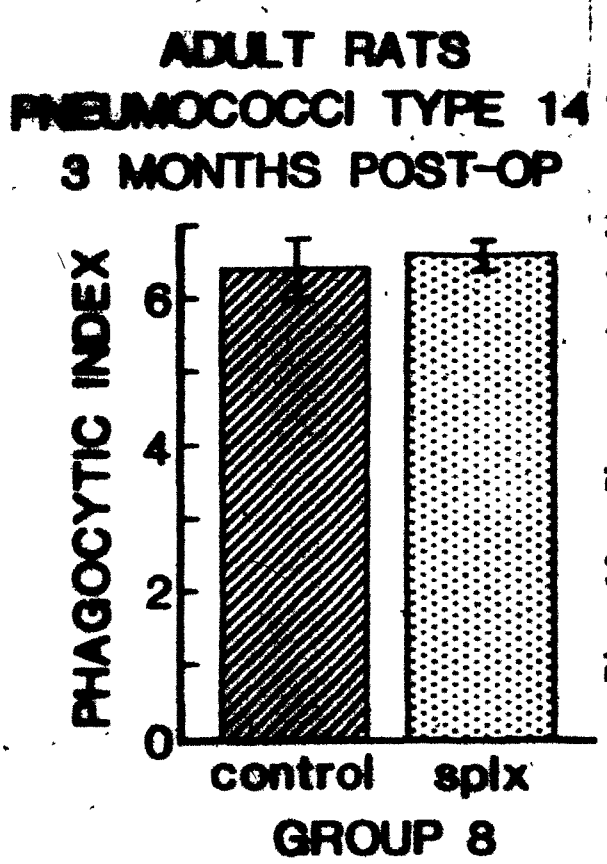


Fig. 18: Phagocytic indices of alveolar macrophages from splenectomized, auto-implanted or sham operated rats.

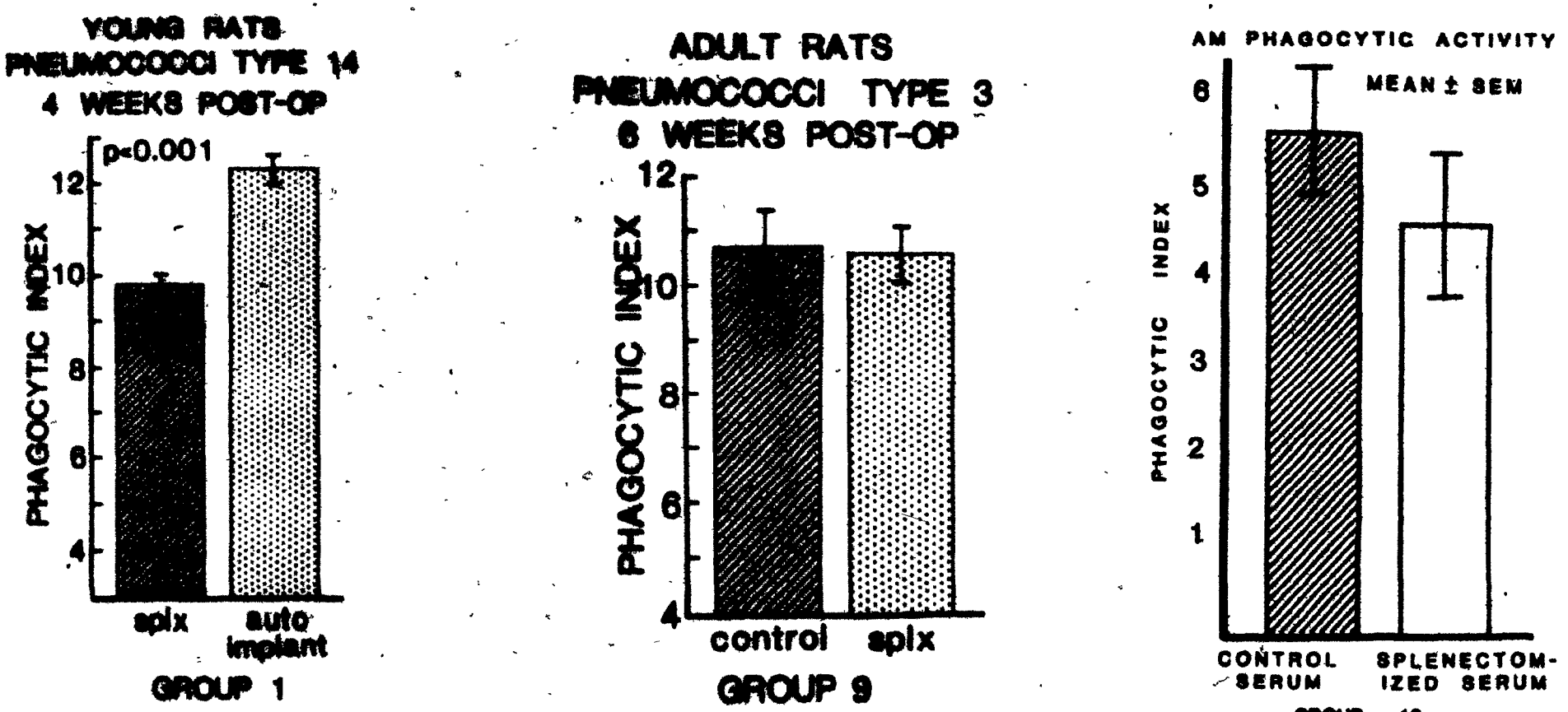


Fig. 19: Phagocytic indices of alveolar macrophages from splenectomized, auto-implanted or sham operated rats. Group 10 shows phagocytic index of normal alveolar macrophages when control or splenectomized rat serum is used as opsonin.

type 3 pneumococci (Group V: Control 19.56±1.14/Splx 18.89±0.4/ autoimplant 18.61±0.4) (Figure 18).

In the autoimplanted groups, only in 13 of 18 animals were obvious autoimplanted splenic tissues detected in the abdominal cavity. Histological examination of these implanted tissues 4 weeks following the procedure revealed a normal general splenic architecture with an equal distribution of white and red pulps, good vascularization, and occasional areas of granulation tissue formation, necrosis and cyst formation. In the splenectomized non-autoimplanted rats, no evidence of splenosis was detected.

DISCUSSION

The occurrence of pneumococcal infection is frequently associated with two important observations. First is the concommitant viral infection or body chilling (400,501). The second is that although certain types of pneumococci are frequently found in the respiratory tract of healthy individuals, other types were often responsible for the pneumococcal disease. In recent reports, although type 3 pneumococcus was frequently found in the upper respiratory tract of healthy children (127,180,269), itwas rarely isolated from the blood and cerebro-spinal fluid of children with pneumococcal disease (78,180, 233,269). While three out of five recent investigations showed that type 14 was the principal cause of the invasive disease, its presence as an inhabitant of the respiratory tract of healthy children and adults was uncommon (269). These studies suggest that the host immune defenses may respons differently to these two different types of pneumococci. While host defenses would resist one type of pneumococcus, they could fail with the other.

For more than a decade now, splenectomized children are known to be more susceptible to overwhelming pneumococcal sepsis. The incapacity of the splenectomized patients to mount an adequate protective response is thought to be bacterial species specific (74,125,141) and may, in fact, by type specific as well.

Most of the studies on the immunological changes in splenectomized animals were directed to the ability of these animals to clear cir-

culating pneumococci (57,74,514) without much attention being paid to the mechanism of primary invasion of such organism from their frequent site of inhabitation into the circulation.

In our experiments, we were able to identify a defect in the local respiratory system host defense, namely, the alveolar macrophages' ability to phagocytize bacteria after the animals were subjected to splenectomy. This defect was noticed to be bacterial species, type specific, to manifest in the younger animals, and to disappear with the further maturation of the animals (Fig. 20) a finding which may explain in part the higher susceptibility of splenectomized infants and young patients compared to splenectomized adults (140,141,497), although the latter were still found to be relatively at a risk of post-splenectomy infection when compared to the population at large (187,291,361,403).

The observed failure of the splenectomized rat alveolar macrophages to engulf type 14 pneumococci as efficiently as it did in type 3 pneumococci remains unexplained, and indeed, may present a model of how the alteration of the immune function as by splenectomy may promote the occurrence of one type of bacterial invasion rather than another.

The decreased phagocytic activity of splenectomized rat alveolar macrophages may result in an increased availability of the rapdily proliferating pneumococci to invade the circulation. The AM dysfunction would also alter the local specific lymphocyte response to the pneumococcus by deferring its supply of pneumococcal antigen determinants, eventually resulting in a reduced lung immune response. This may partially explain the frequently observed phenomenon of overwhelming pneumococcal bacteremia with minimal inflammatory lung signs (43,104,175).

The fact that AM from splenectomized animals were unable to phagocytize Ps. aeruginosa, an organism which is not frequently observed to be the pathogen in the post-splenectomy sepsis, indicates that the AM dysfunction may not be directly to the pneumococcus alone, and that perhaps the exposure to and the availability of one kind of organism or another are both important factors in the eventual

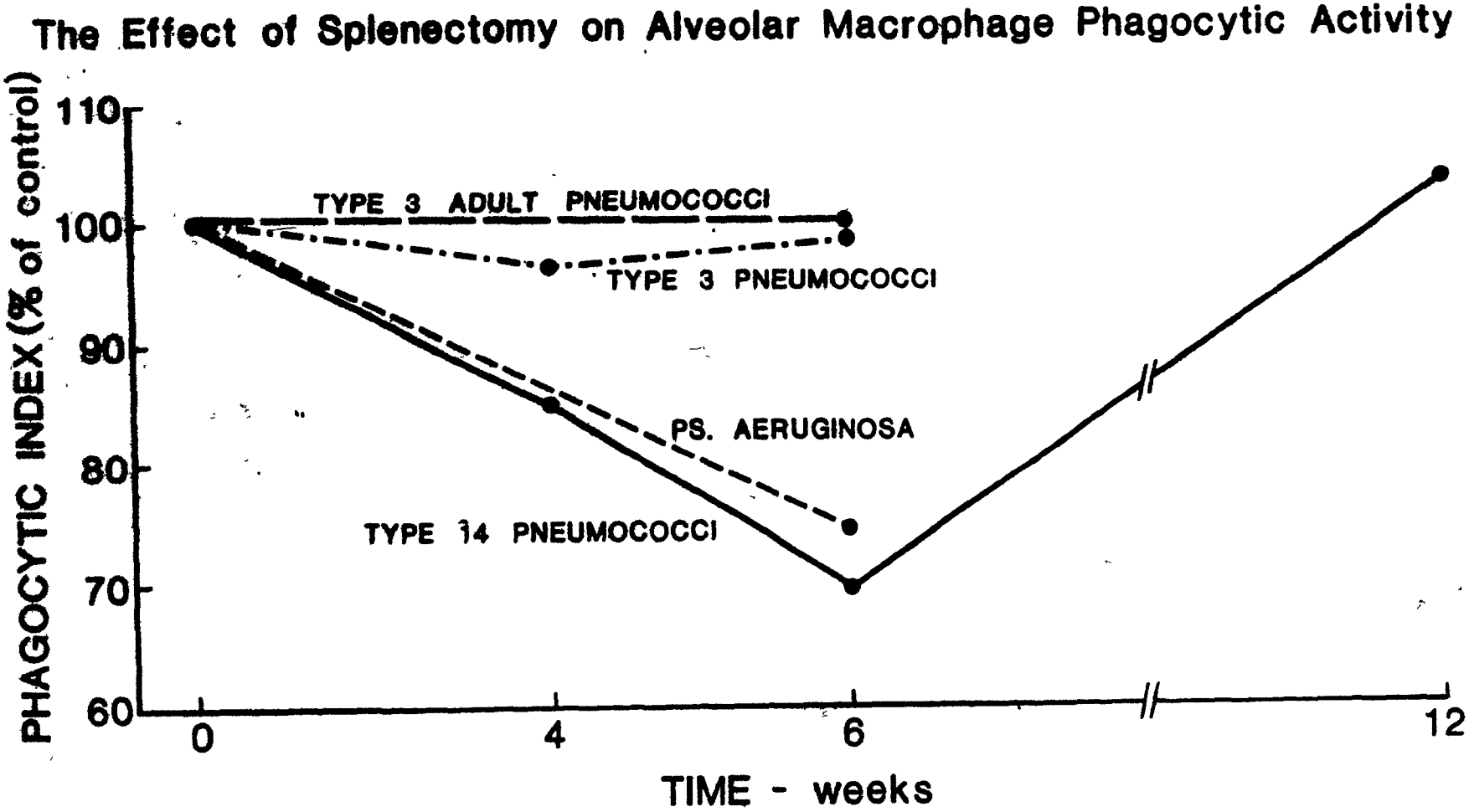


Fig. 20: Note change in the phagocytic activity of splenectomized rat AM varies with species, type of bacteria, duration after splenectomy and the age of the animal. N.B. Results on young animals except when indicated.

determination of the frequency with which a bacterium becomes responsible for post-splenectomy sepsis.

Our observation that there was no significant difference in the opsonic activity of serum from splenectomized animals as compared to that from controls suggest that the failure in AM phagocytic activity detected in splenectomized animals is not due to a deficiency of circulating factors; but is rather a cellular defect.

The mechanism of the modulatory effect of spleen on AM remains uncertain. The decreased phagocytic activity of the AM is not a generalized phenomenon as it affects specific species and types of bacteria, suggesting only a partial change in the characterization of AM which occurs when splenectomy is performed. Whether such change affects a part or the whole of the AM population could not be determined from our experiment. One can only speculate that such changes of the AM function may be induced by 1) an effect of the spleen on the hemopoetic system responsible for the production of mononuclear phagocytes, which disappears with splenectomy, to be functionally replaced by an alternative tissue, or 2) by an early in life production of a part of the AM precursor mononuclear phagocyte population by the spleen, a function which would gradually be replaced by the bone marrow in the grown animals, or 3) by the presence of a splenic cell pool responsible for specific stimulation or suppression of certain AM functions.

In the auto-implant experiments, we chose to examine whether auto-implanting part of the spleen could reverse the depressed alveolar macrophage function. We selected the duration of 4 weeks post-splenectomy for AM assessment, since we were able to confirm the previously described observation that this period of time is sufficient for complete splenic reconstruction (480). In our experiments, auto-implantation of the spleen resulted in the preservation of the AM phagocytic activity against pneumococci type 14 and an insignificant change in its activity against pneumococci type 3 (Fig. 21), indicating a protective role of such a procedure.

The failure of most investigators to demonstrate the protective effects of implants in animals against pneumococcal infection might be related to the intravenous route of bacterial challenge used in these studies which circumvented the lung host defenses.

The Effect of Spleen Autoimplantation on Rat Alveolar Macrophage Phagocytic Activity

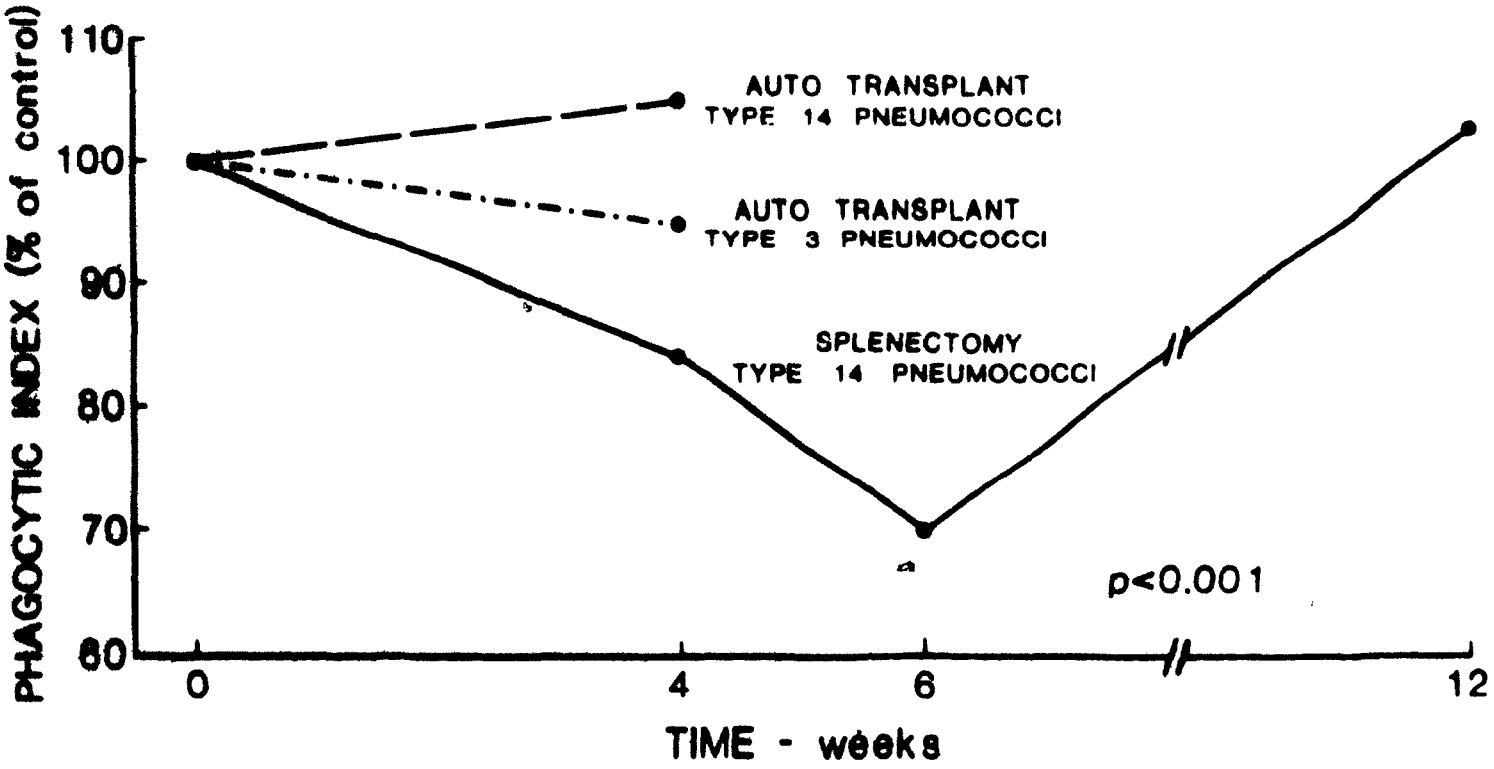


Fig. 21: Note auto-implantation of part of the spleen can prevent the deterioration of AM phagocytic activity against type 14 pneumococci as early as 4 weeks after splenectomy.

TABLE 2

EFFECT OF SPLENECTOMY AND AUTOIMPLANTATION ON AM PHAGOCYTIC INDICES

GROUPS	AGE	BACTERIA	PHAGOCYTIC INDEX			TIME OF AM HARVEST (AFTER PROCEDURE)
			CONTROL (SHAM)	SPLENECTOMY	AUTOIMPLANTATION	
I	YOUNG	PNEUMOCOCCI, TYPE 14	11.62±0.71	9.83±0.19*	12.27±0.27†	4 WEEKS
II	YOUNG	PNEUMOCOCCI, TYPE 14	12.1±0.6	8.3±0.5*		6 WEEKS
III	YOUNG	PNEUMOCOCCI, TYPE 14	8.0±0.5	5.7±0.8*		6 WEEKS
IV	YOUNG	PNEUMOCOCCI, TYPE 3	16.7±1.28	16.1±0.33		4 WEEKS
V	YOUNG	PNEUMOCOCCI, TYPE 3	19.56±1.14	18.89±0.4	18.61±0.4	4 WEEKS
VI	YOUNG	PNEUMOCOCCI, TYPE 3	19.86±0.85	19.94±0.36		6 WEEKS
VII	YOUNG	PSEUDOMONAS AERUGINOSA	6.67±0.48	5.03±0.17*		6 WEEKS
VIII	ADULT	PNEUMOCOCCI, TYPE 14	6.4±0.4	6.6±0.2		12 WEEKS
IX	ADULT	PNEUMOCOCCI, TYPE 3	10.66±0.58	10.61±0.48		6 WEEKS

			PHAGOCYTIC INDEX USING SERUM OPSONINS FROM		
GROUP	AGE	BACTERIA	CONTROL RATS	SPLX RATS	TIME
X	YOUNG	PNEUMOCOCCI, TYPE 14	5.7±0.7	4.7±0.8	6 WEEKS

N=6 in all groups. Numbers are mean±SEM.

*P<0.05 compared to control. †P<0.001 compared to splenectomy.

Group VIII Surgical procedure performed in young animals, phagocytic assay done on the same animals in adulthood.

TABLE 3

RELATIONSHIP BETWEEN THE PHAGOCYTIC INDEX (PI)

AND THE NUMBER OF BACTERIAE INGESTED (NBI) BY AM

NBI=P.Ix(CFU)*

GROUP 2 (EXAMPLE)

Total bacteria in the tube=2.1×10^7 CFU

	Control	Splenectomized
PI	12.1	8.3
NBI	2.5×10^6	1.7×10^6

*CFU= colony forming units as obtained by growing bacteria in blood agar plates.

Note = the difference in PI between the control and splenectomized rats (12.1-8.3 = 3.8) reflects a difference of $2.5 \times 10^6 - 1.7 \times 10^6 = 8 \times 10^5$ bacteria ingested by 2×10^5 AM.

THE EFFECT OF SHORT-TERM PHARMACOLOGICAL DOSE OF METHYLPREDNISOLONE ON ALVEOLAR MACROPHAGE FUNCTION

The use of pharmacological doses of corticosteroids in the management of septic shock and ARDS patients has been gaining increasing support (435). Benefits obtained by the early use of different regimes of high dose synthetic corticosteroids have been documented (435,456). However, because the use of corticosteroids on a long-term basis is associated with detrimental effects on the host immune defenses, a similar effect was anticipated when using pharmacological doses in the acute management of already jeopardized traumatic patients (208,220). Such an impairment in host immune responses, if occurs, would render these critical patients further susceptible to grave infections.

In the lungs, the longterm use of different corticosteroids resulted in a decrease in both lung ability to clear bacteria (174) (220) and alveolar macrophage phagocytic activity.

In the following experiment, we examined the effect of using pharmacological doses of methylprednisolone for short terms on alveolar macrophage function.

MATERIAL & METHOD

Four groups of Sprague Dawley rats (n=6 each) weighing 250 mg each were studied. The first group (MP_{30}) was subjected to intra-muscular injection of Solumedrol (Methylprednisolone) (Upjohn) in a dose of 30 mg/kg/day in 3 divided doses for 2 days. The second group (MP_{50}) was given 50 mg/kg/day in 3 divided doses for 2 days. The third and fourth groups were given normal saline injections and served as simultaneous controls. Four hours following the last dose, the rats were anesthetized by intra-peritoneal injection of 30 mg/kg of Nembutol following which they were subjected to broncho-alveolar lavage.

Alveolar macrophages from lavage fluid were identified, counted, their viability assessed and their phagocytic and bactericidal

activity examined in vitro against P. aeruginosa as previously described.

RESULTS

The total AM cell yield in the broncho-alveolar lavage fluid from control and steroid treated rats was similar (control 3.4±.3 x 10^6; $MP_{(30)}$ 3.6±.6 x 10^6) (control 3.9±.2 x 10^6; $MP_{(50)}$ 3.8±.6 x 10^6) (Figure 22). The number of alveolar macrophages in all the groups remained more than 96% of total cell count.

The phagocytic activity of alveolar macrophages from rats receiving Methylprednisolone 30 mg/kg/day for 2 days decreased to 86% of control values (Figure 22). However, when the phagocytic activity of alveolar macrophages from rats receiving Methylprednisolone 50 mg/kg/day for 2 days was assessed, a significant decrease to 43.6% of control values was noticed ($P<0.001$) (Figure 22). In all groups, controls and steroid treated, no difference in the intra-cellular bactericidal activity was noticed.

Electron microscopy examination of alveolar macrophages challenged with opsonized P. aeruginosa revealed that while alveolar macrophages of control and MP_{30} treated rats had active pseudopodia formation, abundant organelles and active nuclei, those from MP_{50} treated rats showed signs of cytotoxicity with dense osmeophillic inclusion bodies and poor pseudopodia formation (Figure 23).

DISCUSSION

Trauma and sepsis may progress to a stage of compromised hemodynamic and respiratory state. Recently, the use of pharmacological doses of corticosteroids in the acute management of such critical conditions have obtained scientific merits (453,456) Improvement of both hemodynamic and respiratory functions have been documented. However, the adverse effect of longterm corticosteroid administration has raised suspicion of similar immunocompromise occurring with short-term pharmacological corticosteroid treatment.

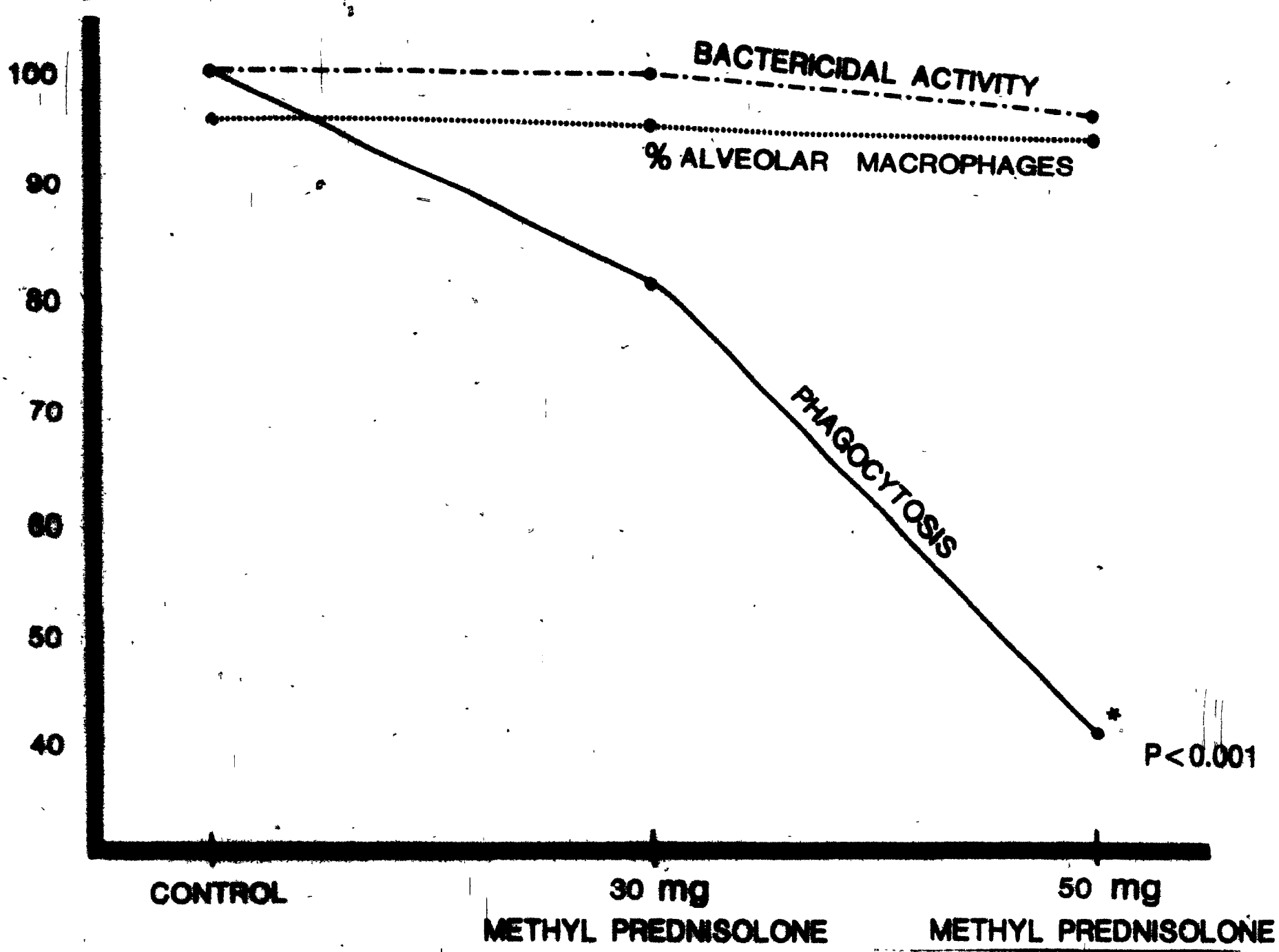


Fig. 22: Effect of parenteral administration of Methylprednisolone 30 or 50 mg/kg/day for 48 hours on rat alveolar macrophages.

Fig. 23: Alveolar macrophage from rat treated with 50 mg/kg/day of Methylprednisolone for 48 hours. Note cytotoxic changes.

Pseudomonas aeruginosa is frequently encountered as a pathogen in post-traumatic pneumonia and septic shock lung (490). Assessment of the host defense mechanism would be more purposeful if aimed towards pathogenic organisms associated with these clinical states.

In this study, the administration of Methylprednisolone parentrally in a dose of 30 mg/kg/day divided q8h for 48 hours resulted in insignificant alteration in the ability of alveolar macrophages to phagocytize or kill a challenging dose of Pseudomonas aeruginosa. However, when the rats were given Methylprednisolone in a dose of 50 mg/kg/day divided q8h for 48 hours, the alveolar macrophages phagocytic activity decreased significantly to less than 50% of normal. This decrease in the alveolar macrophage phagocytic activity was not associated with an alteration in its bactericidal activity. Electron microscopic examination of alveolar macrophages from the later group suggested the occurrence of cytotoxic changes. The apparent preservation of intra-cellular killing ability may be explained by the fact that the decrease in the phagocytic capability would result in less numbers of intra-cellular bacteria requiring less bactericidal activity.

Recently, Gudewicez showed that guinea pigs treated for 7 days with 100 mg/kg/day of cortisone acetate had a decrease in both the number of the alveolar macrophages and its phagocytic activity (190). On the other hand, Hunninghake and Fauci reported an increase in the alveolar macrophage harvest following the administration of exactly the same dose and regime of cortisone acetate (227). In our study, we were unable to detect any change in the number of alveolar macrophages harvested by broncho-alveolar lavage when Methylprednisolone was administered up to 50 mg/kg/day for 2 days.

While studies present contradicting responses in alveolar macrophage functions on the administration of different doses and regimes of corticosteroids (124), it seems that the type of corticosteroid used may determine on few occasions the response of the alveolar macrophage, for, while hydrocortisone showed no effect on PHA induced or antibody dependent cellular

cytotoxicity, cortisone acetate, the depo-preparation caused a marked decrease in the cytotoxic effector function of alveolar macrophages (227).

Corticosteroids bind to macrophage receptors in a specific interaction, the affinity of which correlates with their biological effects (509,511,512). The rate of hormone metabolism, its plasma protein transport mechanism and pre-administration receptor load (510) are important elements in defining the eventual response of macrophages to the administered corticosteroid. Our findings suggested that the use of pharmacological doses of Methylprednisolone in doses of 30 mg/kg/day or less for 48 hours is unlikely to cause an alteration in the ability of alveolar macrophages to phagocytize and kill bacteria. Increasing the administered dose to 50 mg/kg/day may result in a significant depression of their phagocytic activity renderingthe lungs more susceptible to infection.

PERSPECTIVE

In this laboratory, progression of our understanding in the handling of these cells and its examination resulted in the accumulation of significant data. We now have evidence to suggest that nutritional, pharmacological and operative manipulation may alter alveolar macrophage anti-bacterial function and render the lung susceptible to infection.

The surgeon can no longer ignore the cellular alterations which may occur in the lung of traumatic, post-operative, immuno-suppressant, malnourished or splenectomized patients. Broadening the surgeon's understanding of the risks encountered in each of the previous situations would not only allow him to recognize the area at fault, but would afford him/her the opportunity to prevent and treat it with reasonable basic knowledge.

In addition, our laboratory has developed an additional experience in undergoing investigative procedures to assess alveolar macrophage function, which we are now advancing to our critically ill surgical patients.

Initial reports from this laboratory indicate that alterations in the granulocyte/mononuclear cell ratios in the broncho-alveolar wash-outs from our trauma patients may be used as early indicators for the development or improvement of respiratory insufficiency.

Study of alveolar macrophage anti-bacterial function may also be of use in recognizing the critical patients who are immuno-compromised and at risk of pneumonia and overwhelming sepsis. An anecdotal example is that of patient A and B, both with multiple trauma, including the chest, in our Surgical Intensive Care Unit. Patient A showed an AM phagocytic activity three times higher than that of patient B. Patient A was taken off the ventilator the next day, while patient B's vital signs and respiratory function deteriorated progressively and died three days later from overwhelming sepsis with multiple organ failures.

Alveolar macrophage functions may not only be used as indicators of avid lung immune efficiency, but their maturity and accessibility through broncho-alveolar lavage allows us to examine an important immune system in our bodies, the mononuclear-phagocytic system.

Finally, our techniques have now been improved to use the Coulter counter to quantitate the lavage cells and spectrophotometry to standarize bacterial counts in the phagocytic assays.

REFERENCES

1. Adamson, I.Y.R., Bowden, H.D.: Adaptive responses of the pulmonary macrophagic system to carbon. II. Morphologic studies. Lab. Invest. 38:430, 1978.

2. Adamson, I.Y.R., Bowden, D.H.: Derivation of type I epithelium from type 2 cells in the developing rat lung. Lab. Invest. 32:736, 1975.

3. Ainsworth, S.K., Allison, F.: Studies on the pathogenesis of acute inflammation. IX. The influence of hyperosmolality secondary to hyperglycemia upon the acute inflammatory response induced by thermal injury to ear chambers of rabbits. J. Clin. Invest. 49:433, 1970.

4. Alexander, J.W., Meakins, J.L.: A physiological basis for the development of opportunistic infections in man. Ann. Surg. 176:273, 1972.

5. Alexander, J.W., Ogle, C.K., Stinnett, J.D. et al: Relationship between host defense variables and infection in severe thermal injury. Burns 5: 248, 1976.

6. Alexander, J.W., Stinnett, J.D., Ogle, C.K. et al: A comparison of immunologic profiles and their influence on bacteremia in surgical patients with a high risk of infection. Surg. 86:94, 1979.

7. Alexander, P.: The function of the macrophage in malignant disease. Annu. Rev. Med. 27:207, 1976.

8. Allen, J.C.: The diabetic as a compromised host, from Infection and the Compromised Host. Clinical Correlations and Therapeutic Approaches. Ed. J.C. Allen. Waverly Press Inc. Baltimore, USA. 1981.

9. Alper, C.A., Abramson, N., Johnston Jr., R.B. et al: Increased susceptability to infection associated with abnormalities of complement-mediated functions and of the third component of complement (3). N. Engl. J. Med. 282:349, 1970.

10. Altman, L.C., Snyderman, R., Oppenheim, J. et al: A human mononuclear leukocyte chemotactic factor: Characterization specificity and kinetics of production by homologous leukocytes. J. Immunol. 110:801, 1973.

11. Altura, B.M., Hershey, S.G.: RES phagocytic function in trauma and adaptation to experimental shock. Am. J. Physiol. 215:1414, 1968.

12. Altura, B.M., Hershey, S.G.: Sequential changes in reticuloendothelial system function after acute haemorrhage. Proc. Soc. Exp. Biol. Med. 139:935, 1972.

13. Andersen, H.A., Fontana, R.S Transbronchioscopic lung biopsy for diffuse pulmonary diseases Technique and results in 450 cases Chest 62 125, 1972

14 Arend, W.P , Mannik, M The macrophage receptor for IgG Number and affinity of binding sites J Immunol 110 1455, 1973.

15 Arms, R.A., Dines, D.E , Rinstman, T C Aspiration pneumonia Chest 65.136, 1974

16 Armstrong, J.A., Hart, P D.A. Response of cultured macrophages to mycobacterium tuberculosis with observations on fusion of lysosomes with phagosomes J Esp. Med 134 713, 1971

17 Atherton, S T , White, D J Stomach as source of bacteria colonizing respiratory tract during artificial respiration Lancet 2 969, 1978

18 Atkins, E , Bodel, P , Francis, L Release of endogenous pyrogen in vitro from rabbit mononuclear cells J Exp Med 126 357, 1967

19 Austrian, R., Gold, J Pneumococcal bacteremia with especial reference to bacteremic pneumococcal pneumonia Ann Intern Med. 60 759, 1969

20 Axline, S.G , Simon, L.M , Robin, E.C et al Effects of oxygen on metabolic patterns and endocytosis of macrophages, from Mononuclear Phagocytes Part II Ed. R Van Furth, p. 1247-1269, Martinus Nijhoff Publ. Boston 1980

21 Babior, G.M. Oxygen dependent microbial killing by phagocytes N Engl J Med. 298:659, 1978

22 Back, M.K., Brashler, J.R., Brooks, C.D., et al Lonophore A 23187-induced production of slow reacting substance of anaphylaxis (SRS-A) by rat peritoneal cells in vitro: Evidence for production by mononuclear cells. J. Immunol 120:998, 1978.

24 Bagdale, J., Root, R.K., Bulger, R.J. Impaired leukocyte function in patients with poorly controlled diabetes. Diabetes 23:9, 1974

25 Ballow, M , Shira, J.E., Harden, L. et al Complete absence of the third component of complement in man. J Clin. Invest. 56:703, 1975

26. Barber, R.E., Lee, J., Hamilton, W.K. Oxygen toxicity in man: A prospective study in patients with irreversible brain damage. N. Engl. J Med. 283 1478, 1970.

27. Bartlett, J.G., Gorbach, S.L. The triple threat of aspiration pneumonia. Chest 68:560, 1975

28 Battisto, J.R., Cantor, L.C., Borek, F. et al. Immunoglobin synthesis in hereditary spleenless mice. Nature 222:1196, 1969.

29 Beller, D.I., Farr, A.G., Unanue, E.R. Regulation of lymphocyte proliferation and differentiation by macrophages. Fed Proc 37:91, 1978

30 Bengmark, S., Bõjesson, B., Die subcutane milztransposition: eine experimentelle und klinische studie. Langenbeeks Arch Chir 342:189, 1976

31 Bennacerra, F.B., Sebestyen, M., Schlossman, S. A quantitative study of the kinetics of blood clearance of P^{32} labeled Escherichia coli and Staphylococci by the reticuloendothelial system. J Exp Med 110:27, 1959

32 Bensch, K.G., Dominguez, E.A. Studies on the pulmonary air tissue barrier. IV. Cytochemical tracing of macromolecules during absorption. Yale J Biol & Med 43:236, 1971

33 Benveniste, J., Duval, D., Vargaftig, B.B. et al. Libération du "platelet activating factor" par les cellules de lavage broncho alveolaire. Bull Eur Physiopathol Respir 15:26, 1979

34 Berken, A., Sherman, A.A. Reticuloendothelial system phagocytosis in diabetes mellitus. Diabetes 23:218, 1974

35 Berman, L.R. Intravascular microaggregation and the respiratory distress syndrome. Pediatr Clin North Am. 22:275, 1975

36 Berman, L.R., Smulson, M.E., Pattengale, P. et al. Pulmonary microembolization after soft tissue injury on primates. Surg 70:246, 1971

37 Bhaskaram, P. Macrophage function in severe protein energy malnutrition. Indian J. Med. Res. 71:247, 1980.

38 Bianco, C., Eden, A., Cohen, E.A. The induction of macrophage spreading: Role of coagulation factors and the complement system. J. Exp. Med. 144:1531, 1976.

39 Bienenstock, J., Johnston, N., Perey, D.Y.E. Bronchial lymphoid tissue. I. Morphologic characteristics. Lab. Invest. 28:686, 1973

40 Bienenstock, J., Johnston, N., Perey, D.Y.E. Bronchial lymphoid tissue. II. Functional characteristics. Lab. Invest. 28:693, 1973

41 Biggar, W.D., Bogart, D., Holmes, B. et al. Impaired phagocytosis of pneumococcus type 3 in splenectomized rats. Proc. Soc. Exp. Biol. & Med. 139:903, 1972.

42 Biggar, W.D., Sturgess, J.M. Hydrogen peroxide release by rat alveolar macrophages: Comparison with blood neutrophils. Infect. & Immunity 19:621, 1978.

43. Bisno, A., Freeman, J.C. The syndrome of asplenia, pneumococcal sepsis and disseminated intravascular coagulation. Ann. Intern Med. 72:389, 1970.

44. Bitter-Suermann, H. Surgical aspects of spleen grafting in rats. Eur. Surg: Res. 7:34, 1975.

45. Blanden, R.V., Hapel, A.J., Doherty, P.C. et al Lymphocyte macrophage interactions and macrophage activation in the expression of antimicrobial immunity in vitro, in immunobiology of the Macrophage. Ed. D.S. Nelson, p. 367-400, Academic Press, New York, 1976.

46. Blanden, R.V., Lefford, M.J., MacKaness, G.: The host response to Calmette-Gurein Bacillus infection in mice. J Exp. Med. 129:1029, 1969.

47 Blondin, J., Resenberg, R., Janoff, A. An inhibitor in human lung macrophages active against human neutrophil elastase Am. Rev. Resp. Dis. 106:477, 1972.

48. Bloom, W. Immune reactions in tissue culture. I Reaction of the lungs from normal and immunized rabbits to pigeon erythrocytes. Arch. Pathol. 3:608, 192?.

49 Blue, J., Weiss, L.. Electron microscopy of the red pulp of the dog spleen including vascular arrangements, periarterial macrophage sheaths (ellipsoids) and the contractile, innervated reticular meshword. Am. J. Anatomy 161:189, 1981.

50. Blue, J., Weiss, L. Periarterial macrophage sheaths (ellipsoids) in cat spleen - an electron microscope study. Am. J. Anatomy 161:115, 1981

51. Blumenstock, F.A., Saba, T., Roccario, E. et al. Opsonic fibronectin after trauma and particle injection determined by a peritoneal macrophage monolayer assay. J. Reticuloendothelial Soc. 30:61, 1981.

52. Blussé van oud Alblas, A., Van der Linden-Schrever, B., Mattie, H. et al:The effect of glucocorticoids in the kinetics of pulmonary macrophages. J. Reticuloendothelial Soc 30:11, 1981.

53. Blussé van oud Alblas, A., Van der Linden-Schrever, B., Van Furth, R.. Origin and kinetics of pulmonary macrophages during an inflammatory reaction induced by intravenous administration of heat-killed Bacillus Calmette-Guerin. J. Exp. Med. 154:235, 1981.

54. Blussé van oud Alblas, A., Van Furth, R.: Origin, kinetics and characteristics of pulmonary macrophages in the normal steady state. J. Exp. Med. 149:1504, 1979

55. Bode, F.R., Pare, J.A., Fraser, R.G.: Pulmonary diseases in the compromised host. Medicine 53: 255, 1974.

56. Bodel, P.: Studies on the mechanism of endogenous pyrogen production III. Human blood monocytes. J. Exp. Med. 140:554, 1974.

57. Bogart, D., Bigger, W.D., Good, R.A.: Impaired intravascular clearance of pneumococcus type-3 following splenectomy. J. Reticuloendoth. Soc.11:77, 1972.

58. Bolen, G.: Phagocytosis-associated oxidative metabolism in the rabbit alveolar macrophage. Senior thesis , Yale University School of Medicine, New Haven, Ct., 1977.

59. Boltz-Nitulescu, G., Bazin, H., Spiegelberg, H.L.: Specificity of Fc receptors for IgG_2a, IgG_1b and IgE on rat macrophages. J. Exp. Med. 154: 374, 1981.

60. Bowden, D.H., Adamanson, I.Y.R.: Pulmonary macrophages in the reticuloendothelial system - a comprehensive treatise, vol. 1, Morphology (I. Carr, W.T. Daemsied), p. 607, Plenum Press, New York, 1980.

61. Bowden, D.H., Adamson, I.Y.R.. The pulmonary interstitial cell as immediate precursor of the alveolar macrophage. Am. J. Pathol. 68:521, 1972.

62. Bowden, D.H., Adamson, I.Y.R., Grantham, W.G. et al: Origin of the lung macrophage - evidence derived from radiation injury. Arch Pathol. 88: 540, 1969.

63. Bowers, T.K., Craddock, P.R., Jacob, H.S.: Acquired granulocyte abnormality during drug allergic reactions - possible role of complement activation. Blood 49: 3, 1977.

64. Boyden, S.J.: The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leukocytes. J. Exp. Med. 115: 453, 1962.

65. Brain, J.D., Godleski, J.G., Sorokin, S.P. in Lung Biology in Health and Disease, Vol. 5, Respiratory defense mechanisms, J.D. Brain, D.F. Proctor, L. Reid, eds., Dekker, New York, pp. 849-885, 1977.

66. Brayton, R.G., Stokes, P.E., Schwartz, M.S. et al: Effect of alcohol and various diseases on leukocyte mobilization, phagocytosis and intracellular bactericidal killing. New Engl. J. Med. 282: 123, 1970.

67. Brigham, K.L., Bowers, R.E., Haynes, J.: Increased sheep lung vascular permeability caused by Escherichia coli endotoxin. Circ. Res. 45: 292, 1979.

68. Brigham, K.L., Bowers, R.E., McKeen, C.R.: Methylprednisolone prevention of increased lung vascular permeability following endotoxemia in sheep. J. Clin. Invest. 67: 1103, 1981.

69. Brigham, K.L., Snell Jr. J.D., Harris, T.R. et al: Indicator dilution lung water and vascular permeability in humans: Effects of pulmonary vascular pressure. Circ. Res. 44: 523, 1979.

70. Brigham, K.L., Woolverton, W.C., Blake, L.H. et al: Increased sheep lung vascular permeability caused by Pseudomonas bacteremia. J. Clin. Invest. 54: 792, 1974.

71. Brody, A.R., Craighead, J.E.: Cytoplasmic inclusions in pulmonary macrophages of cigarette smokers. Lab. Invest. 32: 125, 1975.

72. Brooke, M.S., Hechter, O:, Kass, E.H.: Antiendotoxic activity of corticosteroids. Endocrinol. 69: 867, 1961.

73. Broome, C.V., Facklam, R.R.: Epidemiology of clinically significant isolates of Streptococcus pneumoniae in the United States. Rev. Infect. 2: 277, 1981.

74. Brown, E.J., Hosea, S.W., Frank, M.M.: The role of the spleen in experimental pneumococcal bacteremia. J. Clin. Invest. 67: 975, 1981.

75. Brown, J.P., Leedom, J.M.: Pneumonias, bacterial in Communicable and Infectious Diseases F.H. Top, P.F. Wehrle, eds., St. Louis, C.V. Mosby, pp. 608, (ed.8), 1976.

76. Brubaker, L.H., Evans, W.H.: Separation of granulocytes, monocytes, lymphocytes, erythrocytes and platelets from human blood and relative tagging with diisopropylfluorophosphate (DFP), J. Lab. Clin. Med. 73: 1036, 1969.

77. Bucher, U., Reid, L.: Development of the mucus-secreting elements in human. Thorax 16: 219, 1961.

78. Burke, J.P., Klein, J.O., Gezon, H.M. et al: Pneumococcal bacteremia. Am. J. Dis. Child. 121: 353, 1971.

79. Cameron, J.L., Reynolds, J., Zuidema, G.D.: Aspiration in patients with tracheostomies. Surg. Gyn. & Obs. 136: 68, 1973.

80. Campbell, E.J., White, R.R., Rodriguez, R.J. et al: Specific binding of human leukocyte elastase by human alveolar macrophages. Am. Rev. Resp. Dis. 117: 319, 1978.

81. Carlson, R.W., Schaeffer, R.C., Michaels, E.G. et al: Pulmonary edema fluid - spectrum of features in 37 patients. Circ. 60: 1161, 1979.

82. Carr, I., Daems, W.T.: The macrophage,a bird's eye view in The Reticuloendothelial System, a Comprehensive Treatise, Vol. 1, pp. 1-17, Plenum Press, New York,1980.

83. Cashner, F.M., Schyler, M.R., Salvaggio, J.E.: The effect of cortisone acetate on pulmonary lesions in a rabbit model of hypersensitivity pneumonitis (cortisone and hypersensitivity pneumonitis), Clin. Exp. Immunol. 36: 266, 1979.

84. Center for Disease Contol: National Nosocomial Infection Study Report, Annual Summary, 1977.

85. Center for Disease Control:National Nosocomial Infection Study Report, Annual Summary, 1978.

86. Chandhary, S., Hughes, W.T., Feldman, S. et al: Percutaneous transthoracic needle aspiration of the lung. Am. J. Dis. Child. 131: 902, 1977.

87. Chaudry, I.H., Tabata, Y., Schleck, S. et al: Effect of splenectomy on reticuloendothelial fnction and survival following sepsis. J. Trauma 20: 649, 1980.

88. Chernew, I., Braude, A.I.: Depression of phagocytosis by solutes in concentrations found in the kidney and urine. J. Clin. Invest. 41: 1945, 1962.

89. Chomet, B., Gach, B.M.: Lobar pneumonia and alcoholism: An analysis of thirty-seven cases. Am. J. Med. Sci. 253: 300, 1967.

90. Christou, N.V., McLean, A.P., Meakins, J.L.: Host defense in blunt trauma: Interrelationship of kinetics of energy and depressed neutrophil function, nutritional status, and sepsis. J. Trauma 20: 833, 1980.

91. Clark, J.M., Lambertsen, C.H.: Pulmonary oxygen toxicity: A review. Pharmacol. Rev. 23: 37, 1971.

92. Clowes, G.H.A., Farrington, G.H., Zuschneid, W. et al: Circulating factors in the etiology of pulmonary insufficiency and right heart failure accompanying severe spesis (peritonitis). Ann. Surg. 171: 663, 1970.

93. Cohen, A.B.: Interrelationships between the human alveolar macrophage and alpha-1-antitrypsin. J. Clin. Invest. 52: 2793, 1973.

94. Cohen, A.B., Batra, G.K.: Bronchoscopy and lung lavage induced bilateral pulmonary neutrophil influx and blood leukocytosis in dogs and monkeys. Am. Rev. Resp. Dis. 122: 239, 1980.

95. Cole, P., Turton, C., Lamyon, H., Collings, J.: Bronchoalveolar lavage for the preparation of free lung cells: Technique and complications. Br. J. Dis. Chest 74: 273, 1980.

96. Comroe Jr., J.H., Dripps, R.D., Dumke, P.R. et al: Oxygen toxicity The effects of inhalation of high concentration of oxygen for twenty-four hours on normal men at sea level and at a simulated altitude of 18,000 feet. JAMA 128: 710, 1945.

97. Constantopoulos, A., Najjar, V.A.: Tuftsin deficiency syndrome, a report of two new cases. Act. Paediatr. Scand, 62:645, 1973.

98. Constantopoulos, A., Najjar, V.A., Wish, B. et al: Defective phagocytosis due to tuftsin deficiency in splenectomized subjects Am. J. Dis. Child. 125: 663, 1973.

99. Coonrod, J.D., Yoneda, K: Complement and opsonins in alveolar secretions and serum of rats with pneumonia due to streptococcus pneumonia. Rev. Infect. Dis. 3 310, 1981.

100. Crocker, S.H., Eddy, D.O., Obenauf, R.N. et al: Bacteremia: Host specific lung clearance and pulmonary failure. J. Trauma 21: 215, 1981.

101. Crocker, S., Eddy, D., Wismer, B. et al: Pulmonary clearance of blood borne Pseydomonas aeruginosa in pigs. Am. Rev. Resp. Dis. 121: 123, 1980 (Suppl.).

102. Currie, G.A.: Activated macrophages kill tumour cells by releasing arginase. Nature 273: 758, 1978.

103. Currie, G.A., Hedley, D.W.: Monocytes and macrophages in malignant melanoma. I. Peripheral blood macrophage precursors. Br. J. Cancer 36: 1, 1977.

104. Dailey, M.O., Coleman, C.N., Kaplan, H.S.: Radiation-induced splenic astrophy in patients with Hodgkins disease and non-Hodgkins lymphomas. New. Engl. J. Med. 302: 215, 1980.

105. Dannenberg Jr., A.M., Burstone, M.S., Kinsley, J.W. et al: A Histochemical study of phagocytic and enzymatic function of rabbit mononuclear and polymorphonuclear exudate cells and alveolar macrophages. I. Survey and quantitation of enzymes and states of cellular activation. J. Cell. Biol. 17: 465, 1963.

106. Dauber, J.H., Daniele, R.D.; Chemotactic activity of guinea pig alveolar macrophages. Am. Rev. Reso. Dis. 117: 673, 1978.

107. Dauber, J.H., Daniele, R.: Secretion of chemotaxis by guinea pig lung macrophages. I. The spectrum of inflammatory cell responses. Exp. Lung Res. 1. 23, 1980.

108. David, J.R.:Delayed hypersensitivity in vitro: Its mediation by cell-free substances formed by lymphoid cell-antigen interaction. Proc. Natl. Acad. Sci. USA 56: 72, 1966.

109. David, J.R., Remold, H.G.: Macrophage activation by lymphocyte mediators and studies on the interaction of macrophage inhibitory factor (M.I.F.) with its target cell in Immunobiology of the Macrophage, pp. 401-427, D.S. Nelson, ed., Academic Press, New York, 1976.

110. Davies, P., Sornberger, G.C., Huber, G.L.: A method for the collection of alveolar macrophages for microscopy. Am. Rev. Resp. Dis. 116: 1113, 1977.

111. Davis, G.S., Brody, A.R., Adler, K.B.: Functional and physiologic correlates of human alveolar macrophage cell shape and surface morphology. Chest 75: 280, 1979.

112. Davis, J.H.:Surgical aspects of diabetes mellitus in Textbook of Surgery, The Biological Basis of Modern Surgical Practice, D.C. Sabiston, W.B. Saunders, eds., Philadelphia, PA. 1977.

113. DeBruyn, P.P.H., Farr, A.G.: Lymphocyte-RES interactions and their fine-structural correlates in The Reticuloendothelial System - Comprehensive treatise, Vol. 1, pp. 499-523, Morphology, I. Carr, W.T. Daems, eds., Plenum Press, New York, 1980.

114. Dee, T.H., Schiffman, G., Sottile, M.I. et al: Immunologic studies in pneumococcal disease. J. Lab. Clin. Med. 89: 1198, 1977.

115. DeMeo, A.N., Anderson, B.R.: Defective chemotaxis associated with a serum inhibitor in cirrhotic patients. N. Engl. J. Med. 286: 735, 1972.

116. Deneke, S.M., Bernstein, S., Fanburg, B.L.: Absence of inductive effect of hyperoxia on superoxide dismutase activity in rat alveolar macrophages. Am. Rev. Resp. Dis. 118: 105, 1978.

117. Deneke, S.M., Fanburg, B.L.: Normobaric oxygen toxicity of the lung. N. Engl. J. Med. 303: 76, 1980.

118. Dent, R.G., Cole, P.: In vitro monocyte maturation and survival in lung cancer. Proceedings of the British Thoracic Association and the Thoracic Society. Thorax 36: 229, 1981.

119. Dessaint, J.P., Capron, A., Joseph, M. et al: Cytophilic binding of IgE and anti-IgE in the rat: II. Immunologic release of lysosomal enzyme from macrophages by IgE and anti-IgE in the rat. A new mechanism of macrophage activation. Cell. Immunol. 46: 24, 1979.

120. Dessaint, J.P., Torpier, G., Capron, M. et al: Cytophilic binding of IgE to the macrophage. I. Binding characteristics of IgE on the surface of macrophages in the rat. Cell. Immunol. 46:12, 1979.

121. Diamond, L.K.: Splenectomy in childhood and the hazard of overwhelming infection. Pediatrics 43: 886, 1969.

122. Dickermann, J.D., Horner, S.R., Coil, J.A. et al: The protective effect of intraperitoneal splenic autotransplants in mice exposed to an aerosolized suspension in type III streptococcus pneumoniae. Blood 54: 354, 1979.

123. Distelhorst, C.W., Benutto, B.M.: Glucocorticoid receptor content of T-lymphocytes: Evidence for heterogeneity. J. Immunol. 126: 1630, 1981.

124. Douby, W.R., Whitcomb, M.E.: The effects of corticosteroid administration on the bronchoalveolar cells obtained from guinea pigs by lung lavage. Am. Rev. Resp. Dis 117: 893, 1978.

125. Donaldson, S.S., Moore, N.R., Rosenberg, S.A. et al: Characterization of post-splenectomy bacteremia among patients with and without lymphoma. N. Engl. J. Med. 287: 69, 1972.

126. Douglas, S.D., Schopfer, K.: Phagocyte function in protein-calorie malnutrition. Clin. Exp. Immunol. 17: 121, 1974.

127. Dowling, J.N., Sheehe, P.R., Feldman, H.A.: Paryngeal pneumococcal acquisitions in "normal" families - a longitudinal study. J. Infect. Dis. 124: 9, 1971.

128. Downey, E.C., Catanzaro, A., Ninnemann, J.C.: Longterm depressed immunocompetence of patients splenectomized for trauma. Surg. Forum 31: 41, 1980.

129. Drachman, R.H., Root, E.K., Wood, W.B.: Studies on the effect of experimental nonketotic diabetes mellitus on antibacterial defense. I. Demonstration of a defect in phagocytosis. J. Exp. Med. 124: 227, 1966.

130. Dressler, D.P., Skornik, W.A.: Alveolar macrophage in the burned rat. J. Trauma 14: 1036, 1974.

131. Dumonde, D.C., Kelly, R.H., Preston, P.M. et al: Lymphokines and macrophage function in the immunological response in Mononuclear Phagocytes in Immunity, Infection and Pathology, pp. 675-699, R. van Furth, ed. Blackwell, London, 1975.

132. Eddy, D., Crocker, S., Wismer, B. et al: Dose-dependent effects of Pseudomonas aeruginosa bacteremia in pigs. Fed. Proc. 39: 1067, 1980.

133. Eickhoff, T.C.: Pulmonary infections in surgical patients. Surg. Clinics of N. Amer. 60: 175, 1980.

134. Einstein, C.P., Schneeberger, E.E., Colton, H.R.: Synthesis of the second component of complement by long-term primary cultures of human monocytes. J. Exp. Med. 143: 114, 1976.

135. Eisemann, B., Beart, R., Norton, L.: Multiple organ failure. Surg. Gyn. & Obs. 144: 323, 1977.

136. Elfenbein, G.J., Kallman, C.H., Tutschka, P.J. et al: The immune system in 40 aplastic anemic patients receiving conventional therapy. Blood 53: 652, 1979.

137. Elfenbein, G.J., Parrish, J.S.: Immune effector function in vitro proximate to antimicrobial defense mechanisms in vivo in allogeneic marrow transplant patients. Exp. Haematol. (Suppl. 6) 7: 19, 1979.

138. Elfenbein, G.J., Saral, R.: Infectious disease during immune recovery after bone marrow transplantation in Infection and the Compromised Host, Clinical Correlations and Therapeutic Approaches, pp. 157-196, J.C. Allen, ed., The Williams and Wilkins Company, Baltimore, USA, 1981.

139. Eliashiv, A., Olumide, F., Norton, L. et al: Depression of cell-mediated immunity in diabetes. Arch. Surg. 113: 1180, 1978.

140. Ellis, E.F., Smith, R.T.: The role of the spleen in immunity. Pediatrics 37: 111, 1966.

141. Erakalis, A.J., Kevy, S.V., Diamond, L.K. et al: Hazard of overwhelming infection after splenectomy in children. N. Engl. J. Med. 276: 1225, 1967.

142. Erdmann, III, A.J., Vaughan Jr., T.R., Brigham, K.L. et al: Effect of increased vascular pressure on lung fluid balance in unanesthetized sheep. Circ. Res. 37: 271, 1975.

143. Eriksson, E., Straube, R.C., Robson, M.C.: White blood cell consumption in the microcirculation after a major burn. J. Trauma 19: 94, 1979.

144. Evans, M.J., Cabral, L.J., Stephens, R.J. et al: Cell division of alveolar macrophages in rat lung following exposure to No_2. Am. J. Pathol. 70: 199, 1973.

145. Evans, R., Alexander, P.: Mechanisms of extracellular killing of nucleated mammalian cells by macrophages in Immunobiology of the Macrophage, pp. 535, D.S. Nelson, ed., Academic Press, New York, San Francisco, London, 1976.

146. Farr, A.G., Dorf, M.E., Unanue, E.R.: Secretion of mediators following T-lymphocyte-macrophage interaction is regulated by the major histocompatability complex. Proc. Natl. Acad. Sci. USA 74:3542, 1977.

147. Fauve, R.M., Pierce-Chase, C.H.: Comparative effects of corticosteroids on host resistance to infection in relation to chemical structure. J. Exp. Med. 125: 807, 1967.

148. Fialkow, P.R., Jacobson, R.J., Papayannopoulou, T.: Chronic myelocytic leukemia: Clonal origin in a stem cell common to the granulocyte, erythrocyte, platelet and monocyte/macrophage. Am. J. Med. 63: 125, 1977.

149. Fishman, A.P., Pietra, G.G.:Handling of bioactive materials by the lung (1st of two parts). N. Engl. J. Med. 291: 884, 1974.

150. Fishman, A.P., Pietra, G.G.: Handling of bioactive materials by the lung (2nd of two parts). N. Engl. J. Med. 291: 953, 1975.

151. Frank, L., Yam, J., Roberts, R.J.: The role of endotoxin in protection of adult rats from oxygen-induced lung toxicity. J. Clin. Invest. 61: 269, 1978.

152. Frank, M.M., Schreiber, A.D., Atkinson, J.P. et al: Pathophysiology of immune haemolytic anaemia. Ann. Intern. Med. 87: 210, 1977.

153. Freidland, J., Selton, C., Silverstein, E.: Induction of angiotensin converting enzyme in human monocytes in culture. Biochem. Biophys. Res. Comm. 83: 843, 1978.

154. Friis, R.R.: Interaction of L-cells and Chlamydia Psittaci: Entry of the parasite and host responses to its development. J. Bacteriol. 110: 706, 1972.

155. Fuchs, P.C.: History from epidemiology of hospital-associated Infections, P.C. Fuchs editor, pp. 1-25, American Society of Clinical Pathologists. Chicago Library of Congress Cataloging in Publication Data, 1978.

156. Fuenfer, M.M., Olson, G.E., Polk, H.C.: Effect of various corticosteroids upon the phagocytic bactericidal activity of neutrophils. Surg. 78: 27, 1975.

157. Fulton, R.L., Jones, C.E.: The cause of post traumatic pulmonary insufficiency in man. Surg. Gyn. & Obs. 140: 179, 1975.

158. Gallin, E.K., Seligmann, B., Gallin, J.I.: Alteration of macrophage and monocyte membrane potential by chemotactic factors from Mononuclear Phagocytes, pp. 505-526, R. van Furth, ed., Martinus Nijhoff Publ., Boston.

159. Garvey, J., Guarneri, J., Khan, F. et al: Clinical evaluation of bronchopulmonary lavage using the flexible fiberoptic bronchoscope. Ann. Thorac, Surg. 30: 427, 1980.

160. Gee, J.B.L., Kaskin, J., Duncombe, M.P. et al: The effects of ethanol on some metabolic features of phagocytosis in the alveolar macrophage. Reticuloendothelial Soc. 15: 61, 1974.

161. Geiger, K., Gallagher, M.L., Hedley-White, J.: Cellular distribution and clearance of aerosolized dipalmitoyllecithin. J. Appl. Physiol. 39, 759, 1975.

162. Gemmell, C.G., Peterson, P.K., Schmelling, D. et al: Potentiation of opsonization and phagocytosis of streptococcus pyogenes following growth in the presence of clindamycin. J. Clin. Invest. 67: 1249, 1981.

163. Gigli, I., Nelson Jr., R.A.: Complement dependent immune phagocytosis. I. Requirements for C_1, C_4, C_2, C_3. Exp. Cell. Res. 51: 45, 1968.

164. Gil, J., Reiss, O.K.: Isolation and characterization of lamellar bodies and tubular myelin from rat lung homogenates. J. Cell. Biol. 58: 152, 1973.

165. Ginsburg, H.B., Schullinger, J.N., Barlow, B.A. et al.: Impaired resistance to infection following splenic irradiation of rats. Surg. Forum 30: 28, 1979.

166. Girre, C., Pelletier, M., Giroud, J.P.: Demonstration of the presence during acute inflammatory reactions of a serum factor mitogenic for macrophages. J. de Pharmacol. 12: 147, 1981.

167. Godleski, J., Brian, J.: The origin of alveolar macrophages in mouse radiation chimeras. J. Exp. Med. 136: 630, 1972.

168. Golde, D.W., Burgaleta, C., Sparkes, R.S. et al.: The Philadelphia chromosome in human macrophages. Blood 49: 367, 1977.

169. Golde, D.W., Byers, L.A., Finley, T.N.: Proliferative capacity of human alveolar macrophage. Nature 247: 373, 1974.

170. Golde, D.W., Drew, W.L., Klein, H.Z. et al.: Occult pulmonary haemorrhage in leukaemia. Br. Med. J. 2: 166, 1975.

171. Golde, D.W., Finley, T.N., Cline, M.J.: Production of colony stimulating factor by human macrophages. Lancet 2: 1397, 1972.

172. Golde, D.W., Finley, T.N., Cline, M.J.: The pulmonary macrophage in acute leukemia. N. Engl. J. Med. 290: 875, 1974.

173. Goldstein, E., Bartlema, H.C., Van Der Ploeg, M. et al: Effect of ozone on lysosomal enzymes of alveolar macrophages engaged in phagocytosis and killing of inhaled Staphylococcus Aureus. J. Infect. Dis. 136: 299, 1978.

174. Goldstein, E., Lippert, W., Warshauer, D.: Pulmonary alveolar macrophage: Defender against bacterial infection of the lung. J. Clin. Invest. 54: 519, 1974.

175. Goldstein, E., Munson, E.S., Eagle, C. et al.: The effects of anesthetic agents on murine pulmonary bactericidal activity. Anesthesiol. 34: 344, 1971.

176. Gopal, V., Bisno, A.L.: Pulminant pneumococcal infections in normal asplenic hosts. Arch. Intern. Med. 137: 1526, 1977.

177. Gordon, S.: Macrophage neutral proteinases and chronic inflammation. Annu. Rec. Biochem. 46: 823, 1976.

178. Gorin, A.B., Kohler, J., Denardo, G.: Non-invasive measurement of pulmonary transvascular protein flux in normal man. J. Clin. Invest. 66: 869, 1980.

179. Gotze, O., Muller-Eberhard, H.J.: The C_3 activation system: An alternate pathway of complement activation. J. Exp. Med. 134 (Suppl): 905, 1971.

180. Gray, B.M., Converse III, G.M., Dillon Jr., H.C.: Serotypes of streptococcus pneumoniae causing disease. J. Infect. Dis. 140: 979, 1979.

181. Graziano, C., Miller, C.L., Lim, R.C.: Role of monocyte function in alteration of the thrombofibrinolytic system after shock. Surg. Forum 31: 24, 1980.

182. Green, G.M., Kass, E.H.: Factors influencing the clearance of bacteria by the lung. J. Clin. Invest. 43: 769, 1964.

183. Green, G.M., Kass, E.H.: The influence of bacterial species on pulmonary resistance to infection in mice subjected to hypoxia, cold stress, and ethanol intoxication. Br. J. Exp. Pathol. 46: 360, 1965.

184. Green, L.H., Green, G.M.: Differential suppression of pulmonary antibacterial activity as the mechanism of selection of pathogen in mixed bacterial infection of the lung. Am. Rec. Resp. Dis. 98: 819, 1968.

185. Grieble, H.G., Colton, F.T., Bird, T.J. et al: Fine particle humidifiers· Source of Pseudomonas aeruginoas infections in a respiratory disease unit. New. Engl. J. Med. 282: 531, 1970.

186. Griffin Jr., F.M., Mullinas, P.J.: Augmentation of macrophage complement receptor function in vitro. III. C_3b receptors that promote phagocytosis migrate within the plane of the macrophage plasma membrane. J. Expr. Med. 154: 291, 1981.

187. Grinblat, J., Gilboa, Y.: Overwhelming pneumococcal sepsis 25 years after splenectomy. Am. J. Med. Sci. 270: 523, 1975.

188. Grogan, J.B.: Transient defect in the bactericidal capacity of rabbit alveolar macrophages following sublethal shock. J. Trauma 19: 52, 1979.

189. Gross, G.N., Rehm, S.R., Pierce, A.K.: The effect of complement depletion on lung clearance of bacteria. J. Clin. Invest 62: 373, 1978.

190. Guarneri, J.J., Laurenzi, G.A.: Effect of alcohol on the mobilization of alveolar macrophages. J.Lab. Clin. Med. 72: 40, 1968.

191. Gudewicz, P.W.: The effects of cortisone therapy on lung macrophage host defense function and glucose metabolism. Circ. Shock 8: 95, 1981.

192. Gudewicz, P.W., Saba, T.M., Coulson, F.: Phagocytic and bactericidal activities of pulmonary macrophages following sublethal traumatic shock. Proc. Soc. Exp. Biol. Med. 153: 262, 1976.

193. Haeckel, E.: Die Radiolarien, pp. 104-105, Georg Reimer, Berlin, 1862.

194. Haffajee, A.A., Angorn, I.B., Brain, P.P. et al: Diminished cellular immunity due to impaired nutrition in oesophageal carcinoma. Br. J. Surg. 65: 480, 1978.

195. Hand, W.L., Cantey, J.R.: Antibacterial mechanisms of the lower respiratory tract. I. Immunoglobulin synthesis and secretion. J. Clin. Invest. 53: 354, 1974.

196. Hammer, J.A., Rannels, D.E.: Effects of halothane on protein synthesis and degradation in rabbit pulmonary macrophages. Am. Rev. Resp. Dis. 124: 50, 1981.

197. Hammerschmidt, D.E., Weaver, L.J., Hudson, L.D. et al: Association of complement activation and elevated plasma C_5a with adult respiratory distress syndrome. Lancet May 3, 1980.

198. Hammerschmidt, D.E., White, J.G., Craddock, P.R. et al: Corticosteroids inhibit complement-induced granulocyte aggregation - a possible mechanism for their efficacy in shock states. J. Clin. Invest. 63: 798, 1979.

199. Harmsen, A., Birmingham, J., Engen, R.: Method of obtaining swine alveolar macrophage by segmental pulmonary lavage. J. Immunol. Methods 27: 199, 1979.

200. Harmsen, A.G., Jeska, E.L.: Surface receptors on porcine alveolar macrophages and their role in phagocytosis. J. Reticuloendothelial Soc. 27: 631, 1980.

201. Harris, G.D., Johanson Jr., W.G., Pierce, A.K. Determinants of lung bacterial clearance in mice after acute hypoxia. Am. Rev. Resp. Dis. 116: 671, 1976.

202. Harris, G.D., Wood, D.E., Rine, R. et al.: The effect of intra-alveolar fluid on lung bacterial clearance. Lung 158 91, 1980.

203. Harris, J.D., Swenson, E.W., Johnson III, J.E. Human alveolar macrophages: Comparison of phagocytic ability, glucose utilization and ultrastructure in smokers and non-smokers. J. Clin. Invest 49: 2086, 1970.

204. Harrow, E., Jakab, G., Brody, A. et al.· The pulmonary response to a bacteremic challenge. Am. Rev. Resp. Dis. 112· 7, 1975.

205. Hart, P.D.A., Armstrong, J.A., Brown, C.A. et al. Ultrastructural study of the behaviour of macrophages toward parasitic mycobacteria. Infect. & Immunity 5:803, 1972.

206. Hartwig, J.H., Yin, H.L., Stossel, T.P. Contractile proteins and the mechanisms of phagocytosis in macrophages from Mononuclear Phagocytes II, pp. 971-996, Ralph Van Furth, ed., Martinus Nijhoff Publ Boston, 1980.

207. Hellmann, A., Goldman, J.M.: Effects of cyclosporin-A on human granulopoiesis in vitro. Transplantation 30: 386, 1980.

208. Hill Jr., R.B., Rowlands Jr., D.T., Rifkind, D.: Infectious pulmonary disease in patients receiving immunosuppresive therapy for organ transplantation. N. Engl. J. Med. 271: 1021, 1964.

209. Hocking, W.G., Golde, D.W.: The pulmonary alveolar macrophage (1st of two parts). N. Engl. J. Med. 301: 580, 1979.

210. Hof. D.G., Repine, J.E., Peterson, P.E. et al.: Phagocytosis by human alveolar macrophages and neutrophils: Qualitative differences in the opsonic requirements for uptake of Staphylococcus Aureus and Streptococcus pneumoniae in vitro. Am. Rev. Resp. Dis. 121: 65, 1980.

211. Hoffmann, M.K.: Macrophages and T cells control distinct phases of B cell differentiation in the humoral immune response in vitro. J. Immunol. 125:2076, 1980.

212. Hoidal, J.R., White, J.G., Repine, J.E.: Impairment of human alveolar macrophage oxygen consumption and superoxide anion production by local anesthetics used in bronchoscopy. Chest 75: 243, 1979.

213 Holland, F , Holland, N [illegible] The [illegible] of macrophage phagocytic receptors by anti membrane [illegible] J Exp Med 135 458, 197[illegible]

214 Homan, W P , Fabre, W , Millard, P [illegible] Effect of cyclosporin A upon second set rejection of rat renal allografts Transplantation 30 354, 1980

215 Horovitz, J H , Carrico, [illegible] Shires, [illegible] Pulmonary response manor injury Arch Surg 108 346 1974

216 Hosea, S W , Brown, E , Hamburger, M I Opsonic requirements for intravascular clearance after splenectomy N Eng Med 304, 245, 1981

217 Howard, R.J Host defense against infection Part [illegible] Surg 17 267, 1980

218 Howard, R.J Host defense against infection Part [illegible] Surg 17 319, 1980

219 Hsueh, W , Gonzalez-Crussi, F , Hanneman, E Prostaglandin synthesis indifferent phases of phagocytosis in lung macrophages Nature 283 80, 1980

220 Huber, G L , LaForce, F M , Mason, R.M et al Impairment of pulmonary bacterial defense mechanisms by immunosuppressive agents Surg Forum 21 285, 1970

221 Huber, G L , Porter, S L , Burley, S W et al The effect of oxygen toxicity on the inactivation of bacteria by the lung Chest 61 (Suppl) 665, 1972

222. Huber, H , Fudenberg, H.H. Receptor sites of human monocytes for IgG. Int Arch Allergy Appl Immunol 34 18, 1968

223. Hughes, W.T Pneumocystis Carinii pneumonia from Infection and the Compromised Host, Clinical Correlations and Therapeutic Approaches, pp. 91-120, J.C Allen, ed Williams and Wilkins, Baltimore, London, 1981

224. Hughes, W.T , Feldman, S., Aur, R.J.A. et al. Intensity of immunosuppressive therapy and the incidence of pneumocystis Carinii pneumonitis. Cancer 36 2004, 1975.

225 Hughes, W.T., Price, R.A., Kin, H.K. et al. Pneumocystis Carinii pneumonitis in children with malignancies. J. Pediatr. 82 404, 1973.

226. Hughes, W.T., Smith, D.R.. Infection during induction of remission in acute lymphocytic leukemia. Cancer 31: 1008, 1973.

227. Hunninghake, G.W., Fauci, A.S.: Immunologic reactivity of the lung. III. Effects of corticosteroids on alveolar macrophage cytotoxic effector cell function. J. Immunol. 118: 146, 1977.

[illegible] Neutrophil-generated [illegible] macrophages [illegible] Am Rev Resp Dis [illegible] 1978

2[illegible] [illegible] Oropharyngeal [illegible] normal adults and patients [illegible] depressed [illegible] Am [illegible] Med 64 564, 1978

23[illegible] [illegible] pneumonia Lancet [illegible] 1980

231. [illegible] Complement induced [illegible] leukostasis [illegible] tissue injury Arch Pathol Lab Med 104 [illegible] 1980

232. Jacob, H [illegible] Granulocyte complement interaction [illegible] a beneficial antimicrobial mechanism that can cause disease Arch Int Med 138 461, 1978

233. Jacobs, N M , Lerdkachornsuk, S , Metzger, W Pneumococcal bacteremia in infants and children A ten year experience at the Cook County Hospital with special reference to pneumococcal serotypes isolated Pediatrics 64 296, 1979

234. Jandl, J H , Jones, A R , Castle, W B. The destruction of red cells by antibodies in man I Observations on the sequestration and lysis of red cells altered by immune mechanisms J Clin Invest 36 1428, 1957

235. Jasin, H E , Orozco, J H , Ziff, M Serum heat labil opsonins in systemic lupus erythematosis J Clin Invest 53 343, 1974

236. Jay, S.J , Johanson, W G , Pierce, A K. The radiographic resolution of Streptococcus pneumoniae pneumonia N. Engl J. Med. 293 789, 1975

237. Johanson Jr , W G , Pierce, A K., Sanford, J P. et al. Nosocomial respiratory infections with gram-negative bacilli Ann. Intern. Med. 77 701, 1972

238. Johnston Jr , R.B The host response to invasion by Streptococcus pneumoniae Protection and the pathogenesis of tissue damage. Rev. Infect. Dis. 3 282, 1981.

239. Johnston Jr , R.B., Chadwick, D.A., Pabst, M.J.: Release of superoxide anion by macrophages Effect of in vivo or in vitro priming from Mononuclear Phagocytes Part II, pp. 1143-1163, R. Van Furth, ed., Martinus Nijhoff Publ., Boston, 1980.

240. Johnston Jr., R.B., Klemperer, M.R., Albert, C.A. et al.: The enhancement of bacterial phagocytosis by serum: The role of complement components and two cofactors. J. Exp. Med. 129: 1275, 1969.

243 Johnston, R.B., Newman, S.L., Struth, A.G. An abnormality of the alternate pathway of complement activation in sickle cell disease. N. Engl. J. Med. 288: 803, 1973.

244 Jones, T.C., Hirsch, J.G. The interaction between Toxoplasma gondii and mammalian cells. II. The absence of lysosomal fusion with phagocytic vacuoles containing living parasites. J. Exp. Med. 136: 1173, 1972.

243 Joseph, M., Tonnel, A.B., Capron, A. et al. Enzyme release and superoxide anion production by human alveolar macrophages stimulated with immunoglobulin E. Clin. Exp. Immunol. 40: 416, 1980.

244 Kabat, E.A. Structural concepts in Immunology and Immunochemistry, pp. 244. Holt, Rinehart and Winston, New York, 1968.

245 Kaltreider, H.B. Expression of immune mechanisms in the lung. Am. Rev. Resp. Dis. 113: 347, 1976.

246 Kapanci, Y., Weibel, E.R., Kaplan, H.P. et al. Pathogenesis and reversibility of the pulmonary lesions of oxygen toxicity in monkeys. II. Ultrastructural and morphologic studies. Lab. Invest. 20: 101, 1969.

247 Kaplan, A.M., Morhanakumar, T. Expression of a new cell surface antigen on activated murine macrophages. J. Exp. Med. 146: 1461, 1977.

248 Kaplan, A.P., Kay, A.B., Austen, K.F. A prealbumin activator of prekallikrein. III. Appearance of chemotactic activity for human neutrophils by the conversion of human prekallikrein to kallikrein. J. Exp. Med. 135: 81, 1972.

249 Kaplan, E.L., Lasedal, T., Quie, P.G.: Studies of polymorphonuclear leukocytes from patients with chronic granulomatous disease of childhood - Bactericidal capacity for streptococci. Pediatrics 41:591, 1968.

250 Kaplan, J.E., Saba, T.M.. Humoral deficiency and reticuloendothelial depression after traumatic shock. Am. J. Physiol. 230: 7, 1976.

251. Kaplan, R., Sahn, S., Petty, T.: Incidence and outcome of the respiratory distress syndrome in gram negative sepsis. Arch. Intern. Med. 129: 867, 1979.

252. Karnovsky, M.L.: Metchnikoff in Messina, a century of studies on phagocytosis. New. Engl. J. Med. 304: 1178, 1981.

253. Karnovsky, M.L., Drath, D., Lazdins, J.: Biochemical aspects of the function of the reticuloendothelial system in The Reticuloendothelial System in Health and Disease: Functions and Characteristics, pp. 121-130, S.M. Reichard, M.R. Escobar, H. Friedman, eds., Plenum Press, New York, 1976.

254 Karnovsky, M.L., Lazdins, J., Simmons, S.R. Metabolism of activated mononuclear phagocytes at rest and during phagocytosis in Immunity, Infection and Pathology, pp. 423-438, R. Van Furth, ed., Blackwell, London, 1975

255 Karrer, H.E. The ultrastructure of mouse lung. The alveolar macrophage. J. Biophys. Biochem. Cytol. 4: 693, 1958

256 Kass, E.H. Hormones and host resistance to infection. Bacteriol. Rev. 24: 177, 1960

257 Katz, D.R. Macrophages in non-Hodgkin lymphomas and hairy cell leukaemia. J. Pathol. 134: 117, 1981

258 Kazmierowski, J.A., Gallin, J.I., Reynold, H.Y. Mechanisms for the inflammatory response in primate lungs. Demonstration and partial characterization of an alveolar macrophage-derived chemotactic factor with preferential activity forpolymorphonuclear leukocytes. J. Clin. Invest. 59: 273, 1977.

259. Kazura, J.W., Negendank, W., Guerry, D. et al: Human monocyte-lymphocyte interaction and its enhancement by levamisole. Clin. Exp. Immunol. 35: 258, 1979.

260 Keeling, P., Winchurch, R.A., Munster, A.M. Immunomodulation in major burns: an in vitro system for assaying the effect of modulators. J. Trauma 20: 830, 1980.

261. Keller, H.U., Wilkinson, P.C., Abercrombie, M. et al:A proposal for the definition of terms related to locomotion of leukocytes and other cells. Clin. & Exp. Immunol. 27: 377, 1977.

262 Keller, R., Keist, R., Ivatt, R.J.: Functional and biochemical parameters of activation related to macrophage cytostatic effects on tumor cells. Int. J. Cancer 14: 675, 1974.

263. Kemp, A.S., Cripps, A.W., Brown, S.: Suppression of leukocyte chemokinesis and chemotaxis by human IgA. Clin. Exp. Immunol. 40: 388, 1980.

264. Keusch, G.T., Douglas, S.D., Hammer, G. et al: Antibacterial functions of macrophages in experimental protein calorie malnutrition. II. Cellular and humoral factors for chemotaxis, phagocytosis and intracellular bactericidal activity. J. Infect. Dis. 138: 134, 1978.

265. Kielian, M.C., Cohn, Z.A.: Phorbol myristate acetate stimulates phagosome - Lysosome fusion in mouse macrophages. J. Exp. Med. 154: 101, 1981.

266. Kim, M., Goldstein, E., Lewis, J.P. et al: Murine pulmonary alveolar macrophages - Rates of bacterial ingestion, inactivation and destruction. J. Infect. Dis. 133: 310, 1976.

267. Klebanoff, S.J.: Oxygen intermediates and the microbicidal event from Mononuclear Phagocytes Part II, pp. 1105-1137, R. Van Furth, ed., Martinus Nijhoff Publ., Boston, 1980.

268 Klein, J. Biology of the mouse histocompatibility 2 complex. Principles of immunogenetics applied to a single system Springer Verlag, New York, 1975

269 Klein, J.O. The epidemiology of Pneumococcal disease in infants and children Rev Infect. Dis. 3 246, 1981

270 Kolobow, T., Solco, M., Gattinoni, L. et al. Adult respiratory distress syndrome (ARDS) Why did ECMO fail? Internat. J Artif Organs 4: 58, 1981

271. Kovacs, K.F., Chaudry, I.H., Bane, A.E.. Improved reticuloendothelial function and initial survival after sepsis with autotransplanted splenic tissue. Surg. Forum 31 65, 1980.

272. Krivit, W. Overwhelming postsplenectomy infection Am. J. Hematol. 2 193, 1977.

273. Kuhn, C., Finke, E.H.: The topography of the pulmonary alveolus. Scanning electron microscopy using different fixations. J. Ultrasctruct. Res. 38: 161, 1972.

274. Kung, J.T., Brooks, S.B., Jackway, J.P.et al: Suppression of in vitro cytotoxic response by macrophages due to induced arginase. J. Exp. Med. 146: 665, 1977.

275. Kurland, J.I., Bockman, R.S.: Prostaglandin E production by human monocytes and mouse macrophages. J. Exp. Med. 147: 952, 1978.

276. Kurland, J.I., Moore, M.A.S.: Regulatory role of the macrophage in normal and neoplastic hemopoiesis in Experimental Hematology Today, pp. 51-62, S. Baum,G. Ledney, eds., Springer Verlag, New York,1977.

277. LaForce, F.M.: Effect of alveolar lining material on phagocytic and bactericidal activity of lung macrophages against Staphylococcus aureus. J. Lab. Clin. Med. 88: 691, 1976.

278. LaForce, F.M., Mullane,, J.F., Boehme, R.F. et al: The effect of pulmonary oedema on antibacterial defenses of the lung. J. Lab. Clin. Med. 82: 634, 1973.

279. Lamm, M.E.: Cellular aspects of immunoglobulin A. Adv. Immunol. 22: 223, 1976.

280. Laster, C.E., Gleich, G.J.: Chemotaxis of oesinophils and neutrophils by aggregated immunoglobulins. J. Allergy Clin. Immunol. 48: 297, 1971.

281. Laurenzi, G.A., Guarneri, J.J.: A study of the mechanisms of pulmonary resistance to infection - the relationship of bacteria clearance to ciliary and alveolar macrophage function. Am. Rev. Resp. Dis. 93: 134, 1966.

282. Laurenzi, G.A., Guarneri, J.J., Endriga, R.B. Important determinants in pulmonary infections Med. Thorac 22 48, 1965.

283. Laurenzi, G.A., Potter, R.T , Kass, E.H. Bacteriologic flora of the lower respiratory tract. New. Engl. J. Med. 265: 1273, 1961.

284. Law D.K , Dudrick, S.J., Abdou, N.I. Immunocompetence of patients with protein calorie malnutrition. Ann. Intern. Med. 79: 545, 1973.

285. Law, D.K., Dudrick, S.J., Abdou, N.I.: The effects of protein-calorie malnutrition on immune competence of the surgical patient. Surg. Gyn. & Obs. 139: 257, 1974.

286. Leake, E.S., Wright, M.J., Myrvik, Q.N.: Differences in surface morphology of alveolar macrophages attached to glass and to millipose filters. A scanning electron microscope study. J. Reticuloendothelial Soc. 17: 370, 1975.

287. Lehrer, R.I., Ferrari, L.G., Patterson, J. et al: Fungicidal activity of rabbit alveolar and peritoneal macrophages against Candida albicans. Infect. & Immu. 28: 1001, 1980.

288. Leibovitch, S.J., Ross, R.: A macrophage dependent factor that stimulates the proliferation of fibroblasts in vitro. Am. J. Pathol. 84: 501, 1976.

289. Leijh, P.C.J., Vanden Barselaar, M. Th., Van Zwer, T.L. et al: Extracellular stimulation of intracellular killing from Mononuclear Phagocytes, Punctional Aspects Part II, pp. 1467-1489, R. Van Furth, ed., Martinus Nijhoff Publ., Boston, 1980.

290. Leijh, P.C.J., Vanden Barselaar, M. Th., Van Zwet, Th. L. et al: Requirement of extracellular complement and immunoglobulin for intracellular killing of microorganisms by human monocytes. J. Clin. Invest. 63: 772, 1979.

291. Leonard, A.S., Giebink, G.S., Baesl, T.J. et al: The overwhelming postsplenectomy sepsis problem. World J. Surg. 4: 423, 1980.

292. Leonard, A.S., Giebink, G.S., Baesle, T.J. et al: Sequestration of heat treated autologous red cells in the spleen. J. Lab. Bin.Med. 90: 666, 1977

293. Levine, H.: Needle biopsy diagnosis of tuberculous peritonitis. Am. Rev. Resp. Dis. 97: 889, 1968.

294. Likhite, V.V.: Immunological impairment and susceptibility to infection after splenectomy. JAMA 236: 1376, 1976.

295. Likhite, V.V.: Opsonin and leukophilic γ-globulin in chronically splenectomized rats with and without heterotopic autotransplanted splenic tissue. Nature 253: 742, 1975.

296. Likhite, V.V.: Protection against fulminant sepsis in splenectomized mice by implantation of autochthonous splenic tissue. Exp. Hematol. 6: 433, 1978.

297. Lindquist, O., Saldeen, T., Sandler, H.: Pulmonary damage following pulmonary microembolism in the dog. Acta. Chir. Scand, 142: 15, 1976.

298. Lindquist, O., Schwartz, S.: Pulmonary platelet trapping during shock and pulmonary embolism. J. Surg. Res. 18: 559, 1975.

299. Lindsay, P.A., Kurnick, N.B.: Preparation of tissues for liquid scintillation radioactivity counting. Internatl. J. Appl. Radiation & Isotopes 20: 97, 1969.

300. Lohmann-Matthes, M.L.: Induction of macrophage mediated cytotoxicity in Immunobiology of the Macrophage, pp. 463-486, D.S. Nelson, ed., Academic Press, New York, 1976.

301. Lowerie, D.B., Jackett, P.S., Aber, V.R. et al: Cyclic nucleotides and phagosome-lysosome fusion in mouse peritoneal macrophages from Mononuclear Phagocytes Part II, pp. 1057-1075, E. Van Furth, ed., Martinus Nijhoff Publ., Boston, 1980.

302. Lowrie, D.B., Jackett, P.S., Ratcliffe, N.A.: Mycobacterium microti may protect itself from intracellular destruction by releasing cyclic AMP into phagosomes. Nature 254: 600, 1975.

303. MacCuish, A.C., Urbaniak, S.J., Campell, C.J. et al: Phytohemagglutinin transformation and circulating lymphocyte subpopulations in insulin-dependent diabetic patients. Diabetes 23: 708, 1974.

304. Mackowiak, P.A., Martin, R.M., Jones, S.R. et al: Pharyngeal colonization of gram-negative bacilli in aspiration pron persons. Arch. Intern. Med. 138: 1224, 1978.

305. Mahmoud, A.A., Rodnaman, H.M., Mandel, M.A. et al: Induced and spontaneous diabetes mellitus and suppression of cell mediated immunologic responses. J. Clin. Invest. 57: 362, 1976.

306. Malangoni, M.A., West, K., Grosfeld, J.L.: Response to pneumococcal challenge after ligation of the splenic artery. Surg. Forum 31: 44, 1980.

307. Manawada, B.R., LaForce, F.M.: Impairment of pulmonary antibacterial defense mechanisms by halothane anaesthesia. Chest (Suppl.) 75: 242, 1979.

308. Mann, P.E.G., Cohen, A.B., Finley, T.N. et al: Alveolar macrophages: Structural and functional differences between non-smokers and smokers of marijuana and tobacco. Lab. Invest. 25: 111, 1971.

309. Martin, S., Kerby, G., Holland, B.: A method for measuring removal of bacteria from the blood by the various organs of the intact animan. Soc. Exp. Biol. Med. 72: 63, 1949.

310. Martinez-Tello, F.J., Braun, D.G., Blanc, W.A.: Immnoglobulin production in bronchial mucosa and bronchial lymph nodes, particularly in cystic fibrosis of the pancreas. J. Immunol. 101: 989, 1968.

311. Mason, R.J., Williams, MC., Greenleaf, R.D.: Isolation of lung cells from Lung Cells in Disease, A. Bouhuys, ed., North Holland Publ. Co. Amsterdam, 1976.

312. Massaro, D., Kelleher, K., Massaro, G.: Alveolar macrophages: Depression of protein synthesis during phagocytosis. Am. J. Physiol. 218: 1533, 1970.

313. McEwen, C.R., Stallard, R.W., Juhos, E.T.: Separation of biological particles by centrifugal elutriation. Anal. Biochem. 23: 369, 1968.

314. McIntosh, L., Thomson, A.W.: Activity of the mononuclear phagocyte system in cyclosporin-A treated mice. Transplant. 30: 384, 1980.

315. McKay, D.G.: Trauma and disseminated intravascular coagulation. J. Trauma, 9: 646, 1969.

316. McVay, L.V., Sprung, D.H., Stein, D.N.: Antibiotic prophylaxis in chronic congestive heart failure. Am. J. Med. Sci. 226, 491, 1954.

317. Meakins, J.L., McLean. A.P., Kelly, R. et al: Delayed hyersensitivity and neutrophil chemotaxis. Effect of trauma. J. Trauma 18: 240, 1978.

318. Meltzer, M.S., Jones, E.E., Boetcher, D.A.: Increased chemotactic responses of macrophags from B.C.G.-infected mice. Cell. Immunol. 17: 268, 1975.

319. Mendelson, C.L.: The aspiration of stomach contents into the lungs during obstetric anaesthesia. Am. J. Obs. & Gyn. 52: 171, 1946.

320. Merkow, L., Pardo, M., Epstein, S.M. et al: Lysosomal stability during phagocytosis of Aspergillus flavus spores by alveolar macrophages of cortisone-treated mice. Science 160: 79, 1968.

321. Metchnikoff, E.: Immunity in infective diseases, translated by F.G. Binnie, Cambridge University Press, 1905.

322. Michl, J., Unkeless, J.C., Silverstein, S.C.: Modulation of macrophage plasma membrane receptors for IgG and complement from Mononuclear Phagocytes, Functional Aspects Part II, pp. 921-940. R. Van Furth, ed., Martinus Nijhoff Publ. Boston, 1980.

323. Miles, A.A.: Local defences against infection and their relation to thegeneral reticuloendothelial defenses in Physiopathology of Research, B.M. Halptern, ed., Springfield III, Thomas, 1957.

324. Miller, R.G., Phillips, R.A.: Separation of cells by velocity sedimentation. J. Cell. Comp. Physiol. 73: 191, 1969.

325. Modell, J.H., Moya, F.: Postoperative complications, incidence and management. Anesth. Analg. 45: 432, 1966.

326. Modry, D.L., Chiu, R.C.J.: Pulmonary reperfusion syndrome. Ann. Thorac. Surg. 27: 206, 1979.

327. Modry, D.L., Chiu, R.C.J.: The roles of ventilation and perfusion in lung metabolism surgical implications. J. Thorac. & Cardiovasc. Surg. 74: 275, 1977.

328. Moen, J.K., Reimann, H.A.: Immune reactions in diabetes. Arch. Intern. Med. 789, 1933.

329. Mohagheghpour, N., Parhami, B., Dowlatshabi, K. at al: Immunoregulatory properties of human esophageal tumor extract. J. Immunol. 122: 1350, 1979.

330. Moore, M.A., Merson, M.H., Chrach, P. et al: The characteristics of out-patient acquired pneumonia. Johns Hopkins Med. J. 140: 9, 1977.

331. Morgan, E.L., Weigle, W.O.: Regulation of the immune response:III. The role of macrophages in the potentiation of the immune response by Fc fragments. J. Immunol. 126: 1302, 1981.

332. Morland, B., Kaplan, G.: Macrophage activation in vivo and in vitro. Exp. Cell. Res. 108: 279, 1977.

333. Morrow, P.E.: Some physical and physiological factors controlling the fate of inhaled substances. I. Deposition. Health Phys. 2: 366, 1960.

334. Motohashi, S.J.: The effect of splenectomy on the production of antibodies. Med. Res. 43: 473, 1972.

335. Mowat, A.G., Baum, J.: Chemotaxis of polymorphonuclear leukocytes from patients with diabetes mellitus. Diabetes 20: 424, 1971.

336. Munster, A.M.: Alteration of the host defense mechanism in burns. Surg. Clin. N. Amerc. 50: 1217, 1970.

337. Murphey, S.A., Hyams, J.S., Fisher, A.B. et al: Effects of oxygen exposure on in vitro function of pulmonary alveolar macrophages. J. Clin. Invest. 56: 503, 1975.

338. Murphey, S.A., Root, R.K., Schreiber, A.D.: The role of antibody and complement in phagocytosis by rabbit alveolar macrophages. J. Infect. Dis. 140: 896, 1979.

339. Murray, H.W., Cohn, Z.A.: Activated mononuclear phagocyte functions., INFLO 12: 1, 1979

340. Mustafa, M.G., Tierney, D.F.: Biochemical and metabolic changs in the lung with oxygen, ozone and nitrogen toxicity. Am. Rev. Resp. Dis. 118: 1061, 1978.

341. Myrvik, Q.N., Leake, E.S., Fariss, B.: Studies on pulmonary alveolar macrophages from the normal rabbit:A technique to procure them in a high state of purity. J. Immunol. 86: 128, 1961.

342. Najjar, V.A.: Biochemistry and physiology of tuftsin Thr-Lys-Pro-Arg. In The Reticuloendothelial System, a Comprehensive Treatise Vol. II Biochemistry and Metabolism, pp. 45-71, A.J. Sharra, R.R. Strauss, editors, Plenum Press, New York, 1980.

343. Najjar, V.A.: Defective phagocytosis due to deficiencies involving the tetrapeptide tuftsin. _. Pediatrics 87: 1121, 1975.

344. Najjar, V.A., Constantopoulos, A.: A new phagocytosis stimulating tetrapeptide hormone, tuftsin and its role in disease. J. Reticuloendothelial Soc. 12: 197, 1972.

345. Najjar, V.A., Fidalgo, B.V., Stitt, E.: The physiological role of the lymphoid system. VII, The disappearnce of leukokinin activity following splenectomy. Biochem, 7: 2376, 1968.

346. Najjar, V.A., Nishioka, K., Constantopoulos, A. et al: The function and interaction of erythrophilic -globulin with autologous erythrocytes. Sechste Internationales Symposium iiber Structur and Punktion der Erythrozyten, pp. 615-627, S.M. Rapaport, F. Jung, editors, Akademie Ver Lag, East Berlin, 1972.

347. Naimark, A.: Cellular dynamic and lipid metabolism in the lung. Fed. Proc. 32: 1967, 1973.

348. Nash, G., Blennerhassett, J.B., Pontoppidan, H.: Pulmonary lesions associated with oxygen therapy and artificial ventilation. N. Engl. J. Med. 276: 368, 1967.

349. Naum, Y.: Growth of pulmonary alveolar macrophages in vitro: Responses to media conditioned by lung cell lines. Cytobios. 14: 211, 1975.

350. Neff, T.A.: Percutaneous trephine biopsy of the lung. Chest 61: 18, 1972.

351. Nelson, D.S: Macrophages-progress and problems. Clin. & Exp. Immunol. 45: 225, 1981.

352. Nelson, R.D., Quie, P.G., Simmons, R.L.: Chemotaxis under agarose: A new simple method for measuring chemotaxis and spontaneous migration of human polymorphonuclear leukocytes and monocytes. J. Immunol. 115: 1650, 1975.

353. Nerurkar, L.S., Zeligs, B.J., Bellanti, J.A.: Maturation of the rabbit alveolar macrophage during animal development. II. Biochemical and anzymatic studies. Pediatric Res. 11: 1202, 1977.

354. Newhouse, M., Sanchis, J., Bienenstock, J.: Lung defense mechanisms (first of two parts). N. Engl J. Med. 295, 990, 1976.

355. Newhouse, M., Sanchis, J., Bienenstock, J.: Lung defense mechanisms (second of two parts). N. Engl. J. Med. 295: 1045, 1976.

356. Niehaus, G.D., Schumacker, P.T., Saba, T.M.: Influence of opsonic fibronectin deficiency on lung fluid balance during bacterial sepsis. J. Appl. Physiol. 49: 693, 1980.

357. Norton, J.M., Munck, A.: Glucose transport in murine macrophages In vitro characterization of the monosaccharide transport system of the thioglycollate-elicited mouse peritoneal macrophage. J. Immunol. 125: 252, 1980.

358. Norton, J.M., Munck, A.: In vitro actions of glucocorticoids on murine macrophages: Effects on glucose transport and metabolism growth in culture and protein synthesis. J. Immunol. 125: 259, 1980.

359. Ogmundsdottir, H.M., Weir, D.M.: Mechanisms of macrophage activation. Clin. & Exp. Immunol. 40. 22, 1980.

360. Olsen, G.N., Harris, J.O., Castle, J.R. et al: Alpha-1-antitrypsin content in the serum, alveolar macrophages and alveolar lavage fluid of smoking and normal subjects. J. Clin. Invest. 55: 427, 1975.

361. Oren, R.A., Farnham, A.E., Saito, K.: Metabolic patterns in three types of phagocytizing cells. J. Cell. Biol. 17: 748, 1963.

362. Oren, R., Farnham, A.E., Saito, K. et al: Metabolic patterns in three types of phagocytizing cells. J. Cell. Biol. 17: 487, 1963.

363. Palmer, D.L.: Alcoholism infection and immunity from Infection and the Compromised Host Clinical Correlations and Therapeutic Approaches, J.C. Allen, editor, Williams and Wilins, Baltimore, London, 1981.

364. Pantalone, R., Page, R.C.: Enzyme production and secretion by lymphokine-activated macrophages. J. Reticuloendothelial Soc. 21: 343, 1977.

365. Paterson, N.A.M., Wasserman, S.I., Said, J.W. et al: Release of chemical mediators from partially purified human lung mast cells. J. Immunol. 117: 1356, 1976.

366. Pearce, M.L., Yamashita, J., Beazell, J.: Measurement of pulmonary edema. Circ. Res. 16: 482, 1965.

367. Pennington, J.E.: Bronchoalveolar cell response to bacterial challenge in the immunosuppressed lung. Am. Rev. Resp. Dis. 116: 885, 1977.

368. Pennington, J.E.: Differential effects of cyclophosphamide and cortisone acetate on bronchoalveolar phagocytic cell population. Am. Rev. Resp. Dis. 118: 319, 1978.

369. Pennington, J.E.: Quantitative effects of immunosuppression of bronchoalveolar cells. J. Infect. Dis. 136: 127, 1977.

370. Pennington, J.E., Mathews, W.J., Marino, J.T. et al: Cyclophosphamide and cortisone acetate inhibit complement biosynthesis by guinea pig bronchoalveolar macrophages. J. Immunol. 123: 1318, 1979.

371. Penrod, K.E.: Nature of pulmonary damage produced by higher oxygen pressures. J. Appl. Physiol. 9: 1, 1956.

372. Perillie, P.E., Nolan, J.P., Finch, S.C. Studies of the resistance to infection in diabetes mellitus - local exudative cellular response. J. Lab. Clin. Med. 59: 1008, 1962.

373. Philpott, D.E., Harrison, G.A., Turnbill, C. et al Ultrastructural changes in tracheal epithelial cells exposed to oxygen. Aviat. Space Environ. Med. 48: 812, 1977.

374. Pierce, A.K., Reynolds, R.C., Harris, G.D. Leykocytic response to inhaled bacteria. Am. Rev. Resp. Dis. 116: 679, 1977.

375. Pierce, A.K., Sanford, J.P.: Aerobic gram-negative bacillary pneumonias. Am. Rev. Resp. Dis. 110: 647, 1974.

376. Pierce, C.W., Kapp, J.A.: The role of macrophage in antibody responses in vitro in Immunobiology of the Macrophage, pp. 2-33, D.S. Nelson, ed., Academic Press, New York, 1976.

377. Poduslo, S.E., Norton, W.T.. Isolation of specific brain cells. Methods Enzymology 35: 561, 1975.

378. Polk, H.C., Fry, D., Flint, L.: Dissemination and causes of infection. Surg. Clin. North Am. 56: 817, 1976.

379. Polverini, P.J., Cotran, R.S., Gimbrone, M.A. et al: Activated macrophages induce vascular proliferation. Nature 269: 804, 1977.

380. Poulter, L.W., Turk, J.L.: Studies on the effect of soluble lymphocyte products (lymphokines) on macrophage physiology. II. Cytochemical changes associated with activation. Cell. Immunol. 20: 25, 1975.

381. Pratt, S.A., Finley, T.N., Smith, M.H. et al: A comparison of alveolar macrophages and pulmonary surfactant obtained from the lungs of human smokers and non-smokers by endobronchial lavage. Anat. Rec. 163: 497, 1969.

382. Quie, P.G.: Bactericidal function of human polymorphonuclear leukocytes. Pediatrics 50: 264, 1972.

383. Quie, P.G., Davis, A.T.: Phagocyte and granulocytic disorders in Immunologic Disorders in Infants and Children, pp. 273-288, E.R. Stiehm, V.A. Fulginiti, eds., W.B. Saunders Co., Philadelphia, 1973.

384. Quie, P.G., White, J.G., Holmes, B. et al: In vitro bactericidal capacity of human polymorphonuclear leykocytes: Diminished activity in chronic granulomatous disease of childhood. J. Clin. Invest. 46: 668, 1967.

385. Rabinovitch, M., Destefano, M.J.: Particle recognition by cultivated macrophages. J. Immunol. 110: 695, 1973.

386 Rachmilewitz, M., Schlesigner, M. Production and release of transcobalamin II A vitamin B-12 transport protein by mouse peritoneal macrophages Exp Hemat 5 (Suppl 2) 108, 1977

387 Raffin, T.A., Simon, L M., Douglas, W.H.J et al The effect of variable O_2 tension and exogenous superoxide dismutase on type II pneumocytes exposed to paraquat Lab. Invest 42 205, 1980

388 Rapaport, R.T , Bachvaroff, R., Nemirovsky, M et al Pulmonary micro-embolization in severe thermal injury. Proc Soc. Exp. Biol. Med 139· 1013, 1972.

389 Ravry, M , Maldonaldo, N., Velez-Garcia, E. et al Serious infection after splenectomy in Hodgkins disease. Ann. Int. Med. 77 11, 1972.

390. Reimann, H.A. Microbic pneumonias secondary to other diseases, shock, senility and mechanical causes, pp. 167, The Pneumonias, H.A. Reimann, editor Warren H. Green. Publishing Inc., St. Louis, Missouri, 1972

391 Reinarz, J.A., Pierce, A.K , Mays, B.B. et al· The potential role of inhalation therapy equipment in nosocomial pulmonary infection. J Clin Invest. 44· 831, 1965

392 Reynolds, H.Y Lung host defenses a status report. Chest 75 239, 1979.

393. Reynolds, H.Y., Kazmierowski, J.A , Dale, D.C.:Changes in the composition of canine respiratory cells obtained by bronchial lavage following irradiation or drug immunosuppression. Proc. Soc. Exp. Biol. Med. 151: 756, 1976.

394 Reynolds, H.Y., Kazmierowski, J.A., Newball, H.: Specificity of opsonic antibodies to enhance phagocytosis of Pneudomonas aeruginosa by human alveolar macrophages J. Clin. Invest. 58: 376, 1975.

395. Reynolds, H.Y., Newball, H.H.: Analysis of proteins and respiratory cells obtained from human lungs by bronchial lavage. J. Lab. Clin. Invest. 84:,559, 1974.

396. Reynolds, H.Y., Thompson, R.E.: Pulmonary host defences. II. Interaction of respiratory antibodies with Pseudomonas aeruginosa and alveolar macrophages. J. Immunol. 111: 369, 1973.

397. Rhoades, E.R., Ringrose, R., Mohr, J.A. et al: Contamination of ultrasonic nebulization equipment in nosocomial pulmonary infection. J. Clin. Invest. 44: 831, 1965.

398. Richardson, J.D., Fry, D.E., Arsdall, L.V. et al: Delayed pulmonary clearance of gram-negative bacteria - The role of intraperitoneal sepsis. J. Surg. Res. 26: 499, 1979.

399. Richardson, J.D., Woods, D., Johanson Jr., W.G. et al: Lung bacterial clearance following pulmonary contusion. Surg. 86: 730, 1979.

400 Riley, I.D., Douglas, R.M. An epidemiologic approach to pneumococcal disease. Rev. Infect Dis.3· 233, 1981

401. Rumland, D., Hand, W.L. The effect of ethanol on adherence and phagocytosis by rabbit alveolar macrophages J Lab. Clin. Med. 95. 918, 1980.

402. Ringrose, R.E., McKown, B., Felton, F G. et al A hospital outbreak of serratia Marscescens associated with ultrasonic nebulizers. Ann. Intern, Med. 69. 719, 1968.

403 Robinette, C.D., Frauman, J.F. Splenectomy and subsequent mortality in veterans of 1939-45 war. Lancer 2 127, 1977.

404. Rodriguez, R.J., White, R.R., Senior, R.M. et al Elastase release from human alveolar macrophages - Comparison between smokers and non-smokers Science 198: 313, 1977.

405. Rogers, D.. Host mechanisms which act to remove bacteria from the blood stream. Bacteriol. Rev. 24: 50, 1960.

406. Rogers, D., Melly, M.: Studies on bacteremia - III. The blood stream clearance of Escherichia coli in rabbits. J. Exp. Med. 105: 113, 1957.

407. Rosen, P.P., Martini, N.,Armstrong, D." Pneumocystis carinii pneumonia - Diagnosis by lung biopsy. Am. J. Med.58. 794, 1975.

408. Rosenow III, E.C.: The spectrum of drug-induced pulmonary disease. Ann. Intern. Med. 77 977, 1972.

409. Rosenthal, A.S., Shevach, E.M.: Function of macrophages in antigen recognition by guinea pig T-lymphocytes. I. Requirements for histocompatible macrophages and lymphocytes. J. Exp. Med. 138: 1194, 1973.

410. Rosenwasser, L.J., Werdelin, O., Braendstrup, O. et al: Physical and functional macrophage lymphocyte interactions in antigen recognition by T-lymphocytes from Mononuclear Phagocytes Functional Aspects Part II, pp. 1887-1904, R. Van Furth, ed., Martinus Nijhoff Publ., Boston, 1980.

411. Ross, B.B., Gramiak, R., Rahn, H.: Physical dynamics of the cough mechanism. J. Appl. Physiol. 8: 264-: 1955.

412. Rossi, F., Bellavite, P., Dobrina, A. et al: Oxidative metabolism of mononuclear phagocytes from Mononuclear Phagocytes Part II, pp. 1187-1217, R. Van Furth, ed., Martinus Nijhoff Publ., Boston, 1980.

413. Rossi, F., Patriarca, R., Romeo, D. et al: The mechanism of control of phagocytic metabolism in The Reticuloendothelial System in Health and Disease: Function and Characteristics, pp. 205-223, S.M. Reichard, M.R. Escobar, H. Friedman, eds., Plenum Press, New York, 1976.

414. Roubin, R., Kennard, J., Foley, D. et al: Markers of macrophage heterogeneity - Altered frequency of macrophage subpopulations after various pathologic stimuli. J. Reticuloendothelial Soc. 29: 423, 1981.

415. Rousseau, G.G., Baxter, J.D., Higgins, S.J. et al: Steroid-induced nuclear binding of glucocorticoid receptors in intact hepatoma cells. J. Mol. Biol. 79: 539, 1973.

416. Rowley, D.A.: Formation of circulating antibody in splenectomized human being following intravenous injection of heterologous erythrocytes . J. Immunol. 65: 515, 1960.

417. Rudzik, R., Clancy, R.L., Perey, D.Y.E. et al:Repopulation with IgA-containing cells of bronchial and interstitial lamina propria after transferof homologous Peyer's patch and bronchial lymphocytes. J. Immunol. 114: 1599, 1975.

418. Rylander, R., Mattsby, I., Snella, M.C.: Airway cellular and immune response after exposure to inhaled endotoxins. Chest 75 (Suppl.) 278: 1979.

419. Rylander, R., Snella, M.C., Garcia I.: Pulmonary cell response patterns after exposure to airborne bacteria. Scand. J. Resp. Dis. 56: 195, 1975.

420. Saba, T.: Physiology and physiopathology of the reticuloendothelial system. Arch. Int. Med. 126: 1051, 1970.

421. Saba, T.M.: Prevention of liver reticuloendothelial systemic host defense failure after surgery by intravenous opsonic glycoprotein therapy. Ann. Surg. 188: 142, 1978.

422. Saba, T.M.: Reticuloendothelial system host defense after surgery and traumatic shock (symposium review). Circ. Shock 2: 91, 1976.

423. Saba, T.M.: The effect of surgical trauma on the clearance and localization of blood-borne particulate matter. Surg. 71: 675, 1972.

424. Saba, T.M., Scovill, W.A., Powers J.R., S.R.: Human host defense mechanisms as they relate to surgery and trauma. Surg. Annual 12: 1, 1980.

425. Sackner, M.A., Landa, J., Hirsch, J. et al: Pulmonary effects of oxygen breathing - a 6 hour study in normal men. Ann. Int. Med. 82: 40, 1975.

426. Sacks, T., Moldow, C.F., Craddock, P.R. et al: Endothelial damage produced by toxic oxygen radicals released from complement-triggered granulocytes. Prog. Clin. Biol. Res. 21: 719, 1978.

427. Sacks, T., Moldow, C.F., Craddock, P.R. et al: Oxygen radicles mediate endothelial cell damage by complement-stimulated granulocytes. An in vitro model of immune vascular damage. J. Clin. Invest. 61: 1161, 1978.

428. Sandberg, A.L., Snyderman, R., Frank, M.M. et al: Production of chemotactic activity by guinea pig immunoglobulins following activation of the C_3 complement shunt pathway. J. Immunol. 108: 1227, 1972.

429. Sandritter, W., Mittermayer, C., Riede, V.N. et al: Shock lung syndrome (a general review). Pathol. Res. Pract. 162: 7, 1978.

430. Sanford, J.P.: Klebsiella and other gram-negative bacterial pneumonias in Text Book of Medicine, Edition 14, pp. 286-288, P.B. Beeson, W. McDermott, eds., W.B. Saunders Co., Philadelphia, 1975.

431. Satoh, P.S., Constantopoulos, A., Nishioka, K. et al: Tuftsin, threonyl-lysyl-prolyl-arginine, the phagocytosis stimulating messenger of the carrier cytophilic γ-globulin leucokinin in Chemistry and Biology of Peptides, pp. 403-408, J. Meinhoffer, ed., Ann Arbor Science Publisher, Ann Arbor, 1972.

432. Schiffman, G.: Immune response to pneumococcal polysaccharide antigens: A comparison of the murine model and the response in humans. Rev. Infect. Dis. 3: 224, 1981.

433. Schroter, R.C., Sudlow, M.F.: Flow patterns in models of the human bronchial airways. Resp. Physiol. 7: 341, 1969.

434. Schulkind, M.L., Ellis, E.F., Smith, R.T.: Effect of antibody upon clearance of I^{125} labeled pneumococci by the spleen and liver. Pediatr. Res. 1: 178, 1967.

435. Schumer, W.: Steroids in the treatment of clinical septic shock. Ann. Surg. 184: 333, 1976.

436. Schwartz, A.D., Goldthorn, J.F., Winkelstein, J.A.: Lack of protective effect of autotransplantation in Sprague-Dawley rats. Blood 51: 475, 1978.

437. Schwartz, R.H., Bimco, A.R., Handwerger, B.S. et al: Demonstration that monocytes rather than lymphocytes are insulin binding cells in preparations of human peripheral blood mononuclear leukocytes - Implications for study of insulin-resistance states in man. Proc. Nat. Acad. Sciences (USA) 72: 474, 1975.

438. Schwartz, R.H., Yano, A., Paul, W.E.: Interaction between antigen presenting cells and primed T-lymphocytes - An assessment of Ir gene expression in the antigen presenting cell. Immunol. Rev. 40: 153, 1978.

439. Scrimshaw, N.S.: Synergism of malnutrition and infection: Evidence from field studies in Guatemala. JAMA 212: 1685, 1970.

440. Scrimshaw, N.S., Taylor C.E., Gordon, J.E.: Interactions of nutrition and infection. W.H.O. Monogr. Series 57: 1, 1968,

441. Sedaghat, B.R., Masse, J.C., Lafuma, N.J. et al: Biologic cellulaire, evaluation de la population total des macrophages alveolaires chez le rat. C.R. Acad. Science, Ser. D. 273: 229, 1971.

442. Selinger, S.L., Bland, R.D., Demling, R.H. et al: Distribution volumes of (^{131}I) albumin (^{14}C) sucrose, and ^{36}Cl in sheep lung. J. Appl. Physiol. 39: 773, 1975.

443. Sell, S.: Immunology, Immunopathology and Immunity, 2nd Ed., Harper and Row, Hagerstown, 1975.

444. Shellito, J., Murphy, S.: The effect of experimental acid aspiration on alveolar macrophage function in rabbits. Am. Rev. Resp. Dis. 122: 551, 1980.

445. Schwartz, L.W., Christman, C.A.: Lung lining material as a chemotactant for alveolar macrophages. Chest 75: 284, 1979.

446. Sieger, L: Effects of chronic methotrexate therapy on numbers and function of rabbit alveolar macrophages. Proc. Soc. Exp. Biol. & Med. 156: 406, 1977.

447. Silverstein, S.C.: The militant macrophage, even the best of cellular defenses can be bested by a clever opponent. The Sciences 21: 18, 1981.

448. Simon, L.M., Liu, J., Theodore, J. et al: Effect of hyperoxia hypoxia and maturation on superoxide dismutase activity in isolated alveolar macrophages. Am. Rev. Resp. Dis. 115: 279, 1977.

449. Simon, L.M., Robin, E.D., Phillips, J.R. et al: Enzymatic basis for bioenergetic differences of alveolar versus peritoneal macrophages and enzyme regulation by molecular O2. J. Clin. Invest. 59: 443, 1977.

450. Simon, P.L., Willoughby, W.F.: The role of subcellular factors in pulmonary immune function - Physicochemical characterization of two distinct species of lymphocyte-activating factor produced by rabbit alveolar macrophages. J. Immunol. 126" 1534, 1981.

451. Singer, D.B.: Postsplenectomy sepsis. Perspect. Pediatr. Path. 1: 285, 1973.

452. Singer, M.M., Wright, E, Stanley, L.K. et al: Oxygen toxicity in man - A prospective study in patients after open-heart surgery. N.Engl. J. Med. 283: 1473, 1970.

453. Skamene, E., Chayasirisobhon, W.: Enhanced resistance of splenectomized mice infection with Listeria monocytogenes. Immunol. 33: 851, 1977.

454. Skamene, E., Chayasirisobhon, W., Konshavn, P.: Inceased phagocytic activity of splenectomized mice challenges with Listeria monocytogenes. Immunol. 34: 901, 1978.

455. Skornik, W., Dressler, D.P.' Alveolar macrophage function in immunized burned rats. Arch. Surg. 108: 715, 1974.

456. Sladen, A.: Methyl prednisolone pharmacological dose in shock lung syndrome. J. Thorac. & Cardiovasc. Surg. 71: 800, 1976.

457. Slapak, M., Lee, H.M., Hume, D.M.: Transplant lung and lung complications in renal transplantation. Adv. Transplant. 49: 769.

458. Smith, K.A., Crabtree, G.R., Kennedy, S.J. et al: Glucocorticoid receptors and glucocorticoid sensitivity of mitogen stimulated and unstimulated human lymphocytes. Nature 267: 523, 1977.

459. Smith, L.J., Brody, J.S.: Influence of methyl prednisolone on mouse alveolar type 2 cell response to acute lung injury. Am. Rev. Resp. Dis. 123: 459, 1981.

460. Smith, M.R., Shin, H.S. Wood Jr., W.B.: Natural immunity to bacterial infections - The relation of complement to heat labile opsonins. Proc. Nat. Acad. Sciences (USA) 63: 1151, 1969.

461. Smith, T.J., Wagner, R.R.: Rabbit macrophage interferons. I. Conditions for biosyntesis by virus infected and uninfected cells. J. Exp. Med. 125: 559, 1967.

462. Soderland, S.C., Naum, Y.: Growth of pulmonary alveolar macrophages in vitro. Nature 245: 150, 1973.

463. Sorkin, E., Stecher, V.J., Borel, J.F.: Chemotaxis of leucocytes and inflammation. Series Haematol. 3: 131, 1970.

464. Spector, W.G.: Pulmonary fibrosis due to chemicals and particles Ann. N.Y. Acad. Science 221: 309, 1974.

465. Spirer, Z., Zakuth, V., Diamant, S. et al: Decreased tuftsin concentrations in patients who have undergone splenectomy. Br. Med. J. 2: 1574, 1977.

466. Spray, S.B., Zuidema, G.D., Cameron, J.D.: Aspiration pneumonia: Incidence of apiration with endotracheal tubes. Am. J. Surg. 131: 701, 1976.

467. Staub, N.C.: Pulmonary edema. Physiol. Rev. 54: 678, 1974.

468. Staub, N.: Pulmonary edema due to increased microvascular permeability to fluid and protein. Circ. Res. 43: 143, 1978.

469. Stecher, V.J.: Synthesis of proteins by mononuclear phagocytes in Mononuclear Phagocytes, pp. 133-147, R. Van Furth, ed., Blackwell, Oxford, 1970.

470. Stecher, V.J., Morse, J.H., Thorbecke, G.J.: Sites of production of primate serum proteins associated with the complement system. Proc. Soc. Exp. Biol. Med. 124: 433, 1967.

471. Stein-Streilein, J., Frazier, J.L., Gross, G.N. et al: Splenic influence on the development of a local pulmonary immune response. Infect. & Immunity 24: 139, 1979.

472. Stevens, R.M., Teres, D., Skillman, J.J. et al: Pneumonia in intensive care unit: A 30 month experience. Arch. Intern. Med. 134: 196, 1974.

473. Stossel, T.P.: On the mechanism of action of heat-labile (HLO) and heat-stable (HSO) opsonins. Blood 40: 969, 1972.

474. Stossel, T.P.: Phagocytosis (first of three parts) N. Engl. J. Med. 290; 717, 1974.

475. Stossel, T.P.: Quantitative studies of phagocytosis - Kinetic effects of cations and of heat-labile opsonin. J. Cell. Biol. 58: 346, 1973.

476. Stossel, T.P., Alper, C.A., Rosen, F.S.: Serum dependent phagocytosis of paraffin oil emulsified with bacterial lipopolysaccharide. J. Exp. Med. 137: 690, 1973.

477. Straatsma, B.R., Zimmerman, L.E., Gass, J.D.: Phycomicosis A clinicopathologic study of fifty-one cases. Lab. Invest. 11:693, 1962.

478. Sueishi, K., Tanaka, K., Oda, T.: Immuno-ultrastructural study of surfactant system - Distribution of specific protein of surgace active material in rabbit lung. Lab. Invest. 37: 136, 1977.

479. Suskind, R. (ed): Malnutrition and The Immune Response, pp. 468, Kroc Foundation Series Vol. 7, Raven Press, New York, 1977.

480. Tavassoli, M., Ratzan, R.J., Crosby, W.H.: Studies on regeneration of heterotopic splenic autotransplants. Blood 41: 701, 1973.

481. Tesluk, G.C., Thomas Jr., C.G.: Prevention of postsplenectomy pneumococcal sepsis in rats Surg. Forum 30: 35, 1979.

482. Thomaidis, T.S., Fidalgo, B.V., Harshman, S. et al: The physiological role of lymphoid system. IV. The separation of γ- globulin into physiologically active components by cellulose phosphage chromatography. Biochem. 6: 3369, 1967.

483. Thomas, E.D., Ramberg, R.E., Sale G.E.: Direct evidence for a bone marrow origin of the alveolar macrophages in man. Science 192:1016, 1976.

484. Tillotson, J.R., Lerner, A.M.: Characteristics of non-bacteremic Pseudomonas pneumonia. Ann. Int. Med. 68: 295, 1968.

485. Tourville, D.R., Adler, RH., Bienenstock, J. et al: The human secretory immunoglobulin system - Immunohistological localization of γA, secretory "piece" and lactoferrin in norman human tissues. J. Exp. Med. 129: 411, 1969.

486. Unanue, E.R.: Secretory function of mononuclear phagocytes. Am. J. Pathol. 83: 396, 1976.

487. Van Furth, R.: The origin and turnover of promonocytes, monocytes and macrophages in normal mice in Mononuclear Phagocytes, pp. 151-165, R. Van Furth, ed., Blackwell, Oxford, 1970.

488. Van Waarde, D., Hulsing-Hesselink, E., Van Furth, R.: Properties of a factor increasing monocytopoiesis (FIM) occurring in the serum during the early phase of an inflammatory reaction. Blood 5: 727, 1977.

489. Verhoef, J., Peterson, P.K., Quie, P.G.: Kinetics of Staphylococcus opsonization, attachment, ingestion and killing by human polymorphonuclear leukocytes - A quantitative assay using ^{3}H thymidine labelled bacteria. J. Immunol. Methods 14: 303, 1977.

490. Vito, L., Dennis, R.C., Wisel, R.D. et al: Sepsis presenting as acute respiratory insufficiency. Aneg. Gyn. & Obs. 138: 896, 1974.

491. Voisin, C., Aerts, C., Jakubczak, E. et al: La culture cellulaire en phase gazeuse. Un nouveau modèle experimentale d'étude in vitro des activités des macrophages alveolaires. Bull. Eur. Physiopathol. Resp. 13: 69, 1977.

492. Voisin, C., Aerts, C., Tonnel, A.B. et al: Etude in vitro de l'activité bactericide (Staphylococcus aureus) et fongicide (Candida Albicans) des macrophages alveolaires de cobaye en presence de dipropionate de beclamethasone. La Nouv. Presse Med. 15: 1326, 1977.

493. Völkman, A.: Disparity in origin of mononuclear phagocyte populations. J. Reticuloendothelial Soc. 19: 249, 1976.

494. Volkman, A., Gowmans, J.L.: The production of macrophages in the rat. Br. J. Exp. Pathol. 46: 50, 1965.

495. Vreim, C.E., Staub, N.C.: Protein composition of lung fluids in acute alloxan edema in dogs. Am. J. Physiol. 230: 376, 1976.

496. Wagle, S.R., Lugebretsen, W.R.: Isolation, purification and metabolic characteristics. Methods in Enzymol, 35: 579, 1975.

497. Walker, W.: Splenectomy in childhood: Reveiw in England and Wales. 1960-64. Br. J. Surg. 63: 36, 1976.

498. Walker, W.S.: Functional heterogeneity of macrophages in Immunobiology of the Macrophage, pp. 91-110, D.S. Nelson, ed., Academic Press, New York, 1976.

499. Walter, J.B., Israel, M.S.: General Pathology 4th Edition, pp. 88 Churchill Livingstone, London, 1974.

500. Walzer, P.D., Perl, D.P., Krogstad, D.J. et al: Pneumocystis carinii pneumonia in the United States. Ann. Int. Med. 80: 83, 1974.

501. Wara, D.W.: Hose defense against Streptococcus pneumoniae - The role of the spleen. Rev. Infect. Dis. 3: 299, 1981.

502. Ward, P.A.: Chemotaxis of mononuclear cells: J. Exp, Med. 128: 1201, 1968.

503. Ward, P.A., Chapitis, J., Conroy, M.C. et al: Generation by bacterial proteinases of leukotactic factors from human serum and human C_3 and C_5. J. Immunol. 110: 1003, 1973.

504. Warr, G.A., Martin, R.R., Sharp, P.M. et al: Normal human bronchial immunoglobulin and proteins. Effects of cigarette smoking. Am. Rev. Resp. Dis. 116: 25, 1977.

505. Weisdorf, D.J., Hammerschmidt, D.F., Jacob, H.S. et al: The role of endogenous proteases circulating granulocytes and the spleen in clearance of activated C_5 components - Possible relevance to the shock lung syndrome, abstracted. Clin. Res. 27: 649a, 1979.

506. Weiss, S.M., Rosato, F.E., Stewart, E. et al: Splenic autotran$plant function in experimental bacteremia. Surg. Forum 30: 30, 1979.

507. Weksler, B.B., Coupal, C.E.: Platelet-dependent generation of chemotactic activity in serum. J. Exp. Med. 137: 1419, 1973.

508. Wenzel, R.P., Osterman, C.A., Hunting, K.J.: Hospital acquired infections. II. Infection rates by site, service, and common procedures in a university hospital. Am. J. Epidemiol. 104: 645, 1976.

509. Werb, Z.: Biochemical actions in glucocorticoids on macrophages in culture. Specific inhibition of elastase, collagenase and plasminogen activator secretion and effects on other metabolic functions. J. Exp. Med. 148: 1695, 1978.

510. Werb, Z.: Hormone receptors and hormonal regulation of macrophage physiological functions in Mononuclear Phagocytes - Functional Aspect, Part II, pp. 809-829, R. Van Furth, ed., Martinus Nijhoff, Publ., Boston, 1980.

511. Werb, Z., Foley, R., Munck, A.: Glucocorticoid receptors and glucocorticoid-sensitive secretion of neutral proteinases in a macrophage line. J. Immunol. 121: 115, 1978.

512. Werb, Z., Foley, R., Munck, A.: Interaction of glucocorticoids with macrophages. Identification of glucocorticoid receptors in monocytes and macrophages. J. Exp. Med. 147: 1684, 1978.

513. Westion, W.L., Carson, B.S., Barkin, R.M. et al: Monocyte-macrophage function in the newborn. Am. J. Dis. Child. 131: 1241, 1977.

514. Whitaker, A.N.: The effect of previous splenectomy on the course of pneumococcal bacteremia in mice. J. Pathol. Bacteriol. 95: 357, 1968.

515. White, R., Janoff, A., Godfrey, H.P.: Secretion of alpha-2-macro-globulin by human alveolar macrophages. Lung. 158: 9, 1980.

516. White, R., Lee, D., Habicht, G.S. et al: Secretion of alpha-1-proteinase inhibitor by cultured rat alveolar macrophages. Am. Rev. Resp. Dis. 123: 447, 1981.

517. White, R., Lin. H.S., Kuhn, C.: Elastase secretion by peritoneal exudative and alveolar macrophages. J. Exp. Med. 146: 802, 1977.

518. Whiteside, D.C., Thomas, C.G.: Effect of splenectomy and autologous splenic implantation on a Streptococcus pneumoniae challenge. Surg. Forum 30: 32, 1979.

519. Whittaker, M.G., Clark, C.G.: Depressed lymphocyte function in carcinoma of breast. Br. J. Surg. 58: 717, 1971.

520. Wichterman, K.A., Baue, A.E., Chaudry, I.H.: Sepsis and septic shock. A review of laboratory models and a proposal J. Surg. Res. 29: 189, 1980.

521. Wilkinson, P.C., Allan. R.B.: Assay systems for measuring leukocyte locomotion: an overview in Leukocyte Chemotaxis, pp. 1-24, J.I. Gallin, P.G. Quie, eds., Raven Press, New York, 1978.

522. Wilkinson, P.C., Allan, R.B.: The behaviour of human blood monocytes in chemotactic and chemokinetic enviroments and the role of substratum in monocyte locomotion on Mononuclear Phagocytes, Part I, pp. 475-500, R. Van Furth, ed., Martinus Nijhoff Publ. Boston, 1980.

523. Wilson, I.D.:Studies in the opsonic activity of human secretory IgA using an in vitro phagocytosis system. J. Immunol. 108: 726, 1972.

524. Winkelstein, J.A.: The role of complement in the hosts defense against Streptococcus pneumoniae. Rev. Infect. Dis. 3: 289, 1981.

525. Winlove, C.P., Davis, J., Rubin, B.T. et al: The use of radioiodinated human serum albumin in measurements of arterial permeability. Cardiovasc. Res. 12: 609, 1978.

526. Wistreich, G.A., Lechtman, M.D.: Selected bacterial diseases of the respiratory tract, pp. 643, Microbiology and Human Disease, G.A. Wistreich, editor, Glenco Press, Beverly Hills, California, 1976.

527. Wynne, J.W., Modell, J.H.: Respiratory aspiration of stomach contents. Ann. Int. Med. 87: 446, 1977.

528. Yeager, H., Lussier, L., Prashad, J.: Alveolar macrophage lymphocyte interaction. Chest 75: 289, 1979.

529. Young. L.S., Armstrong, D: Human immunity to Pseudomonas aeruginosa - (1) In vitro interaction of bacteria, polymorphonuclear leukocytes, and serum factors. J. Infect. Dis. 257, 1972.

530. Zakhireh, B., Malech, H.L.: The effect of colchicine and vinblastine on the chemotactic response of human monocytes. J. Immunol. 125: 2143, 1980.

531. Zavala, D.C., Bedell, G.N.: Percutaneous lung biopsy with a cutting needle, Am. Rev. Resp. Dis. 106, 186, 1972.

532. Zeligs, B.J., Nerurkar, L.C., Bellanti, J.A.: Maturation of the rabbit alveolar macrophage during animal development. I. Perinatal influx into alveoli and ultrastructural differentiation. Pediatric Res. 11: 197, 1977.

533. Zigmond, S.H.: Gradients of chemotactic factors in various assay systems in Mononuclear Phagocytes, Part II, pp. 461-473, R. Van Furth, ed., Martinus Nijhoff Publ., Boston, 1980.

534. Zipunsky, A., Brown, E.J., Bienestock, J.: Lack of opsonization potential of 11-S human secretory A. Proc. Soc. Exp. Biol. Med. 142: 181, 1973.