# PANSPECIFIC IMMUNOSUPPRESSION AND NEOPLASTIC ENHANCEMENT

# PANSPECIFIC IMMUNOSUPPRESSION AND NEOPLASTIC ENHANCEMENT

A Thesis

by

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## ABSTRACT

This study was divided into three separate sections.

PART I examined the production of a state of hypogammaglobulinemia within a murine model. By sequential treatment of newborn mice with heterologous anti-mouse immunoglobulin M (IgM), panspecific immunosuppression ensued. This was documented by assaying serum immunoglobulin levels, and by comparing these values to macroscopic and microscopic parameters.

PART II examined the growth characteristics of two solid neoplasms. By careful selection of the relevant conditions, a change in tumor growth was achieved by immunological manipulation. With the tumor, P-815-X2, this satisfied the definition of neoplastic enhancement. With the tumor T1699 enhancement of tumor growth was vaguely suggested but not documented.

PART III represented an attempt to synthesize these seemingly disparate areas. By removing the immunological factors involved (via panspecific immunosuppression), and by employing an enhancible neoplasm, abrogation or prevention of this phenomenon was attempted. Results were not specific. They suggested that this approach warrants further investigation, but failed to document any definitive relationship.

## RESUME

Cette étude comprend trois parties.

La première consiste en l'induction d'un état d'hypogammaglobulinémie chez la souris. Une immunosuppression panspécifique a été obtenue par le traitement répété de souriceaux nouveaux-nés par du sérum hétérologue anti-IgM de souris. Cet état a été étudié par la détermination des taux d'Immunoglobulines sériques et par leur comparaison avec divers paramètres macroscopiques et microscopiques.

La deuxième partie concerne la croissance de deux tumeurs solides. En faisant varier soigneusement les conditions expérimentales, une modification de la croissance tumorale a été obtenue par manipulation immunologique. Dans le cas de la tumeur P-815-X2, cette modification satisfait à la définition de la Facilitation d'une tumeur maligne. Dans celui de la tumeur T1699, une facilitation de la croissance tumorale est plus ou moins suggérée mais pas établie.

La troisième partie représente une tentative de synthèse entre ces domaines apparemment disparates. En utilisant la tumeur "facilitable", nous avons essayé de prévenir ou d'inhiber la facilitation en éliminant certains des facteurs immunologiques impliqués (au moyen de l'immunosuppression panspécifique). Les résultats obtenus ne sont pas spécifiques. Ils suggèrent que cette approche nécessite de plus amples investigations mais ne permettent d'établir aucune conclusion définitive.

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## ABBREVIATIONS USED THROUGHOUT THIS THESIS

| | |
|---|---|
| ALS | - anti-lymphocyte serum |
| APS | - anti-plasma cell serum |
| B cell | - thymus independent bursa derived (bone marrow derived) lymphocyte |
| GVH | - graft versus host reaction |
| HBSS | - Hank's balanced salt solution |
| $^{125}I$ | - iodine 125 |
| IP | - intraperitoneal |
| NZB | - New Zealand Black |
| NZW | - New Zealand White |
| SC | - subcutaneous |
| SRBC | - sheep red blood cells |
| T cell | - thymus dependent lymphocyte |
| $T_{1/2}$ | - half-life |
| TSA | - tumor specific antigen |

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# INTRODUCTION

With the realization that the immune response could be considered as having two rather separate components, came the first attempts at selective immunosuppression (53). These in turn helped to unravel the complex interactions, and produced a complementary unified concept of the control mechanisms involved both in the phylogeny and ontogeny of humoral antibody function (96). Current experimentation, is directed towards the production of a panspecific immunosuppression by modulation of the regulatory controls (118).

About the same time, the role of antibodies in the enhancement of tumor growth was postulated (88). Subsequently it has been shown, depending on the conditions, that antibodies can exert either inhibitory or protective functions (83). To manipulate these parameters and thus prevent tumor enhancement has not yet been possible. Recent studies have indicated that an absence of antibodies may have a beneficial effect on tumor growth (82, 118). However, their relation to the enhancement problem is by no means clear.

The object of this study has of necessity been divided into three sections. In PART I the production of agammaglobulinemia is described for a murine model. In PART II enhancement of neoplasms indigenous to the same strain is detailed. In PART III these areas become synthesized in order to evaluate the relevance of panspecific immunosuppression to the abrogation of neoplastic enhancement.

# PART I: IMMUNOSUPPRESSION

# CHAPTER I - BACKGROUND

## A. IMMUNE SYSTEM - NORMAL

### 1. Duality of the System

It is generally accepted that animals possess a pool of multipotential stem cells (200), which may be either thymus processed or "Bursa" processed, to become immunocompetent T and B lymphocytes respectively (35). This imparts a duality of response to the immune mechanism.

#### a. "Bursa" Dependent System

In 1956 Glick et al identified the Bursa of Fabricius as serving a central role in the developing immune system (56). Subsequent studies using extirpation and irradiation of this organ have more precisely defined its function.

By employing a chromosome marker technique, Moore and Owen (141) found that blood-borne progenitor cells, later identified as stem cells (189), enter the Bursa and within this specific microenvironment, independent of foreign antigens, develop into multiple clones of B lymphocytes (37, 189).

There is a rapidly growing body of evidence, from antigen-coated column (196), autoradiographic (8), immunofluorescent (98, 161, 162), and radioiodination studies (16, 174, 192) to establish the presence of immunoglobulins on the surface of Bursa-processed lymphocytes in the chicken, the mouse and man. Apparently, during the stage of clonal development

(37) within the Bursa, or mammalian Bursa-equivalent, the lymphocytes acquire the ability to synthesize immunoglobulins (161, 174) which subsequently become expressed on the cell surface, and can thus serve as cell markers.

In chickens, the ontogenetic sequence is relatively well-defined. IgM is the first immunoglobulin to be detected, and it appears at day 14 of gestation (36, 97). This is followed sequentially by IgY (which is probably the equivalent of mammalian IgG) (36, 97, 109), and IgA (99, 109, 119). By bursectomizing chickens at different stages of development, and by comparing immunoglobulins and histology, Cooper et al (36) were able to suggest that a developmental switch occurred within the Bursa. This has been amply corroborated in studies employing class specific anti-immunoglobulins, and the intraclonal switch has been defined as the sequence IgM → IgG → IgA (96, 97, 99, 109, 110).

There is random cell migration from the Bursa (99) to the peripheral lymphoid tissues at all stages of differentiation, but once B cells have left the microenvironment of the Bursa the swtiching becomes a rare occurrence (96, 189).

However, even though the Bursa of Fabricius plays a major role in clonal development of B cells, it is not essential for this to occur (106). This may be analogous to the situation in mammals, where a Bursa has not yet been identified, and where the ontogenetic sequence is not as well-defined. Here a Bursa-equivalent microenvironment is postulated, and again from

studies employing class specific immunoglobulin anti-sera, it appears that the developmental sequence IgM→IgG→IgA is followed (74, 103, 104, 116, 117, 153, 154).

Once seeded to peripheral lymphoid structures, the B cells respond to antigenic challenge by undergoing clonal proliferation, to become either memory or antibody producing cells (37). The latter appear to secrete immunoglobulins of the same heavy chain class as is present in the cell surface receptor (196, 197). They apparently also lose their receptors during this process (14). This response is probably initiated by antigenic steric "stretching" (48) or "stabilizing" (13) of the receptor immunoglobulin molecules located on the cell surface. Perhaps it could also explain why the fate of immunoglobulin producing cells is not determined for more than forty-eight hours post-stimulation (114). Therefore, those cells destined to become humoral antibody producers follow a complex, ordered and sequential pattern of development. In order to achieve immunosuppression, methods for modulation of this pathway are required.

b. "Thymus" Dependent System

A comprehensive review of the data outling thymic involvement in the immune response has been done by Miller and Mitchell(128). Subsequently, Owen and Raff (148) postulated a two-stage model for the maturation of T lymphocytes: initially stem cells migrate to the thymus, and within its microenvironment, differentiate into thymocytes with a θ-alloantigen marker; they then mature into T lymphocytes which are able to leave the thymus. In

peripheral tissues they mediate cellular immunity in response to antigenic stimulation, via a cytotoxic action (30, 31).

2. Interrelationship of Systems

The compartmentalization of immunocompetent lymphocytes into T and B classes was interpreted as an efficient product of evolution in the development of sophisticated immune systems. Recently, however, it has been established that, although these distinct lymphoid lines perform different roles, they are not mutually independent. Instead they interact in the development of certain immune responses, notably in the humoral immune response to various antigens.

A considerable body of evidence has accumulated demonstrating the existence of specific receptors for antigen on lymphocytes. For B lymphocytes these are unequivocally established as immunoglobulin in nature (151), and are present in high concentrations on the cell surface (163, 191).

Evidence for receptors on T cells has been more difficult to obtain. Perhaps this is because the quantity of T cell receptors is significantly lower than the sensitivity of the direct techniques employed (146, 191). Indirect methods could be interpreted to indicate that they consist, at least, of subunits of classically defined immunoglobulin structures (61, 166, 167). These observations, however, are not without controversy, based on the quality and specificity of the anti-immunoglobulin reagents employed by different investigators (120).

Thus B lymphocytes possess antigen-specific receptors which are

immunoglobulin in nature. T lymphocytes appear to possess antigen-specific receptors, but whether or not these are immunoglobulins is not definitely established.

The interaction of T and B classes in response to antigenic stimuli was first suggested by Miller (126). Over the past decade a wealth of data on this topic has accumulated, and is succinctly reviewed by Katz and Benacerraf (91). To summarize: The mechanism whereby T cells regulate B cell function could be by a transfer of genetic information (92, 132), by "antigen focusing" (95, 132), or by mediators produced and secreted by T cells (93); this order probably reflects the increasing relevance of the succeeding mechanisms (91). This regulatory role is then expressed as either "helper" or "suppressor" functions.

"Helper" functions were initially suggested by Claman et al (32), and the interpretation corroborated by independent workers (60). The problem was further clarified in a series of elegant experiments by Miller and Mitchell, which demonstrated the importance of interaction in the production of humoral antibody (127, 129, 145). Subsequently it has been shown that, where T cells participate in an antibody response, they enhance B cell antibody responses of all classes of immunoglobulin, but this effect is most pronounced in the IgG classes, and in the switch from IgM→IgG (130).

A negative, or "suppressor" function, of T cells on antibody formation has also been demonstrated. Evidence for this has come from two lines

of investigation. The first involved the depletion of T lymphocytes, either by thymectomy (77) or by treatment with ALS (6,15) and resulted in elevated serum immunoglobulin levels, and enhanced primary immune responses. Kerbel and Eidinger combined the techniques and found that IgM, but not the switch to the IgG phase, was suppressed (94). This raises the possibility that suppressive regulatory T cell function may involve promotion of early IgG synthesis with a feedback suppression of IgM (91, 94).

The second involved investigations of antigenic competition. This phenomenon was recognized by Michaelis (125), and has been reviewed by Adler (2). The underlying mechanism is still unknown, but favors the existence of soluble inhibitory factors released from T cells (139, 159). The best evidence for a T cell released inhibitory substance comes from the recent studies of Gershon and Kondo (54, 55). However, whether this factor is related to, or identical with, other T cell mediators active in the regulation of antibody production is not yet known.

The concept of duality within the immune system is still accepted and basically correct. However, there is, in addition, a cooperative interaction between antigen, B cells and T cells which can be considered as a regulatory function of T cells on the conditions and manner in which B cells respond to antigen bound to their immunoglobulin receptors. This interrelationship ensures several levels of control, and perhaps even an evolutionary safety mechanism. These controls are crucial to an under-

standing of immunotherapy. Hopefully, selective immunosuppression will help in unravelling the problem.

## B. IMMUNE SYSTEM: SUPPRESSED

Numerous methods have been employed to achieve immunosuppression. They vary qualitatively in their degree of selectivity, and quantitatively in the success achieved. In order to maintain perspective, a brief classification, and description, follows.

### 1. Types of Immunosuppression

#### a. Non-selective

These methods are non-specific in their action on immune systems because they either randomly deplete the animal of all types of immunocompetent cells, or produce generalized hematopoietic tissue destruction. Examples are irradiation, metabolic inhibitors, and alkylating agents. A detailed review of this area has been done by Mannick (115).

#### b. Selective

Here the specificity is directed against only one of the limbs of the immune system. This allows them to be discussed separately.

"Thymus" Dependent System

Levey and Medawar (111) found that a heterologous antiserum, raised against T cells and called anti-lymphocyte sera (ALS), could suppress cellular immunity. However, this effect is not totally selective because it can also suppress the primary humoral response to SRBC (140), probably via its action on the antigen-sensitive T "helper" cells (12, 22, 65).

"Bursa" Dependent System

Within this limb of the immune system, there is a gradation of antibody specificity, which appears to be related to the difficulty required in achieving and maintaining antibody suppression.

Nisonoff et al (66, 152) studied the effect of heterologous anti-idiotype antibodies in inbred mice. Suppression of the idiotype could be complete and permanent, provided the anti-idiotype serum was given far enough in advance of the antigenic challenge. The escape found with close temporal proximity was interpreted as being due to the generation of new precursor cells, as opposed to the reactivation of cells that were already suppressed. In fact, if the antiserum was given post-antigenic challenge, no suppressive effect could be detected. This indicates that if a cell is idiotypically suppressed, the situation is probably irreversible.

When immunoglobulin allotype suppression was examined in $F_1$ hybrid mice, by following an allotype product, it was found that induction of suppression was delayed, but after on initial burst of activity of the allotype, the suppression remained chronic (75, 80). A compensatory increase in the alternate allotype occurred, when suppression was achieved in rabbits (40, 113). T cells may play a role in this phenomenon because *in vitro* studies have shown that lymphocytes undergo blast transformation when treated with anti-allotype serum (173), and *in vivo* results indicate that a suppressor cell, whose effect can be destroyed with anti-$\theta$ serum, is required for maintainence of suppression (75, 81). Instead of raising antiserum

against specific immunoglobulin idiotypic or allotypic markers, one can be formed against the components represented only in the B cell lineage (187). This is done by using a plasmacytoma (B cell neoplasm) as the immunizing agent, followed by absorption with T cells. The product, designated as anti-plasma cell serum (APS), is specifically cytotoxic for B cells. However, even at cytotoxic concentrations, it did not completely inhibit antibody formation, and plaque forming cells (66, 187). The mechanism has not yet been determined. Cell-mediated immunity, as assessed by skin grafting, appeared to remain intact (66).

Thus, as the specificity of the segment of the immunoglobulin population broadens, there occurs a corresponding increase in the difficulty with which specific immunosuppression is achieved. This becomes most apparent when immunoglobulin class specific antisera are employed.

2. Immunosuppression with Class Specific Antisera

Even when antisera are prepared against immunoglobulin molecules, the results obtained depend upon the portion of the molecule used as the antigenic stimulus. As light chains are common to all immunoglobulin classes, antisera against them would be expected to have diffuse suppressive effects.

a. Immunosuppression with Anti-light Chain Sera

In vitro studies, using antiserum against murine Kappa chains (these are the predominant light chain class in mouse immunoglobulins), found that the primary response to SRBC could be inhibited and that the inhibition

would be permanent if complement was added to the test system (107). In vivo studies, again using antiserum to mouse Kappa chains (this time raised against human Lambda chains, which share antigenic determinants with mouse Kappa chains), were able to demonstrate repression of synthesis of murine Kappa chains, but this was not a permanent effect (170).

Greaves et al have postulated that the anti-light chain sera binds to sites on the lymphocyte surface, and blocks combination of the receptor sites with antigen either by steric hindrance, or by causing a configurational change in the receptor site itself (61).

Whatever the mechanism, this appears to be an extension of the broadening of function mentioned previously. Repression, rather than suppression, was obtained and even this was not long lasting (170).

b. Immunosuppression with Anti-heavy Chain Sera

Most of these studies have used a murine model. As the immunoglobulins of mice have been classifiable, and subclassifiable (44, 45), with regard to the immunological and physico-chemical properties expressed in the heavy chains, antisera specific for heavy chain classes would be expected to vary in their effects. Indeed, this has been found, and a pattern appears to be emerging.

In Vitro

Fuji and Jerne first examined the effect of heterologous anti-immunoglobulin antiserum on the primary immune response to SRBC. They found that inhibition could be induced, and that it could be reversed simply by the addition of excess murine immunoglobulin. Unfortunately, their antiserum was

not class specific, so that class differences could not be detected (53). This work was extended by Pierce et al who found a gradation of effectiveness within the heavy chain classes: anti-IgM suppressed all classes where as anti-IgG or anti-IgA, suppressed their respective classes only (67, 153, 154). They also found IgM suppression to be dose dependent, temporally dependent (in that the suppressive effect on other classes diminished directly with the duration of incubation before the anti-IgM was added), and reversible (not due to cytotoxic action). To insert a note of caution, Hartman et al demonstrated a stimulating effect on immunoglobulin production if the incubation persisted longer than three days before the addition of the antiserum (67).

In Vivo

In the mouse, immunosuppression has been evaluated by continuing treatment with class specific antisera, starting at birth. In both conventional, and germ-free animals, it was found that anti-IgM suppressed all other heavy chain classes, while anti-$IgG_1$, anti-$IgG_2$ and anti-IgA suppressed their respective classes only (103, 104, 116, 117, 143). In one case, anti-$IgG_1$ caused a corresponding increase in the other classes (104). Again a dose dependent relationship was noted in that animals receiving large doses of anti-IgM either died or were runted (143). A temporal relationship was evident for if the injections of anti-IgM were delayed, the suppressive effect was reduced, or not attained (117). This suppression is probably a reversible phenomenon because some of the immunoglobulin classes recover

(117) (this did not occur with IgM), or are not completely suppressed (143).

However, as a subtle reminder of the complex controls involved, it was found that anti-IgM given during the course of a primary immune response enhanced, rather than suppressed, the IgG fraction (1), and that adult animals treated with anti-IgM produced excessive serum levels of a monomeric form of IgM (116).

The results of this therapy on cell-mediated immunity are not clear; assessment by the graft versus host reaction (GVH) has yielded conflicting results (8, 104).

Lawton et al feel the findings are not inconsistent with the two-stage model that they have proposed for plasma cell development (37, 104). During the stage of clonal development, stem cells within a specific inductive microenvironment proliferate and begin to synthesize IgM. Some, or all, of this antibody is incorporated into the cell membrane to function as a recognition antibody. Anti-IgM antiserum given at this stage should react with the IgM surface receptors (74, 195), and prevent clonal maturation via the switching phenomenon, whereby the other immunoglobulins also become surface receptors. The sequence suggested is IgM → IgG → IgA. Thus, treatment with a specific antiserum during this process could prevent switching occurring with subsequent deletion of clones with specific immunoglobulin receptors. The above sequence is hypothesized to occur within the inductive microenvironment for B lymphocytes, and is independent of antigenic stimulation.

The second stage, that of clonal proliferation, begins on contact with specific antigens. Antiserum treatment of this stage may be unable to completely block antigen access to the surface immunoglobulins, and thus a breakthrough effect would be seen (117). At present, most of this proposed model is purely speculative, although it does form a working framework.

The mechanism by which anti-immunoglobulin prevents an immunoglobulin producing cell from either producing antibody, or switching to produce another heavy chain class is also unknown. That the antiserum combines with specific B cell surface receptors is generally accepted (116, 195). Then perhaps via steric hindrance (166) or modulation of the immunoglobulin receptor (188), (either of which may be Fc dependent) (105, 188), the process of cap formation and pinocytosis ensues (42, 188). Whether this is the mechanism by which control of immunoglobulin production is regulated is, at present, not known.

## C. SELECTION OF METHOD OF IMMUNOSUPPRESSION

Since the object of Part I is the induction of a state of agammaglobulinemia, or hypogammaglobulinemia, in a murine system, one of the above methods of immunosuppression will have to be utilized. When all the parameters are considered, suppression using anti-IgM should prove to be the most efficacious. It combines ease of induction, effectiveness in the suppression of other heavy chain classes, and minimal side effects, with a potential reversibility that may be of clinical significance. Hopefully,

this will be selective enough to either leave cell-mediated immunity intact, or to create a compensatory increase in this limb of the immune response (104).

## CHAPTER II - EXPERIMENTAL DESIGN

### A. PREPARATION OF MATERIALS

#### 1. IgM

The plasmacytoma MOPC 104E (156) was a gift of Dr. M. Potter National Institute of Health, Bethesda, Maryland. This tumor arose in Balb/c mice in 1962, and secretes M heavy chains, and Lambda light chains. This was transplanted to Balb/c mice. When the tumors reached one centimeter diameter, the mice were sacrificed and the serum collected by allowing the blood to clot at 25°C for two hours, followed by clot retraction overnight at 4°C. The sera were subsequently pooled, and the IgM obtained by successive treatment on a Pevikon-Geon block electrophoresis, and a Sephadex G-200 (Pharmacia Fine Chemicals, Inc., Rochester, Minn.) column (155). The concentration of IgM was determined by recording the optical density at 280 nm; the purity was checked by Ouchterlony gel diffusion with commercial (Meloy Laboratories, Springfield, Va.) class or subclass specific antisera, and by Millipore electrophoresis (Millipore Corporation, Bedford, Mass.).

#### 2. Anti-IgM

A monospecific antiserum to mouse IgM was prepared in rabbits (New Zealand Black, NZB; New Zealand White, NZW; High Oak Ranch, Toronto, Ont.) by a method described in detail elsewhere (121, 143). In brief, the

IgM was immunoprecipitated by immunoelectrophoresis (LKB 6800 A, Immunoelectrophoresis Equipment, Stockholm, Sweden) with class specific antisera (gift of Dr. R. Murgita, Buffalo, New York). The precipitin bands were then cut out, and washed daily with normal saline changes to remove non-precipitated protein. This was subsequently homogenized with either complete or incomplete Freund's adjuvant (Difco Laboratories, Detroit, Mich.). The rabbits were immunized by injecting ten precipitin bands with complete Freund's adjuvant on day 0, followed by ten precipitin bands in incomplete Freund's adjuvant on day 14. If no response was detected on day 28, another booster was given. The responders were bled at fortnightly intervals and this blood pooled to form lot #1. At four months, all the responders were bled by cardiac puncture and this pool became lot #2.

A globulin fraction of the pooled sera was obtained by precipitation with 50% $(NH_4)_2SO_4$. This was resuspended in, and exhaustively dialized against, 0.85% NaCl followed with clarification by ultracentrifugation (30,000 g, 30 minutes, 4°C). The protein concentration was adjusted to 130 mg/ml as determined by optical density at 280 nm. Sterilization was by millipore filtration, and then each lot was stored at -20°C. Purity was checked by Ouchterlony gel diffusion with commerical class specific mouse sera (Meloy), and by immunoelectrophoresis against pure murine IgM, and normal mouse serum.

3. Radioiodinated Anti-IgM

To obtain only the 7S immunoglobulin fraction of the anti-IgM

preparation, a portion of lot #1 was eluted via a gradient on a DEAE-Sephadex anion exchange column (Pharmacia Fine Chemicals, Rochester, Minn) (182). The concentration was adjusted to 2 mg/ml using optical density at 280 nm. Purity was checked by Ouchterlony gel diffusion with commerical (Meloy Laboratories, Springfield, Va.) class or subclass specific antisera. Radioiodination of this preparation, was achieved by using 2 mg of the anti-IgM, plus 5 microcuries of $^{125}I$ (Charles E. Frosst & Co., Montreal, Quebec) and following the chloramine-T radioiodination procedure as described by Greenwood (59). This gave a calculated labelling of 0.7 - 0.8 iodine atoms per molecule of anti-IgM immunoglobulin (158).

4. Animals

a. Mice

Both the Balb/c, and the DBA/2, mice were purchased from the Jackson Laboratories (Bar Harbor, Maine). They were maintained in plastic cages and allowed free access to food (Purina Foods, Montreal, Quebec), and water. To obtain DBA/2 newborns, DBA/2 males of breeding age (10 to 30 weeks) were placed in separate cages containing white pine chips (Economic Sawdust Engineering Registered, Montreal, Quebec). After one week DBA/2 females (10 to 30 weeks), were placed, one per male, overnight in the cages. A pregnancy rate of about ten per cent was achieved.

The pregnant DBA/2 females were grouped in cages so that births within cages would occur over a time span of 24 hours. By allowing communal nursing, the attrition rate of newborns was kept to a minimum. The

newborns were weaned on day 21, and then the sexes separated.

b. Rabbits

NZB and NZW rabbits (High Oak Ranch, Toronto, Ontario) were housed in separate cages. Again free access to food and water was permitted.

B. ASSAY SYSTEMS

1. Non-immunological

Before sacrifice, each mouse was to be weighed. At sacrifice (ether plus cervical dislocation) wet splenic weight and total splenic cell count, were to be determined. A portion of the spleen was to be examined histologically. Mean values ± standard deviation for each group were calculated from individual recordings. The Student's T-test was used to compare group differences.

2. Immunological

a. Serum Immunoglobulins

At intervals each animal was bled via the tail, and the blood collected in heparinized capillary tubes. After centrifugation (2500 rpm, 5 minutes, 4°C) the serum immunoglobulin concentrations were determined by the serial dilution Ouchterlony gel diffusion technique described by Arnason et al. (5). These values were reported as the reciprocal of the highest two-fold dilution producing a distinct band against a commerical (Meloy) class or subclass specific antiserum. Mean serum levels ± standard deviation for each experimental group was calculated as the numerical average of the individual values.

b. Immunofluorescence

At sacrifice, the portion of the spleen not used for histology, was employed for preparation of a spleen cell suspension in Hank's Buffered Salt Solution (HBSS). A fraction of this was used to enumerate the number of T and B cells present, by employing the indirect immunofluorscent technique of Coons (34). Anti- Θ serum was a gift of Dr. J. Gordon, McGill University. Anti-mouse immunoglobulin serum, and fluoroscein labelled goat anti-mouse serum were obtained commercially (Meloy). The values obtained for each mouse were used to calculate the mean value ± standard deviation for each group. Group differences were compared by employing the Student's T-test.

c. Hemolytic Plaque Assay

Another fraction of the spleen cell suspension was used for hemolytic plaque assays. The localized hemolysis-in-gel technique was used, with the modification of Wortis and Dresser, to detect and enumerate the cellular synthesis of IgM (198, 199). Fresh frozen guinea pig serum, diluted 1:9 with M199, served as a source of complement. Spleen cells mixed with target erythrocytes were suspended in 0.7% agarose (Bacto-Agar, Difco Laboratories, Detroit, Michigan) for plating.

d. Autoradiography

Separate animals from each experimental group were given to Dr. D. G. Osmond (Professor of Anatomy, McGill University) for autoradiographic studies of the spleen, bone marrow and blood. The $^{125}I$ labelled anti-IgM, previously described, was used as the indicator material in

this assay (149, 150).

C. EXPERIMENTS

1. Anti-IgM Efficacy

To determine if anti-IgM affected serum levels of IgM, and if the effect was dose related, the following protocol was followed. Adult DBA/2 males were exposed to a lethal dose of x-irradiation (Cobalt 60, distance 80 cm, field size 20x20 cm, dose 850 rads). They were then divided into five groups of three animals each: Group I received no further treatment, and served as control. Groups II - V received, at 12 hours post-x-irradiation, 10 mg, 50 mg, 100 mg, and 200 mg of anti-IgM (lot #2) respectively via the IP route. Serum IgM levels were monitored throughout the procedure, and the experiment terminated with death of the control groups.

2. Treatment of Newborns

From the above experiment, and from the experience of Murgita et al (143), a dose and route of administration for the newborns was selected. Starting at 48 hours post-birth, they were to receive, at 48-hour intervals, 10 mg of anti-IgM (lot #2) via the IP route. This schedule was to be followed until death, or sacrifice of the animal. At day 28 they were to be divided into two groups. The males were to receive $5x10^8$ SRBC IP on day 28 followed by sacrifice on day 35. At this time they would be assayed for the immunological and non-immunological parameters (except autoradiography) previously described. The females would be tested only for weight, and serum immunoglobulins, and then autoradiography would be performed on this non-immunized group.

Controls were grouped as above, but were injected with an equivalent volume of normal saline instead of the anti-IgM. An attempt was made to have almost equal numbers in each group, but this proved difficult because of varying litter size, and high attrition rate of newborn DBA/2 mice. Differences between group means were compared by employing the Student's T-test.

## CHAPTER III - RESULTS

### A. MATERIALS

#### 1. IgM

A standard curve, using human serum albumin of known concentration, was plotted for optical density recordings at 280 nm (Figure 1). By extrapolation from this curve the concentration of mouse IgM obtained from the fractionation procedures was 4 mg/ml. By both Ouchterlony double diffusion against class or subclass specific antisera, and Millipore electrophoresis, no contaminants could be detected (Figure 2).

#### 2. Anti-IgM

Ouchterlony gel diffusion with purified IgM revealed an antibody against mouse IgM, and none against IgG or IgA. However, a suggestion that impurities could be present was raised by the extra lines of precipitation that occurred with mouse whole serum. This was verified when an immunoelectrophoresis was performed. Most probably there are antibodies against albumin, although the identity of the remaining bands has not been ascertained (Figure 3).

#### 3. Radioiodinated Anti-IgM

After further fractionation on a DEAE-Sephadex gradient elution

column, a purity analysis revealed that the antibodies against components of whole mouse serum, other than IgM, were no longer detectable (Figure 4). Most of the labelling would then be on the anti-IgM molecule.

B. ASSAY SYSTEMS

1. Immunological

a. Serum Immunoglobulin Levels

Normal mouse IgM levels are between 0.4 - 0.5 mg/ml (90). To correlate the assay method with serum concentrations, serial serum dilutions followed by recording reciprocals of dilutions at which precipitin lines were detectable produced a standard curve (Figure 5). This allows the serum levels to be interpreted in mg/ml, and emphasizes the reliability in the serial dilution technique. Note that a dilution of 1:16 corresponds to the usual serum level of 0.4 - 0.5 mg/ml, and that the lowest level of detectability is 0.03 mg/ml.

C. EXPERIMENTS

1. Anti-IgM Efficacy

The mean values of the five groups are plotted so that the dose relationship can be more fully demonstrated (Figure 6). Note that the half-life (T 1/2) of IgM in the irradiated animal is about 18 hours, and that 10 mg of anti-IgM is almost as effective in reducing serum levels as the 200 mg dose. The gel diffusion recording, for 200 mg, before and after treatment is presented (Figure 6).

2. Treatment of Newborns

A composite picture of the status of control and treatment groups is tabled (Table 1). Because of smaller litter size, and high perinatal

attrition rate, the number of animals in both groups was restricted. Of those receiving normal saline injections, there were five males, and two females. The anti-IgM group was marginally smaller with four males, and two females. The remaining animals had died prior to day 28. However, once immunization had commenced, all animals survived until sacrifice at day 35.

Non-immunological

There was a significant difference in body weight between the two groups ($p < 0.005$). In fact, the treatment group appeared to be uniformly runted with a mean body weight 4.9 gm less than the control animals. The splenic weight, and total splenic cell count, reflected the same pattern. However, this difference was probably secondary to the difference recorded in body weights, and did not reflect on independent change in spleen size. This is, in fact, corroborated when the spleen weight to body weight ratio was calculated, and the difference found not to be significant ($p < 0.10$).

Immunological

Serum immunoglobulin levels of all classes were lower in the treated newborns. Of note, is the total absence of IgM, and almost total absence of IgA. $IgG_1$ and $IgG_2$ were, however, decreased but still present in readily measurable quantities. All differences between control and treatment groups were very significant ($p < 0.005$).

Because of the techniques used, there was some overlap in the values obtained by immunofluorescence. This rendered interpretation of

the data difficult. Yet in the treatment groups, there was a relative paucity of cells bearing surface immunoglobulins ($p < 0.005$).

The reduction of direct hemolytic plaques, almost to background levels, is in accordance with the absence of detectable serum IgM, and reflects the same level of significance ($p < 0.005$).

The autoradiographic results corroborate the above findings. There was a significant decrease ($p < 0.025$) in labelled cells in both spleen, bone marrow and blood. The blood samples were pooled, and as a result were not able to be compared statistically. However, the almost complete absence of cells bearing IgM surface receptors is striking. Almost all of these were pyknotic with homogeneous dark nuclei.

Histological examination of the spleens revealed some morphologic differences between the groups (Figure 7). The treated animals demonstrated no germinal centers. However, no attempt at quantitation was made because of the quality of the sections. Qualitatively, it was noted, both in the routine and the autoradiographic sections, that there was an increase in the number of erythroblasts in the anti-IgM treated mice. These did not label autoradiographically.

Thus the anti-IgM treatment of newborn DBA/2 mice appeared to deplete the population of cells bearing surface immunoglobulin receptors, and this is manifest by decreased serum levels of the immunoglobulins, and inability to mount a primary immune response. Macroscopically, this treatment resulted in partially runted animals.

# CHAPTER IV - DISCUSSION

## A. MATERIALS

In searching for the origin of the impurities in the anti-IgM preparation, there are a number of key places where this could have occurred:

1. IgM

Although the tests of purity revealed no evidence of contaminating serum proteins, it is always possible that they were present in amounts below the level of detectability. To increase the possibility of obtaining a pure sample, a number of additions could be made to the protocol.

(i)

It has recently been found that the plasmacytoma, MOPC 104E, can grow intraperitoneally as a solid tumor (112). However, the ascites formed in reaction to the tumor is extremely rich in IgM. This would serve as a better crude IgM pool from which to start fractionation, because a larger amount of IgM would be available (can repeatedly tap ascites), and there would be less protein contamination.

(ii)

The efficiency of the fractionation procedure could be increased by the addition of two steps: an initial precipitation with ammonium sulphate, and a final separation with DEAE-Sephadex gradient elution (155). These changes, and additions, should result in a purer IgM sample

with which to begin immunizations.

2. Anti-IgM

The use of immunoprecipitated IgM probably served to further purify the immunizing agent. However, this could still be aided by using excessive washing rather than simple daily saline changes (i. e., a large volume of 0. 85% NaCl plus a magnetic stirrer).

With the immunization protocol followed, any antibody response to impurities would be amplified by the repeated injections, and long time span, over which bleeding was performed. This could be circumvented by using smaller doses of the immunizing material, only one booster injection with incomplete Freund's adjuvant followed by an intravenous challenge of whole mouse serum. If the animals were then bled out five days later, the amplifying effect of booster injections should selectively augment the antibody sought.

Unfortunately, the anti-IgM obtained did contain contaminants, probably as a result of a combination of the factors described. Fortunately, it had antibody activity only against mouse immunoglobulins with IgM heavy chains (Figure 3A). That is, it was monospecific for mouse IgM, although antibodies to other mouse serum proteins were present. Because of this it was elected to use the preparation without absorption of the impurities.

3. Radioiodinated Anti-IgM

Although further fractionation of anti-IgM via DEAE-Sephadex gradient elution appeared to remove the impurities detected in the anti-IgM sample,

these were probably present, but below the level of detection. Therefore, most of the labelling would be on the anti-IgM immunoglobulin and this would be adequate for the autoradiographic assay.

## B. EXPERIMENTS

### 1. Anti-IgM Efficacy

Lethal whole body x-irradiation prevents the formation of new immunoglobulin producing cells. Some synthesis continues, but catabolism becomes the major determinant of serum levels (18), with the exception of IgA (19, 20). Bazin et al have studied this phenomenon. By using single radial immunodiffusion they could not detect any catabolism of IgM post irradiation (20). By using a more sensitive radioiodination method they found the half-life of IgM to be 12 hours, and to remain unchanged post-irradiation (18). By using a less sensitive technique, that of serial serum dilutions, we found a half-life for IgM of about 18 hours. This value correlates reasonably with the above data, and with that obtained by Fahey and Sell in normal mice ($T_{1/2}$ = 0.5 days) (46). Differences of this magnitude could be explained solely by strain variations (172), or environmental condtions (171). It also emphasizes the reliability of measurements using serial serum dilutions.

The effect of anti-IgM on serum IgM levels demonstrated several facts. The first is that the intraperitoneal route does work. The second is that, although there does seem to be a dose dependent relationship, the low dosage is almost as efficient as the high. As a runting syndrome had

been documented with the administration of large amounts of anti-IgM (143), these results led to the selection of the 10 mg dose for further experimentation in the newborns.

2. Treatment of Newborns

The results of this section corroborated the findings of other workers, and demonstrated that selective immunosuppression could occur in yet another murine strain. However, there were some variations from, and additions to, the previously observed patterns.

Non-immunological

Runting has been observed in one other laboratory (143), and occurred only with anti-IgM antisera. To avoid this situation a dose of anti-IgM, which was felt to be equivalent to their non-runting dose, was utilized. This resulted in partial runting, manifested by a moderate decrease in body weight, but with no obvious increase in morbidity or mortality. It suggested that this could be a dose related phenomenon, but afforded no further clarification of the mechanism involved.

The decreased mean spleen weight within the anti-IgM group most probably is secondary to the partial runting, as the spleen weight, when compared to body weight, was not significantly different from the control animals.

Immunological

The immunofluorescent, and autoradiographic assays demonstrated a marked reduction in cells bearing surface immunoglobulin receptors.

Because the anti-mouse immunoglobulin, used in the immunofluorescence studies, was not specific for any murine heavy chain class, the results indicated that all classes were suppressed. This, however, even though previously observed in other studies employing anti-IgM, served only as a semi-quantitative assessment of the degree of suppression.

The autoradiographic analysis clarified and quantified this more fully, for the first time, by demonstrating an almost total absence of cells bearing IgM as the surface immunoglobulin. It is likely that the difference in values, obtained by using the two different techniques, could be in part explained by the insensitivity of immunofluorescence compared to autoradiography, and in part to cells bearing surface receptors other than IgM. However, even this latter group of cells must have been partially depleted in order to give low immunofluorscence values.

This pattern of suppression is reflected in the cell products, and functions. IgM was essentially absent in the serum, and was not produced in the primary immune response. The other serum immunoglobulin heavy chain classes were suppressed, although not as much as the IgM. This could be due to modulation of the maturation process described previously, to transplacental transfer of these classes, or to secretion from the mother's mammary glands. Probably their serum levels reflect a combination of the above factors. Similar suppression, with variations as to when the IgA returned to significant levels, have been consistent findings by other workers. This lends further support to the concept of maturation, and switching, within

immunoglobulin classes.

Again IgM appeared to serve a basic regulatory function, both phylogenetically and ontogenetically. Because of this, the suppression obtained by using anti-IgM could be called panspecific. That is, the cells and function of the IgM class were specifically suppressed, while only a relative suppression occurred within the other immunoglobulin heavy chain classes. In effect, the whole B cell population was decreased, producing possibly the mammalian equivalent of bursectomy.

Of interest was the marked increase in erythroblasts observed in the treated newborns. Because of the impurities within the antisera, it is possible that these precipitated hemolysis with a compensatory secondary erythropoiesis. Since both the erythropoietic and granulocytic lines originate from the same stem cell, perhaps treatment has altered a control mechanism with a resultant increase in the erythroid line. Whatever the mechanism, this may be relevant and probably warrants further investigation. Studies of T cell function, such as ability to reject autologous skin, were not assayed. This has been adequately demonstrated in other studies on anti-IgM treated newborn mice, and has been found to be essentially unchanged from control animals (143).

## CHAPTER V - SUMMARY

A method for producing panspecific immunosuppression has been adapted to another immune strain, the DBA/2. The specificity for IgM has, for the first time, been quantitated by using an autoradiographic assay. However, the inability to totally suppress all immunoglobulin heavy chain classes

has again been demonstrated. This partial bursectomy, combined with a syndrome of partial runting could complicate the assessment of abrogation of enhancement, described in Part III.

# PART II: TUMOR ENHANCEMENT

# CHAPTER I - BACKGROUND

## A. TUMOR SPECIFIC ANTIGENS

### 1. Evidence

In the first part of this century, it was demonstrated by such distinguished scientists as Ehrlich (43), that experimental animals could be immunized against transplanted tumors. However, with the introduction of inbred strains of mice circa 1925 - 1930, it became apparent that what was thought to be tumor immunity, was simply an allograft reaction. Enthusiasm subsequently dwindled, and was only renewed with the demonstration that neoplastic tissues contain their own indigenous antigens.

### 2. Types

At present, two classes of tumor specific antigens have been identified.

a.

By employing transplanted tumors, induced by the chemical carcinogen 3-methylcholanthrene, Gross (62), Foley (51), and Prehn and Main (157), demonstrated that tumor specific antigens do exist. Recently, Reiner and Southam (164) reported that the tumor specific antigens in this system are unique for each tumor. This property of non-cross reactivity has subsequently been extended to other inducing agents (101).

b.

In contrast, virus-induced tumors cross react antigenically with all other tumors induced by the same virus, regardless of the morphologic appearance of the tumor, or the similarity of animals bearing the tumor (177).

c.

To extend the concept further, Simmons et al demonstrated non-cross reacting or private, and cross-reacting or public antigens on the same neoplasm. This held for chemically-induced (175), viral-induced and spontaneous murine tumors (176). Such findings demonstrate that tumor specific antigens probably exist for all neoplasms. All that is required are techniques sensitive enough for their detection.

## B. IMMUNE RESPONSE TO TUMOR SPECIFIC ANTIGENS

The existence of tumor specific antigens does not necessarily imply that an immune response will be elicited by them. The evidence now indicates that this, in fact, does occur but not always to the benefit of the host.

### 1. T Dependent System

There is now considerable data showing that lymphocytes of the thymic axis mediate tumor cell destruction. These cytotoxic lymphocytes have been demonstrated in vitro (25, 30, 31) and in vivo (168, 169), both for syngeneic (168) and allogeneic (25, 30, 31, 169) tumors.

### 2. B Dependent System

The role of this level of the immune response is not as well-defined. That circulating antibodies can inhibit the growth of allografted tumor cells

has been shown (100). However, that serum from tumor bearing animals can also protect against the cell-mediated killing of tumor cells has been repeatedly demonstrated (68, 70, 72). The significance of this latter effect is not yet fully established, but it appears that it may contribute to enhancing tumor cell growth (71).

Thus, a neoplasm may incite an immune response to its tumor specific antigens, but, depending on a number of factors not yet fully recognized, this may be deviated towards facilitating tumor growth rather than the production of tumor cell destruction.

## C. ENHANCEMENT

Since the initial observation of the phenomenon in 1906 (49, 50), it has been found to apply not only to transplanted allogeneic tumors, but to non-neoplastic conditions as well (52, 136, 184). We will be concerned only with its implications in the control of neoplastic growth.

### 1. Definition

In order to account for the general significance of the phenomenon, the following definition has been proposed by Voisin: 'Enhancement is the mechanism whereby an antibody promotes the persistence of the corresponding antigen by preventing it from inducing or undergoing (or both) immune rejection' (194). This definition distinguishes enhancement from those conditions involving induced immune unresponsiveness (57).

### 2. Methods of Production

Three procedures have been utilized to produce enhancement. Their

aspects evoke the concept of classical immunization methods.

a. Active Enhancement

This is accomplished by the administration of immunizing injections prior to tumor-challenge. The material used can consist of either normal, or neoplastic tissue, from the prospective graft donor (194).

b. Passive Enhancement

In this case, serum from an actively treated animal can passively transfer the ability to enhance the growth of allogeneic tumors (85, 87).

c. Adoptive Enhancement

Enhancement has also been transferred adoptively to syngeneic animals by means of lymphoid cells from actively pretreated mice (38). In general, active enhancement reflects the clinical situation, while passive enhancement produces more stable results. Indeed, many complex factors control the direction and intensity of enhancement.

3. Factors Influencing

As active enhancement was employed later in this study, a discussion of the factors of variation will be directed towards this aspect of enhancement production.

a. Non-immunological

Many factors of a non-immunological nature are likely to affect the production of enhancement in a given tumor allograft. These include growth rate of the tumor, the degree of histological differentiation, and the intensity and rapidity of its vascularization (194).

b. Immunological

The host's response to immunization probably has a bimodal pattern: this reflects the biphasic immune response, with the cell-mediated response occurring early, followed by a humoral antibody response productive of enhancing antibody (89). Any protocol must be assessed in relation to these considerations.

(i) Immunization Procedure

(a) Antigenic Preparation: Type

Tumor, or normal tissue, can serve as the immunizing material. These can be either modified, but alive (194), or killed and extracted. Of the extracted preparations, supernatants of saline homogenates seem to be the most efficient (39, 88). Repeated freezing and thawing seem to improve the antigenicity so that storage does not present a problem (88). In all, the common denominator seems to be the liberation of soluble antigens.

(b) Antigenic Preparation: Dose

Both Kaliss (86), and Voisin (193) have reported a definite dose relationship: low doses favor inhibition, medium doses behave like untreated controls, while high doses produce enhancement.

(c) Antigenic Preparation: Route of Administration

In rabbits it is known that the intravenous route (181, 193), supercedes intradermal injections (123) for the production of enhancement. In mice the intraperitoneal route appears to be the most efficacious (39).

(d) Antigenic Preparation: Immunization Schedule

Hyperimmunization, or any schedule which utilizes the bimodal nature of the immune response favors enhancement (89). Voisin estimates that this begins 3 - 4 weeks, reaching a maximum about 8 weeks, post-initial immunization (194), in allogeneic systems. However, the enhancing activity may not persist for as long a period in syngeneic models (26).

(ii) Nature of Grafted Tumor

In allogeneic tumor transplants, the ease with which enhancement is induced is related to the in vitro resistance of the tumor cell to the cytotoxic action of the serum (58, 88, 179). This resistance in turn was inversely related to the concentration of surface antigenic receptors (H-2 antigens) (133).

In syngeneic tumor systems, enhancement is specific for tumor specific antigens (73), but any relationship to cytotoxic antibodies has not been demonstrated.

(iii) Nature of Recipient

Four attributes are particularly relevant: the species, strain, sex and site of graft implant.

(a) Species

Most work has been performed on the mouse. However, enhancement can be demonstrated in the rat (49, 50), guinea pig (144), rabbit (29), and dog (142).

(b) Strain

In mice, some strains are more susceptible to enhancement than others. The best are C57Bl/6Ks and C57Br/a (180). This probably reflects strain difference in the biphasic immune response (89).

(c) Sex

Females tend to have stronger immune responses, but exhibit greater difficulty in producing enhancement (58). For this reason females are usually used for production of antisera, while males are preferred for enhancement studies.

(d) Site of Implantation

This is of some relevance in determining the outcome of an experiment. In the BP 8 Sarcoma the intramuscular site is more efficacious than a subcutaneous implant (58). This may be related to antibody, and lymphoid cell, access to the tumor.

4. Mechanism

The theories proposed to explain immunological enhancement, on the basis of whether antibodies modulate the neoplastic tissue or the host's response, can be placed into two categories.

a. Modulation of Neoplastic Tissue

Moller argued that the relevant variable for the induction of enhancement was the presence of antibodies against all major foreign antigens on tumor cells; the specificity of the phenomenon indicated that the antibody interfered with the immunological relation between the tumor and host rather

than produced an antibody-induced alteration in the tumor cell (134). Subsequently, he was unable to demonstrate that antibodies directly change properties of tumor cells (135). Therefore, the hypothesis of tumor cell modulation is unlikely.

b. Modulation of Host Response

If antibodies are to interfere with the host's response to foreign antigens, the modulation could occur at three different levels.

(i) Afferent Stage

This refers to those events involved in the presentation of antigen to the potentially reactive lymphoid cells. For afferent enhancement to exist, the antibodies would have to render foreign antigens inaccessible by "coating" the tumor cells. For some tumor systems, both in vitro (63, 134) and in vivo (63), this appears to be the only relevant mechanism.

(ii) Central Stage

For enhancement to occur at this level, antibodies would have to act directly on the immunologically competent cells and specifically decrease their reactivity (131, 137, 186), or complex with soluble foreign antigen on the surface of the lymphocyte so that target cells would not be recognized (21).

However, since both afferent and central enhancement lead to a decreased number of effector cells, it is not surprising that assay systems are often unable to differentiate between the mechanisms (63).

### (iii) Efferent Stage

Efferent enhancement necessitates antibody attachment to the target cells, thus preventing killing by cytotoxic lymphocytes. This has been well-documented in vitro (24, 122) and may occur with human urinary bladder carcinomas (28).

In any given system, in vivo, it is difficult to ascertain which of these mechanisms is operative. Probably they act in concert and exert a cumulative effect (10, 160).

## D. SELECTION OF METHODS EMPLOYED

As the object is to produce enhancement in syngeneic murine systems, and thus ensure that the phenomenon is due to antibodies to tumor specific antigens rather than transplantation antigens, all of the influencing conditions must be manipulated so that the possibility of producing enhancement will be maximized. These are summarized as follows: active immunization to more closely approximate the clinical situation; weekly intraperitoneal immunizations with large doses of extracted tumor tissue; challenge with viable tumor cells so that tumor growth will coincide temporally with the presence of enhancing antibodies; male recipients with subcutaneous (SC) implants. This combination of the factors involved should favor the production of enhancement.

# CHAPTER II - EXPERIMENTAL DESIGN

## A. PREPARATION OF MATERIALS

### 1. Tumors

Two tumors, both indigenous to the same murine strain as

employed in Part I, were chosen for the study.

a. P-815-X2

This mast cell neoplasm arose (1956) in orchiectomized DBA/2 males probably in response to the chemical carcinogen 3-methylcholanthrene (41). It rapidly developed an ascitic form. Potter and Dunn have tabulated a dose-response relationship for the IP location, and found that the time of death was inversely related to the number of viable cells administered, and that there did not appear to be a sex difference. They did, however, find metastases in the liver, spleen and nodes.

Throughout the course of this study the tumor was passaged in the ascitic form, in DBA/2 males, by weekly transfer of ascitic fluid. SC challenge was performed as follows. Day five (post-IP transplant) ascitic fluid was taken, and viability (varied from 92 - 95%) ascertained by Trypan blue exclusion. Dilutions in HBSS were done to achieve a given viable cell concentration. These cells were then implanted in the SC tissue of the right axilla.

b. T1699

This well-differentiated mammary adenocarcinoma arose in DBA/2 females, as a spontaneous tumor in 1965 (79). It has never converted to the ascitic form, but can be carried either as a SC implant, or in tissue culture. Since the tumor specific antigens are more readily detected when the tumor is maintained in tissue culture (personal communication - Dr. S. Haskell, McGill University), this method was employed for maintenance, and for SC implants. They were performed as for the P-815-X2 except that the location

of implant was the right milk line.

2. Tumor Specific Antigen

Both the P-815-X2, and the T1699, were SC implanted in separate groups of DBA/2 males. When the tumors reached a size of one centimeter, they were aseptically excised and used to make tumor specific antigen (TSA). Two preparative procedures were employed, and the preparations labelled TSA #1 and TSA #2 respectively.

a. TSA #1

This method produced supernatants of a saline-tumor homogenate as described by Day et al (39). The tumor was combined with an equal volume of 0.85% NaCl, and homogenized in a blender. The homogenate was then sequentially centrifuged at 800 g for 30 minutes at 4°C, and at 8,500 g for 30 minutes at 4°C, to produce a supernatant which did not contain particulate material, or viable cells. This corresponded to Day's "full-strength supernatant". Subsequently, it was stored frozen until use (88).

b. TSA #2

In order to extract tumor specific antigens, a method that was described for the production of soluble HL-A antigens (165) was modified. $50 \times 10^9$ tumor cells (viable and unviable) were suspended in HBSS, pH 7.4, containing 3 M KCl. This was mixed for 16 hours at 4°C. The homogenate was then centrifuged at 163,000 g (Rotor 50 T1, Spinco Division, Beckman Instruments, Inc., Palo Alto, California) for one hour at 4°C, followed by a 24-hour dialysis against 0.85% NaCl at 4°C. This formed a gelatinous

precipitate which was removed by centrifugation at 1500 g for 20 minutes at 4°C. The supernatant was considered to contain the TSA and was stored in the frozen state until use (88).

The P-815-X2 had TSA prepared by both methods #1 and #2. As subsequent experimentation demonstrated no difference between the efficacy of these preparations, T1699 TSA was prepared only by method #2.

A portion of T1699 TSA #2 was frozen and thawed, a portion lyophilized, and another portion was suspended in buffer (Glycine 0.5 M, Tris 0.2 M, Mannitol 0.5%, pH 8.0) (84) and fractionated on a Sephadex G-200 (Pharmacia Fine Chemicals AB, Uppsala, Sweden) column. Subsequent immunofluorescent testing for TSA (Dr. S. Haskell, McGill University) revealed that only the crude preparation had satisfactory activity levels. Therefore, only it was used for immunization procedures.

3. Animals

DBA/2 males, six to eight weeks of age, were employed in the following experiments. The remainder of their description is as stated in Part I.

B. ASSAY SYSTEMS

Tumor size, expressed as the product of two diamters at right angles to each other, was measured. Individual values were used to calculate the mean ± standard error for a group.

1. P-815-X2

Because of the consistent growth pattern of this tumor, it was

possible to construct a standard curve comparing the number of cells injected SC to the tumor size obtained on a given day. This allowed individual tumor measurements to be converted to, and expressed as, "tumor cell equivalents". Using these values, group means ± standard deviation could be calculated, and group differences compared via the Student's T-test.

2. T1699

Because of the less consistent growth pattern of this neoplasm, a standard curve was not employed. Instead, groups were compared in regard to the incidence of tumor takes, the ratio of progressors to regressors, and the latent period between challenge and tumor appearance. Again, group means were evaluated by the Student's T-test.

C. EXPERIMENTS

1. P-815-X2

a. Dose-response Relationship

Tumor cells were prepared and injected as previously described. Groups, consisting of five animals, received the following doses of viable tumor cells: 500, 1000, 2500, 5000, 10,000, 50,000, 100,000, 500,000 and 1,000,000. Tumor measurement, as outlined above, was performed. Autopsies were performed to search for metastases in the liver, spleen or lungs.

b. Enhancement

(i)

Mice were divided into two groups of ten animals each. Group I received TSA #1, 1 cc IP on days 0 and 7. On day 14 a challenge of 500 viable

tumor cells was given SC as described. Group II received 1 cc injections of 0.85% NaCl instead of TSA #1 and served as the control.

(ii)

This was identical to (i), except that TSA #2 was used instead of TSA #1. Tumor measurements were performed as usual. Autopsy searches were again conducted.

2. T1699

a. Dose-response Relationship

Tumor cells were prepared and injected as previously described. Groups, consisting of ten animals, received the following doses of viable tumor cells: $10^1$, $10^2$, $10^3$, $10^4$, $10^5$. Routine tumor measurement was done. Autopsies were performed to search for metastases in the liver, spleen or lungs.

b. Enhancement

Mice were divided into two groups of ten animals each. Group I received TSA #2 1 cc IP on days 0 and 7. On day 14 a challenge of $10^5$ viable tumor cells was given SC as described. Group II received 1 cc injections of 0.85% NaCl instead of TSA #2, and served as the control. A search for metastases was made at autopsy.

## CHAPTER III - RESULTS

### A. MATERIALS

1. Tumor Specific Antigen

The Sephadex G-200 separation of T1699 TSA #2 was pooled and

concentrated (Diaflow UM2) as four fractions (Figure 8). The crude TSA #2, the crude TSA #2 frozen and thawed, each of the above fractions, and the resuspended lyophilisate were tested for the presence of TSA via immunofluorescence. Unfortunately, only the crude samples retained adequate activity. For comparison, H-2 antigens were also tested and recorded (Table 2).

B. EXPERIMENTS

1. P-815-X2

a. Dose-response Relationship

The dose-dependent relationship of this tumor extended to all facets of tumor growth: latent period, growth pattern once established, and time of death (Figure 9). Note that the greatest consistency existed in the size range of 0.50 to 1.00 cm. By extrapolating the number of days it would take a given cell challenge to achieve a given size, it was possible to plot a standard curve of size isobars. (Figure 10). This serves to emphasize the regularity of growth pattern that this tumor exhibited, and allowed subtle changes to be detected. Note that this was possible because there were 100% tumor takes. Autopsies revealed no metastases; death was from local invasion.

b. Enhancement

(i) TSA #1

The group which was immunized had a growth pattern that was shifted to the left of the control group (Figure 11). Latent period, rate of growth, and time of death were all affected so that the shape of the curve

did not change. When these values were converted to tumor cell equivalents the control group behaved like a challenge of 13,893±8586 cells, and the immunized group like 114,000±33,246 cells. This difference was significant at the $p < .05$ level.

(ii) TSA #2

The same pattern occurred, with tumor cell equivalent values of 4940±2125 for the non-immunized, and 82,333±16,343 for the immunized (Figure 12). This was significant at the $p < 0.01$ level. Fortunately, a relative comparison can be made between TSA #1 and TSA #2, as they received the same effective cell dose at the same time. Probably no real difference in their effectiveness exists. However, it was decided to continue experimentation with only one preparative method, and because TSA #2 gave higher ratios, it was selected. Autopsies revealed no metastases.

2. T1699

a. Dose-response Relationship

A dose dependent relationship for this neoplasm is evident, but a graded consistency is not readily apparent (Table 3). With a challenge of $10^3$, or less, viable tumor cells no takes occurred. When challenged with $10^4$ and $10^5$ cells, tumor growth followed with a respective increase in tumor takes with increasing challenge size. An exact relationship between challenge dose and subsequent takes cannot be determined from this data. However, a 90% take rate would be expected if $10^5$ viable cells were employed. Note that at this challenge dose a tumor which eventually regressed occurred. There

appears to be a low incidence of this phenomenon (about 11.1%), and a larger group of animals would be required to establish the frequency of this occurring. Whether it occurs with a challenging dose of $10^4$ cannot be assessed because of the small number of animals involved.

In addition to the above parameters, the significant decrease ($p < 0.005$) in latent period with increasing challenge size suggests that a dose-response phenomenon exists.

To further clarify the relationship, the growth patterns were plotted for all tumor takes (Figure 13). Because of the wide variation encountered, mean values were not calculated. Instead the growth curves of individual neoplasms were charted. In addition to reflecting the variables previously discussed, several distinct patterns of growth seemed to emerge:

1. Progressors

All of the $10^4$, and four of the $10^5$ challenge doses progressively increased in size with no evidence of regression at any point in their growth pattern.

2. Progressors with Regression

In three of the $10^5$ challenge dose, progression is delayed by a period of regression which is maximum at 50 to 60 days post-tumor challenge.

After this interval of temporary regression, the tumor continued to enlarge with eventual death of the host.

### 3. Regressors

Although only evident in one animal, this became apparent at the same time at which group #2 was showing maximum regression. This suggested that in a certain proportion of animals, a critical period existed which determined the eventual tumor response, progression versus regression.

Because a challenge of $10^5$ viable cells gave a high percentage of tumor takes, produced a regressor, and demonstrated some variations in growth pattern, this dose was selected for use in subsequent enhancement attempts. However, the inherent variability of the parameters made this tumor more difficult to assess, and a standard growth curve was not constructed. Necropsies revealed no evidence of metastases.

### b. Enhancement

By comparing the results for enhancement (Table 4) with the dose-response relationship for this tumor (Table 3) in regard to the number of tumor takes, and the latent period, it appeared as if the control or non-immunized group had received an effective challenge of between $10^4$ and $10^5$ neoplastic cells. Fortunately, for this control dose, a number of regressors were present, but their relationship to challenge size cannot be interpreted because of the small number of animals involved.

When the treatment group was compared to the control group (Table 4), no real change in the incidence of tumor takes was evident, although the latent period was marginally decreased ($p<0.10$). This indicated that the

treatment group behaved like a slightly higher challenge dose. The absence of regressors was noted.

Again, to see if further clarification was possible, the growth pattern of individual neoplasms was plotted for non-immunized and immunized groups (Figure 14). No attempt to express mean values was made.

Within the control group three growth patterns were evident: there was one progressor, three progressors with regression, and three regressors. Maximum regression occurred between day 30 and 50 post-tumor challenge. In the treatment group five neoplasms demonstrated progressive growth, and one combined regression with progression, maximum at day 30 to 40. This group had a marginally steeper growth pattern than was seen in the controls.

Regressors that were followed for four months never developed further neoplastic growth. Autopsies revealed death from local invasion, without metastases.

## CHAPTER IV - DISCUSSION

### A. MATERIALS

#### 1. Tumor Specific Antigen

A number of attempts have been made to solubilize tumor specific antigens of both carcinogen-and viral-induced neoplasms. Usually, the yield of biologically active material was low, and the materials proved to be labile (7, 23, 76, 124, 147, 178).

As an efficient salt extraction procedure for soluble HL-A antigens has been described (165), and as sufficient biological activity was retained by this method to create "blocking" in an in vitro allogeneic system (201), the technique was adapted to prepare TSA from syngeneic solid tumors.

Subsequent immunofluorescence testing revealed the presence of TSA in the crude preparation obtained. Further fractionation on Sephadex G-200 revealed that TSA eluted in the same area in which H-2 activities are found (183) (Figure 8, Table 2). This entity would then have a high molecular weight (about 50,000). Although immunofluorescence detection of TSA was a semi-quantitative technique, it has adequate sensitivity. Therefore, it would appear that some biological activity was lost upon further processing (lyophilization or fractionation). This loss appeared greater than that found after limited papain digestion plus Sephadex G-150 separation (102). Fortunately, little loss occurred with freezing and thawing of the crude material.

Even though the immunofluorescence testing indicated that there probably was biological activity, the biological efficacy had to be tested in a tumor system.

## B. EXPERIMENTS

### 1. P-815-X2

#### a. Dose-response Relationship

The consistent and reproducible dose-response behaviour of this

neoplasm as a SC implant, has also been shown for the IP route (41). This permitted the construction of a standard growth relationship to facilitate the expression of enhancement. However, it also indicated that changes in growth pattern would be subtle and difficult to detect. For this reason it was decided to use the 500 cell dosage as the challenge in the ensuing enhancement experiments. The longer latent period with this dosage would also allow full advantage to be taken of the time required post first immunization, to develop enhancing antibodies (194).

b. Enhancement

That there has been a change in the growth pattern of this neoplasm was obvious (Figures 11 and 12), and statistically significant. However, did this meet the requirements necessary for its interpretation as enhancement? The immunization procedure with TSA #1 or #2 provided the antigenic stimulus. That this material was in fact tumor specific antigen was accounted for by the fact that H-2 differences were circumvented by employing syngeneic tissue (Moller questioned this because he felt that transplantable neoplasms acquired the properties of allogeneic tissue with the passage of time) (135). This contradicted Brunner's findings that P-815-X2 demonstrated no evidence of TSA. However, these studies were done in vitro (25). Because of the careful selection of parameters involved, the time of tumor growth corresponded to the time when antibodies to the TSA should be present. As other factors remained

constant, the change in tumor cell growth can only be attributed to the effect of the antibody to TSA. These conditions, therefore, met the requirements for enhancement (194). Thus, syngeneic enhancement of P-815-X2 occurred.

From this, however, no attempt to explain the mechanisms involved was warranted.

2. T1699

a. Dose-response Relationship

Consistency in the growth behaviour of this neoplasm has been observed for SC implants in other locations (right shoulder area - personal observation). However, since it is a mammary adenocarcinoma, with probable estrogen dependence (personal communication - Dr. S. Haskell, McGill University) it was elected to evaluate its growth in potential mammary tissue by injecting it SC in the milk line. For consistency only the right side was employed, although there is no reason to expect a different response on the left.

In this location a graded response seemed to occur. When $10^3$, or less, viable cells were employed as challenge, no takes occurred. This reflected tumor cell rejection by the host and Moller's explanation may be valid (135). However, when a crucial size of cell challenge was surpassed, takes occurred. This frequency appeared to reflect the challenging dose, although it was unknown if there was an upper value which would result in 100% takes.

The latent period before tumor appearance also correlated well. Therefore, an estimate of the challenge size could be made by assessing take frequency and latency. The three patterns of tumor growth apparent from the graphs (Figure 13) were more difficult to interpret. Even though this was evident only at a challenge of $10^5$ cells, the small number of animals involved did not negate the possibility that it may have occurred at other cell challenge numbers. Probably it does! Yet, the patterns were intriguing, for there existed a group which appeared to be midway between progressors and regressors. At a particular time the tumor growth regressed, and then plateaued. For unknown reasons, they subsequently progressed. Perhaps this reflected the biphasic nature of the immune response (89). At any rate, this pattern of tumor growth would be expected to be sensitive to immunological manipulation, and may serve to demonstrate enhancement.

As a result of the above factors (takes, latent period, and growth pattern) it was decided to use $10^5$ cells as the challenging dose for further enhancement studies.

The absence of metastases cannot be commented on.

b. Enhancement

Using the above criteria, takes and latency, it appeared that the control, or non-immunized, groups received a cell challenge of between $10^4$ and $10^5$ in number. When the immunized group was examined, there was no real change in take rate, and only minimal decrease in the latency

periods. This behaviour, corresponding to a small increase in the challenging dose, could be considered suggestive of, but not conclusive evidence for enhanced tumor growth.

Further clarification came from the graphs demonstrating individual neoplasm growth characteristics (Figure 14). There appeared to be a shift from the non-immunized to immunized groups in that less regression occurred in the latter. It appeared that, at the critical period mentioned before, the prior manipulation of immunization with subsequent antibody formation exerted some effect so that progression was favored. The mechanism cannot be surmised, but this shift from neoplastic regression to progression was evident.

Thus, when all the above parameters of tumor growth are considered, the effect of an immunization protocol was a shift towards behaviour characteristic of a greater tumor cell challenge, and away from regression. This, however, was only suggestive of enhancement. Clear-cut affirmation would require repetition with larger numbers of animals.

Unfortunately, no metastases occurred in either group.

## CHAPTER V - SUMMARY

By careful selection of all the factors that tended to maximize neoplastic enhancement, one solid syngeneic neoplasm was found to express the phenomenon. However, variations were subtle, and only the constancy of behaviour of P-815-X2 allowed detection. This, in turn, substantiated that salt extracted TSA had biological activity, as proposed by the immuno-

fluorescent studies.

With T1699, identical manipulations failed to produce definitive evidence for any enhancement of tumor growth.

# PART III: SYNTHESIS

# CHAPTER I - BACKGROUND

## A. HYPOTHESIS

Data is accumulating to suggest that serum factors, operating via an enhancement mechanism, are responsible for the inability of a host to reject a neoplasm. However, if these factors were removed would enhancement be abrogated and the balance swing to favor immune rejection? The evidence for this is not completely clear, but indicates the direction to be pursued.

## B. EVIDENCE

This can be classified into two categories:

### 1. In Favor

The serum factors responsible for the production of enhancement have been identified for some tumor-host systems. However, the results are conflicting. In some allogeneic (78, 131, 190) and syngeneic murine models (160), and in human neuroblastomas (83), blocking activity has been found associated only with the IgG class or subclass. In other allogeneic systems the IgG fraction produced enhancement, while the IgM fraction was responsible for inhibition (186). Moller has suggested that this is really not a qualitative difference, but reflects the combination of concentration and avidity (138). If present at appropriate levels, all antibodies should possess enhancing properties (4, 138). The findings of Bubenik support this concept, but stress too that the ratio of 7S to 19S antibodies may exert a modulating influence (27). Certainly this interpretation could

be applied to explain the "blocking" (11, 28, 70, 72) and "unblocking" (9, 11, 69) factors that have been documented. It certainly does not help predict what would happen to the enhancement phenomenon if antibodies were absent.

An indication of what could occur comes from two studies. In the one, Jagarlamoody et al (82) examined the effects of APS on two separate tumor systems. When tumor challenge occurred in hypogammaglobulinemic animals, tumor growth was retarded. In the other, Manning et al (118) employed anti-IgM as the immunosuppressive agent. Again this prevented tumor growth. However, it is not possible to attribute this effect to the suppression of enhancing antibody. To do this the abrogation, or prevention, of enhancement would require documentation.

2. In Opposition

The experiments of nature, however, perhaps serve as a cautionary example. In the primary immunological deficiency diseases, the state of hypo- or agammaglobulinemia is not without sequelae. Usually, they occur in the form of recurrent infections with opportunistic organisms, or lymphoreticular malignancy (3).

C. OBJECT

In Part I, panspecific suppression of the humoral antibody system was achieved by treating newborn mice with repeated injections of anti-mouse IgM. In Part II, reproducible syngeneic enhancement of one neoplasm was demonstrated. In order to evaluate the relevance of enhancing

antibody on an enhanced tumor, it was planned to synthesize Parts I and II. Hopefully, selective abrogation of the immune response would abrogate the enhancement phenomenon.

## CHAPTER II - EXPERIMENTAL DESIGN

### A. PREPARATION OF MATERIALS

#### 1. Anti-IgM

Anti-IgM lot #2, as prepared and standardized in Part I, will be used exclusively.

#### 2. Animals

DBA/2 newborns were obtained, and raised under the same conditions as described in Part I. Weaning and sex separation occurred at three weeks of age.

#### 3. Tumors

For both P-815-X2 and T1699, the immunizing and challenging doses, schedule, and route remained identical to Part II.

#### 4. Immunizing Agents

##### a. Extract of Normal Tissue

A KCl extraction, of DBA/2 liver, spleen, and kidney was prepared according to the regimen used in Part II (165) for the preparation of TSA #2. By comparing optical density, it was standardized to the same concentration as TSA #2.

##### b. Extract of Neoplastic Tissue

TSA #2, as prepared in Part II, was exclusively employed.

B. EXPERIMENT

The format was best presented graphically (Table 5).

1. Anti-IgM Treated Animals

These received anti-IgM lot #2, 10 mg, IP, every 48 hours for the duration of the experiment.

a. Sex

As a shortage of animals necessitated the use of the treated females, it was decided to use the P-815-X2 in this instance, since it apparently shows no sex preference (41). T1699 was subsequently used in the treated males.

b. Immunization

This was performed at four and five weeks of age.

c. Tumor Challenge

This was done at six weeks of age.

2. Control Animals

The immunization and challenge were performed as in the treated group. The interchanging of immunization, and challenge tumor sources, was designed to detect any cross-reactivity between the two tumors.

C. ASSAY

1. Serum Immunoglobulins

In order to correlate the degree of immunosuppression obtained, with that observed in Part I, the serum immunoglobulin levels were determined at day 35. This technique was selected because of its non-invasive character.

Where appropriate, group means ± standard deviation were calculated, and differences compared via the Student's T-test.

2. Tumors

These will be measured and assessed as described in Part II.

## CHAPTER III - RESULTS

Again, the data was most readily expressed in tabled format (Table 6).

### A. IMMUNOSUPPRESSION

Serum immunoglobulin levels on day 35 were again found to be panspecifically suppressed in the anti-IgM treated newborns. IgM was absent, while the mean values ± standard deviation for IgA, $IgG_1$ and $IgG_2$ were 0.32±0.47, 1.50±0.57, and 1.18±0.72 respectively, expressed as serial serum dilutions. This is statistically much lower than that seen in the control groups, and corresponds closely with equivalent results in Part I.

Within the control, or treatment groups, there was minimal variation in serum immunoglobulin levels, across all heavy chain classes.

### B. TUMORS

1. P-815-X2

Unfortunately, the challenging dose of viable tumor cells failed to take, both in the control and treatment groups.

2. T1699

In order to clarify results, the growth pattern of each individual neoplasm has been plotted (Figure 15). Again, because of the wide varia-

tion encountered, no attempt to plot mean values was made. These will be described in conjunction with the tabled data (Table 6).

a. Control Animals

When the incidence of tumor take, and the latency period, are considered relative to the dose-response relationship previously described in detail (Table 3), it appeared that the unimmunized males received an equivalent challenge dose of between $10^4$ and $10^5$ viable tumor cells. The females have a somewhat shorter latency period but did not appear to have an increased number of takes. Their effective challenge dose was probably within the same range.

Upon immunization with tumor tissue, the males had a decrease both in the incidence of tumor takes, and latency period. This was most noticeable when an extract of T1699 was employed as the immunizing agent. The females demonstrated no real change in these parameters.

This was suggestive of enhancement in the males, but not in the females. Further evidence came from the growth patterns of the individual tumors. Those males not immunized, or immunized with extracts of normal tissue demonstrated all three growth patterns: progressors, progressors with regression, and regressors. When extracts of tumor tissue were employed there was a shift toward progressors, and this was most marked with T1699 immunization. The females followed a similar pattern, although there appeared to be no difference between P-815-X2 and T1699 in effecting a shift towards progression.

b. Anti-IgM Treated Animals

When the incidence of takes, and latency period were compared with the standard dose-response relationship (Table 3), the unimmunized group behaved like a challenge of $10^4$ - $10^5$ viable cells. This compares with the challenge dose of the untreated controls previously discussed. Upon immunization there was no marked change in takes, or latency observed. This indicated that there was not a shift to behaviour resembling a larger tumor cell challenge. This was in contrast to the untreated animals where a change was noted. Individual growth patterns could not be employed for clarification, because of the smaller numbers of progressors only.

## CHAPTER IV - DISCUSSION

### A. IMMUNOSUPPRESSION

In order to achieve the same degree of panspecific immunosuppression as was detailed in Part I, the same preparation of anti-IgM, utilizing identical dosage, route and schedule, was employed. To compare efficacy of the technique a non-invasive parameter, that of serum immunoglobulin levels, was assayed. Comparison of results (Table 1 and Table 6) revealed almost identical values. As testing was performed at the same number of days post-natally, the other aspects of immunosuppression measured in Part I, would be expected to apply. They, however, were not measured in Part III, because of their invasive nature. There are, though, several other relevant points. Day 35 was chosen for assay because it fulfilled certain criteria: it was two weeks post-weaning so that

transplacental, and breast feeding influences would be minimized; it coincided temporally the primary response to SRBC, and other immunization procedures; it represented the status of the animal immediately prior to tumor challenge. While the primary immune response to SRBC was found to be affected (at least the IgM portion), this was not documented for the other immunizing methods. Certainly, the behaviour of the other immunoglobulin classes cannot be surmised only from their serum concentrations. Of note is the IgG levels, which were decreased, but never completely suppressed.

The other concern was that these values reflected only one point in time. To assess immune status during tumor growth would require sequential studies. Instead it was decided to achieve a state of panspecific immunosuppression prior to tumor challenge, and then observe tumor growth to ascertain if any effect was noted. If so, further investigation would be warranted.

B. TUMORS

1. P-815-X2

The significance of the complete lack of tumor growth was not known. As it occurred with both the control and treatment groups, it probably reflected some technical probelm in tumor transference. Certainly no speculation was possible.

2. T1699

This neoplasm did take, and although the variations in growth patterns were subtle did indicate possible trends.

a. Control Animals

Essentially, this was a repetition of Part II with added variations. In the males, no change in growth was observed when immunization was performed with extracted syngeneic normal tissue. When tumor tissue was employed, tumor growth mimicked a slightly higher cell challenge. This occurrence, when the immunizing material was an extract of P-815-X2, indicated a possible cross-reactivity between tumors. The mechanism cannot be surmised.

In the females, any change in tumor growth was less obvious, and no cross-reactivity appeared to exist. Whether this sex difference is due to estrogen dependence of T1699 is unknown. These controls established that T1699 does have definite growth characteristics in syngeneic mice. The change of pattern following immunization was also demonstrated. Again there was a trend toward growth characteristics indicative of a larger tumor cell challenge.

b. Anti-IgM Treated Animals

That there was no apparent difference between the unimmunized and immunized animals in their tumor growth patterns was evident. Since the cell challenge was of an equivalent size to the untreated animals above, a suggestion of a trend towards growth characteristic of a greater cell challenge would be expected within the anti-IgM treated, immunized group. This, however, was not observed. Since all animals were male this possibility was increased. However, any definitive statement would be, most certainly,

premature. No attempt to relate this to the abrogation, or prevention of enhancement will be made.

## CHAPTER V - SUMMARY

The ability of anti-IgM to panspecifically suppress a murine model was further demonstrated. This appeared to be consistent and reproducible.

Unforunately, secondary to technical problems, the tumor P-815-X2 failed to take in any group. This resulted in T1699 being left as the only experimental tumor. Again, only a tenuous suggestion of a shift to growth characteristics of a greater cell challenge could be implied from this model.

Therefore, when synthesis of the above two systems was attempted, even less reliable results were obtained. A vague suggestion of the possibility for prevention of the above shift could be entertained. However, any definitive statement would require more concrete evidence. Perhaps this would be most readily achieved by employing larger numbers of experimental animals, within an allogeneic rather than a syngeneic model.

# REFERENCES

1. Abrahams, S., Phillips, R. A., Miller, R. G.

Proc. Soc. Exp. Biol. Med., 141:1073, 1972.

2. Adler, F. L.

Progr. in Allergy, 8:41, 1964.

3. Alexander, J. W., Good, R. A.

Immunobiology for Surgeons, W. B. Saunders Company, Philadephia, pg. 176-177, 1970.

4. Andersson, B., Wigzell, H., Klein, G.

Transplantation, 5:11, 1967.

5. Arnason, B. G., De Vaux St.-Cyr, C., Relyveld, E. H.

Int. Arch. Allergy App. Immunol., 25:206, 1964.

6. Baker, P. J., Barth, R. F., Stashak, P. W., Amsbaugh, D. F.

J. of Immunol., 104:1313, 1970.

7. Baldwin, R. W., Glaves, D.

Clin. Exp. Immunol., 11:51, 1972.

8. Bankhurst, A. D., Warner, N. L.

J. of Immunol., 107:368, 1971.

9. Bansal, S. C., Sjogren, H. O.

Nature (New Biol.), 233:76, 1971.

10. Bansal, S. C., Hargreaves, R., Sjogren, H. O.

Int. J. Cancer, 9:97, 1972.

11. Bansal, S. C., Sjogren, H. O.

Int. J. Cancer, 9:490, 1972.

12. Barth, R. F.

J. of Immunol., 103:648, 1968.

13. Basten, A., Miller, J. F. A. P., Sprent, J., Pye, J.

J. Exp. Med., 135:610, 1972.

14. Basten, A., Warner, N. L., Mandel, T.

J. Exp. Med., 135:627, 1972.

15. Baum, J., Lieberman, G., Frenkel, E. P.

J. of Immunol., 102:187, 1969.

16. Baur, S., Vitetta, E. S., Sherr, C. J., Schenkein, I., Uhr, J. W.

J. of Immunol., 106:1133, 1971.

17. Bazin, H., Micklem, H. S.

Nature, 215:742, 1967.

18. Bazin, H., Malet, F.

Immunology, 17:345, 1969.

19. Bazin, H., Levi, G., Heremans, J. F.

Immunology, 20:563, 1971.

20. Bazin, H., Maldague, P., Schonne, E., Crabbe, P. A., Bauldon, H., Heremans, J. F.

Immunology, 20:571, 1971.

21. Brawn, R. J.

Proc. Nat. Acad. Sci. (U. S. A.), 68:1634, 1971.

22. Brody, T., Papermaster, B. W.

J. of Immunol. 105:139, 1970.

23. Brown, E.G., Potter, C.W., Oxford, J.S.
Europ. J. Cancer, 8:9, 1972.

24. Brunner, K.T., Mauel, J.-C., Cerottini, J.-C., Chapuis, B.
Immunology, 14:181, 1968.

25. Brunner, K.T., Mauel, J.-C., Ruoulf, H., Chapuis, B.
Immunology, 18:501, 1970.

26. Bubenik, J., Koldovsky, P.
Folio. Biol., 11:258, 1965.

27. Bubenik, J., Ivanyi, J., Koldovsky, P.
Folio. Biol., 11:426, 1965.

28. Bubenik, J., Perlmann, P., Helmstein, K., Moberger, G.
Int. J. Cancer, 5:310, 1970.

29. Casey, A.E., Scott, G.M.
Proc. Soc. Exp. Biol. Med., 29:816, 1932.

30. Cerottini, J.-C., Nordin, A.A., Brunner, K.T.
Nature, 227:72, 1970.

31. Cerottini, J.-C., Nordin, A.A., Brunner, K.T.
Nature 288:1308, 1970.

32. Claman, H.N., Chaperon, E.A., Triplett, R.F.
Proc. Soc. Exp. Biol. Med., 122:1167, 1966.

33. Cole, L.J., Maki, S.E.
Nature (New Biol.), 230:244, 1971.

34. Coons, A. H.

In "General Cytochemical Methods", Danielli, J. F. (Ed.), Academic Press, New York, Vol. 1, pg. 399, 1958.

35. Cooper, M. D., Peterson, R. D. A., South, M. A., Good, R. A.

J. Exp. Med., 123:75, 1966.

36. Cooper, M. D., Cain, W. A., Van Alten, P. J., Good, R. A.

Int. Arch. Allergy, 35:242, 1969.

37. Cooper, M. D., Lawton, A. R., Kincade, P. W.

Clin. Exp. Immunol., 11:143, 1972.

38. Cruse, J. M., Germany, W. W., Dulaney, A. D.

Lab. Investigation, 14:1554, 1965.

39. Day, E. D., Kaliss, N., Aronson, A. I., Bryant, B. F., Friendly, D., Gabrielson, F. C., Smith, P. M.

J. Nat. Cancer Inst., 15:145, 1954.

40. Dray, S.

Nature, 195:677, 1962.

41. Dunn, T. B., Potter, M.

J. Nat. Cancer Inst., 18:587, 1957.

42. Elson, C. J., Singh, J., Taylor, R. B.

Scand. J. Immunol., 2:143, 1973.

43. Ehrlich, P.

In "Immunology and Cancer Research", Himmelweit, F. (Ed.), Pergamon Press, London, Vol., 2, pg. 550, 1957.

44. Fahey, J. L., Wunderlich, J., Mishell, R.

J. Exp. Med., 120:223, 1964.

45. Fahey, J. L., Wunderlich, J., Mishell, R.

J. Exp. Med., 120:243, 1964.

46. Fahey, J. L., Sell, S.

J. Exp. Med., 122:41, 1965.

47. Feldmann, M.

J. Exp. Med., 135:735, 1972.

48. Feldmann, M.

J. Exp. Med., 136:582, 1972.

49. Flexner, S., Jobling, J. W.

Proc. Soc. Exp. Biol. Med., 4:156, 1906.

50. Flexner, S., Jobling, J. W.

Proc. Soc. Exp. Biol. Med., 5:16, 1907.

51. Foley, E. J.

Cancer Res., 13:835, 1953.

52. French, M. E., Batchelor, J. R.

Lancet, 2:1103, 1969.

53. Fuji, H., Jerne, N. K.

Ann. Inst. Pasteur, 117:801, 1969.

54. Gershon, R. K., Kondo, K.

J. of Immunol., 106:1524, 1971.

55. Gershon, R.K., Kondo, K.

J. of Immunol., 106:1531, 1971.

56. Glick, B., Chang, T.S., Jaap, R.G.

Poultry Sci., 35:224, 1956.

57. Gorer, P.A.

Adv. in Cancer Res., 4:149, 1956.

58. Gorer, P.A., Kaliss, N.

Cancer Res., 19:824, 1959.

59. Greenwood, F.C., Hunter, W.M., Glover, J.S.

Biochem. J., 89:114, 1963.

60. Gregory, C.J., Lajtha, L.G.

Int. J. Rad. Biol., 17:117, 1970.

61. Greaves, M.F., Torrigiani, G., Roitt, I.M.

Nature, 222:885, 1969.

62. Gross, L.

Cancer Res., 3:770, 1943.

63. Grosser, N.

M.Sc. Thesis, University of Toronto, 1973.

64. Ha, T.-Y., Waksman, B.H., Treffers, H.P.

J. Exp. Med., 139:13, 1974.

65. Harris, N.S., Jagarlamoody, S.M., McKhann, C.F., Najarian, J.S.

J. of Immunol., 108:958, 1972.

66. Hart, D.A., Wang, A., Pawlak, L., Nisonoff, A.

J. Exp. Med., 135:1293, 1972.

67. Hartmann, K.-U., Reeg, S., Mehner, C.
Immunology, 20:29, 1971.

68. Hellstrom, I., Hellstrom, K.E., Evans, C.A., Heppner, G.H., Pierce, G.E., Yang, J.P.S.
Proc. Nat. Acad. Sci. (U.S.A.), 62:362, 1969.

69. Hellstrom, I., Hellstrom, K.E.
Int. J. Cancer, 5:195, 1970.

70. Hellstrom, I., Sjogren, H.O., Warner, G., Hellstrom, K.E.
Int. J. Cancer, 7:226, 1971.

71. Hellstrom, I., Hellstrom, K.E., Sjogren, H.O., Warner, G.A.
Int. J. Cancer, 8:185, 1971.

72. Hellstrom, K.E., Hellstrom, I.
Ann. Rev. Microbiol., 24:373, 1970.

73. Heppner, G.H.
Int. J. Cancer, 4:608, 1969.

74. Herrod, H.G., Warner, N.L.
J. of Immunol., 108:1712, 1972.

75. Herzenberg, L.A., Chan, E.L., Ravitch, M.M., Riblet, R.J., Herzenberg, L.A.
J. Exp. Med., 137:1311, 1973.

76. Holmes, E.C., Kahan, B.D., Morton, D.C.
Cancer, 25:373, 1970.

77. Humphrey, J.H., Parrott, D.M.V., East, J.
Immunology, 7:419, 1964.

78. Irvin III, G. L., Eustace, J. C., Fahey, J. L.
J. of Immunol., 99:1085, 1967.

79. Jackson Laboratory
In "Handbook on Genetically Standardized JAX Mice", Bar Harbor, Maine, 1971.

80. Jacobson, E. B., Herzenberg, L. A.
J. Exp. Med., 135:1151, 1972.

81. Jacobson, E. B., Herzenberg, L. A., Riblet, R., Herzenberg, L. A.
J. Exp. Med., 135:1163, 1972.

82. Jagarlamoody, S. M., McKhann, C. F.
Fed. Proc., 31:767, 1972.

83. Jose, D. G., Skuaril, F.
Int. J. Cancer, 13:173, 1974.

84. Kahan, B. D.
J. of Immun., 99:1121, 1967.

85. Kaliss, N., Molomut, N.
Cancer Res., 12:110, 1952.

86. Kaliss, N.
Cancer Res., 12:379, 1952.

87. Kaliss, N., Molomut, N., Harriss, J. L., Gault, S. O.
J. Nat. Cancer Inst., 13:847, 1953.

88. Kaliss, N.
Cancer Res., 18:992, 1958.

89. Kaliss, N.

Ann. N.Y. Acad. Sci. 129:155, 1966.

90. Kalpaktsoglou, P.K., Hong, R., Good, R.A.

Immunology, 24:303, 1973.

91. Katz, D.H., Benacerraf, B.

Adv. in Immunol., 15:1, 1972.

92. Katz, D.H., Paul, W.E., Goidl, E.A., Benacerraf, B.

J. Exp. Med., 132:261, 1970.

93. Katz, D.H., Unanue, E.R.

J. of Immunol., 109:1022, 1972.

94. Kerbel, R.S., Eidinger, D.

J. of Immunol., 106:917, 1971.

95. Kettman, J., Dutton, R.W.

Proc. Nat. Acad. Sci. (U.S.A.), 68:699, 1971.

96. Kincade, P.W., Lawton, A.R., Bockman, D.E., Cooper, M.D.

Proc. Nat. Acad. Sci. (U.S.A.), 67:1918, 1970.

97. Kincade, P.W., Cooper, M.D.

J. of Immunol., 106:371, 1971.

98. Kincade, P.W., Lawton, A.R., Cooper, M.D.

J. of Immunol., 106:1421, 1971.

99. Kincade, P.W., Cooper, M.D.

Science, 179:398, 1973.

100. Kinsky, R., Chouroulinkou, I., Voisin, G.A.
Transplantation, 10:450, 1970.

101. Klein, G., Sjogren, H.O., Klein, E.
Cancer Res., 23:84, 1963.

102. Law, L.W., Apella, E.
Nature, 243:83, 1973.

103. Lawton III, A.R., Asofsky, R., Hylton, M.B., Cooper, M.D.
J. Exp. Med., 135:277, 1972.

104. Lawton, A., Asofsky, R., Tigelaar, R., Hylton, M., Cooper, M.
Fed. Proc., 31:751, 1972.

105. Lees, R.K., Sinclair, N.R.S.
Immunology, 24:735, 1973.

106. Lerner, K.G., Glick, B., McDuffie, F.C.
J. of Immunol., 107:493, 1971.

107. Lesley, J., Dutton, R.W.
Science, 169:487, 1970.

108. Lesley, J.F., Kettman, J.R., Dutton, R.W.
J. Exp. Med., 134:618, 1971.

109. Leslie, G.A., Martin, L.N.
J. of Immunol., 110:959, 1973.

110. Leslie, G.A., Martin, L.N.
Int. Arch. All. App. Immunol., 45:429, 1973.

111. Levey, R.H., Medawar, P.B.
Ann. N.Y. Acad. Sci., 129:164, 1966.

112. Litton Bionetics

In "Catalogue of Murine Plasma Cell Tumors". Compiled by Sibinovic, K. H., Potter, M. Kensington, Maryland. First Edition, pg. 4, 1973.

113. Mage, R., Dray, S.

J. of Immunol., 95:525, 1965.

114. Makela, O., Nossal, G. J. V.

J. Exp. Med., 115:231, 1962.

115. Mannick, J. A.

Surgery, 67:711, 1970.

116. Manning, D. D., Jutila, J. W.

J. of Immunol., 108:282, 1972.

117. Manning, D. D.

J. of Immunol., 109:1152, 1972.

118. Manning, D. D., Kouttab, N. M., Jutila, J. W.

J. of Immunol., 112:1698, 1974.

119. Martin, L. N., Leslie, G. A.

Proc. Soc. Exp. Biol. Med., 143:241, 1973.

120. Mason, S., Warner, N. L.

J. of Immunol., 104:762, 1970.

121. Mattioli, C. A., Tomasi, T. B.

In Preparation.

122. Mauel, J., Ruoulf, H., Chapuis, B.

Immunology, 18:517, 1970.

123. Medawar, P. B.

J. of Anat., 78:176, 1944.

124. Meltzer, M. S., Leonard, E. J., Rapp, H. J., Borsos, T.

J. of Nat. Cancer Inst., 47:703, 1971.

125. Michaelis, L.

Deut. Med. Wochenschr., 30:1240, 1904.

126. Miller, J. F. A. P.

Lancet, 2:748, 1961.

127. Miller, J. F. A. P., Mitchell, G. F.

J. Exp. Med., 128:801, 1968.

128. Miller, J. F. A. P., Mitchell, G. F.

Transplant. Rev., 1:3, 1969.

129. Mitchell, G. F., Miller, J. F. A. P.

J. Exp. Med., 128:821, 1968.

130. Mitchell, G. F., Mishell, R. I., Herzenberg, L. A.

In "Progress in Immunology", Amos, B. (Ed.), Academic Press, New York, pg. 323, 1971.

131. Mitchell, M. S.

Cancer Res., 32:825, 1972.

132. Mitchison, N. A., Rajewsky, K., Taylor, R. B.,

In "Developmental Aspects of Antibody Formation and Structure", Sterzl, J. and Pike, I. (Ed.), Academic Press, New York, Vol. 2, pg. 547, 1971.

133. Moller, E., Moller, G.

J. Exp. Med., 115:527, 1962.

134. Moller, G.

J. of Nat. Cancer Inst., 30:1153, 1963.

135. Moller, G.

J. of Nat. Cancer Inst., 30:1177, 1963.

136. Moller G.,

Transplantation, 2:281, 1964.

137. Moller, G.

Transplantation, 2:405, 1964.

138. Moller, G.

J. of Immunol., 96:430, 1966.

139. Moller, G., Sjoberg, O.

Cell. Immunol., 1:110, 1970.

140. Monaco, A.P., Wood, M.L., Gray, J.G., Russel, P.S.

J. of Immunol., 96:229, 1966.

141. Moore, M.A.S., Owen, J.J.T.

Dev. Biol., 14:40, 1966.

142. Moseley, R.V., Sheil, A.G.R., Mitchell, R.M., Murray, J.E.

Transplantation, 4:678, 1966.

143. Murgita, R.A., Mattioli, C.A., Tomasi, Jr., T.B.

J. Exp. Med., 138:209, 1973.

144. Nelson, D.S.

Brit. J. Exp. Path., 43:1, 1962.

145. Nossal, G. J. V., Cunningham, A., Mitchell, G. F., Miller, J. F. A. P.
J. Exp. Med., 128:839, 1968.

146. Nossal, G. J. V., Warner, N. L., Lewis, H., Sprent, J.
J. Exp. Med., 135:405, 1972.

147. Oettgen, H. F., Old, L. J., McLean, E. P., Carswell, E. A.
Nature, 220:295, 1968.

148. Owen, J. J. T., Raff, M. C.
J. Exp. Med., 132:1216, 1970.

149. Osmond, D. G., Nossal, G. J. V.
Cell. Immunol., 13:117, 1974.

150. Osmond, D. G., Nossal, G. J. V.
Cell. Immunol., 13:132, 1974.

151. Paul, W. E.,
Transplant. Rev., 5:130, 1970.

152. Pawlak, L. L., Hart, D. A., Nisonoff, A.
J. Exp. Med., 137:1442, 1973.

153. Pierce, C. W., Solliday, S. M., Asofsky, R.
J. Exp. Med., 135:675, 1972.

154. Pierce, C. W., Solliday, S. M., Asofsky, R.
J. Exp. Med., 135:698, 1972.

155. Potter, M.
In "Methods in Cancer Research", Busch, H. (Ed.), Academic Press, New York, Vol. II, pg. 105, 1967.

156. Potter, M.

Physiol. Rev., 52:631, 1972.

157. Prehn, R.I., Main, J.M.

J. of Nat. Cancer Inst., 18:769, 1957.

158. Pye, J.

Hall Institute, Melbourne, Australia. Personal Communication.

159. Radovich, J., Talmage, D.W.

Science, 158:512; 1967.

160. Ran, M., Witz, I.P.

Int. J. Cancer, 9:242, 1972.

161. Raff, M.C., Steinberg, M., Taylor, R.B.

Nature (London), 225:553, 1970.

162. Raff, M.C.

Immunology, 19:637, 1970.

163. Rabellino, E., Colon, S., Grey, H.M., Unanue, E.R.

J. Exp. Med., 133:156, 1971.

164. Reiner, J., Southam, C.M.

Cancer Res., 29:1814, 1969.

165. Reisfeld, R.A., Pellegrino, M.A., Kahan, B.D.

Science, 172:1134, 1971.

166. Riethmuller, G., Rieber, E.-P., Seeger, I.

Nature (New Biol.), 230:248, 1971.

167. Roelants, G., Forni, L., Pernis, B.
J. Exp. Med., 137:1060, 1973.

168. Rosenau, W., Morton, D. L.
J. of Nat. Cancer Inst., 36:825, 1966.

169. Rouse, B. T., Wagner, H., Harris, A. W.
J. of Immunol., 108:1353, 1972.

170. Ruffilli, A., Compere, A., Baglioni, C.
J. of Immunol., 104:1511, 1970.

171. Sell, S., Fahey, J. L.
J. of Immunol., 93:81, 1964.

172. Sell, S.
J. Exp. Med., 120:967, 1964.

173. Sell, S., Gell, P. G. H.
J. Exp. Med., 122:423, 1965.

174. Sherr, C. J., Baur, S., Grundke, I., Zelias, J., Zelias, B.
Uhr, J. W.
J. Exp. Med., 135:1392, 1972.

175. Simmons, R. L., Rios, A., Lundgren, G.
Surgery, 70:38, 1971.

176. Simmons, R. L., Rios, A.
J. of Nat. Cancer Inst., 51:637, 1973.

177. Sjogren, H. O., Hellstrom, I. E., Klein, G.
Exp. Cell Res., 23:204, 1961.

178. Smith, R. W., Morganroth, H. Mora, P. T.

Nature, 227:141, 1970.

179. Snell, G. D.

Cancer Res., 17:2, 1957.

180. Snell, G. D.

Ann. Rev. Microbiol., 11:439, 1957.

181. Snyder, G. B., Kinsky, R., Voisin, G. A.

Plastic Reconstr. Surgery., 33:110, 1964.

182. Sober, H., Peterson, E. A.

Fed. Proc., 17:1116, 1958.

183. Strober, S., Apellae, E., Law, L. W.

Proc. Nat. Acad. Sci. (U. S. A.), 67:765, 1970.

184. Stuart, F. P., Saitoh, T., Fitch, F. W.

Science, 160:1463, 1968.

185. Szenberg, A., Warner, N. L.

Nature (London), 194:146, 1962.

186. Takasugi, M., Hildemann, W. H.

J. of Nat. Cancer Inst., 43:843, 1969.

187. Takahashi, T., Old, L. J., Boyse, E. A.

J. Exp. Med., 132:1325, 1970.

188. Taylor, R. B., Duffus, W. D. H., Raff, M. C., De Petris, S.

Nature (New Biol.), 233:225, 1971.

189. Toivanen, P., Toivanen, A., Good, R.A.

J. of Immunol., 109:1058, 1972.

190. Tokuda, S., McEntee, P.F.

Transplantation, 5:606, 1967.

191. Unanue, E.R., Grey, H.M., Rabellino, E., Campbell, P., Schmidtke, J.

J. Exp. Med., 133:1188, 1971.

192. Vitetta, E.S., Baur, S., Uhr, J.W.,

J. Exp. Med., 134:242, 1971.

193. Voisin, G.A., Kinsky, R., Maillard, J.

Transplantation, 6:187, 1968.

194. Voisin, G.A.

Progr. in Allergy, 15:328, 1971.

195. Warner, N.L., Byrt, P., Ada, G.L.

Nature 226:942, 1970.

196. Wigzell, H., Makela, O.

J. Exp. Med., 132:110, 1970.

197. Wigzell, H., Walters, C.S.

J. Exp. Med., 132:1233, 1970.

198. Wortis, H.H., Dresser, D.W.

Immunology, 14:69, 1968.

199. Wortis, H.H., Dresser, D.W., Anderson, H.R.

Immunology, 17:93, 1969.

200. Wu, A. M., Till, J. E., Siminovitch, L., McCullogh, E. A. – J. Cell. Physiol., 69:177, 1967.

201. Zighelboim, J., Bonavida, B., Rao, S. V., Fahey, J. L. J. of Immunol., 112:433, 1974.

FIGURES

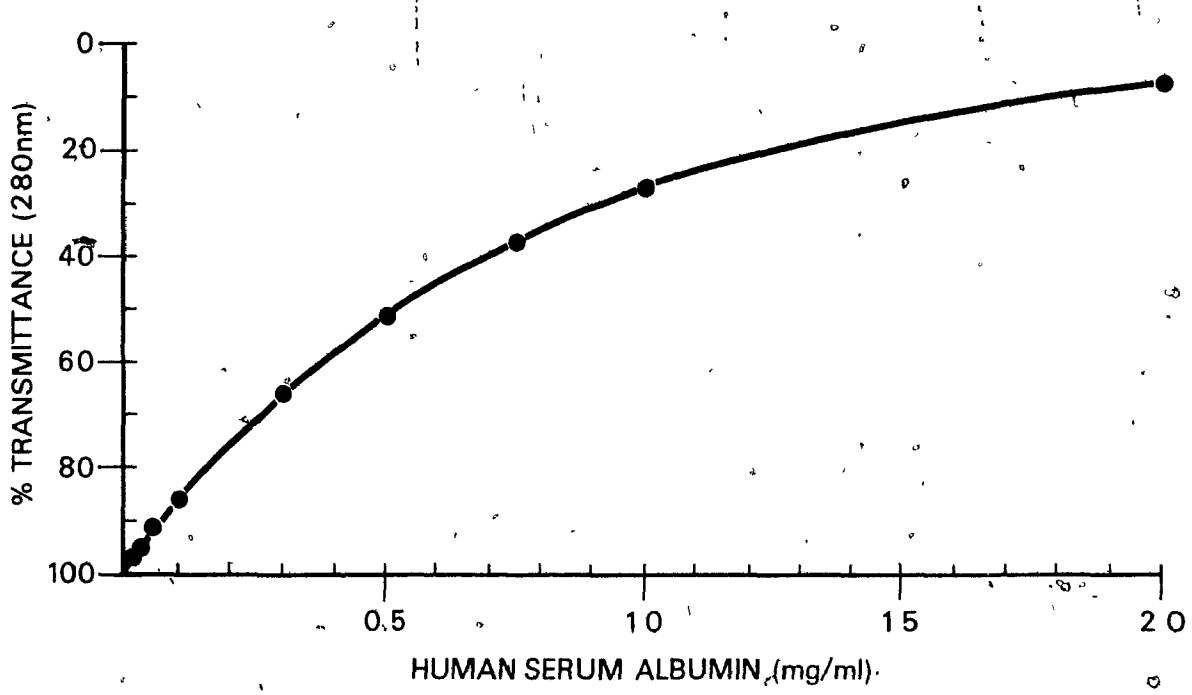



Figure I: STANDARD CURVE - Relationship between transmittance at 280 nm and protein concentration of a solution.

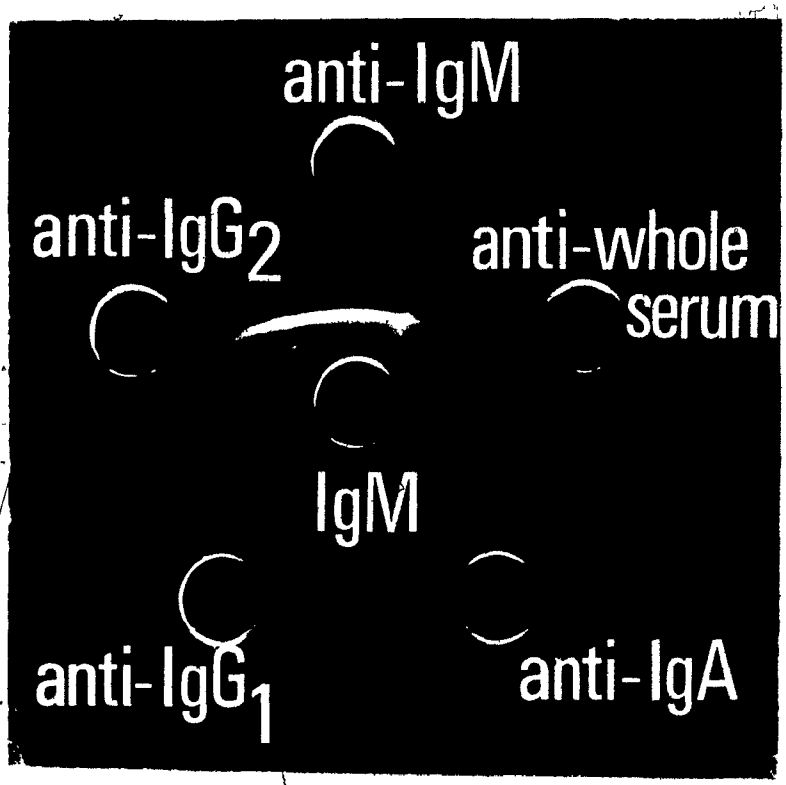
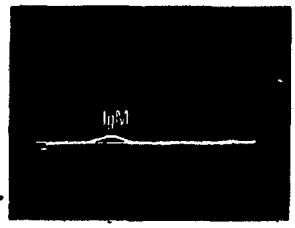
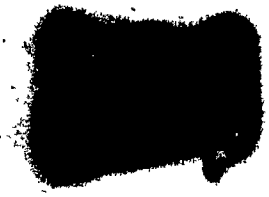



Figure 2A: IgM PURITY - Ouchterlony gel diffusion with class or subclass specific antisera.



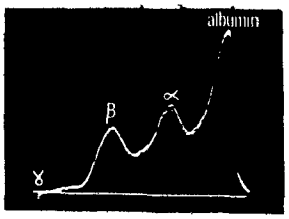



Figure 2B: IgM PURITY - Millipore electrophoresis of sample and normal mouse serum.

The only protein present in the sample is found in the β region. This is where IgM would be found.

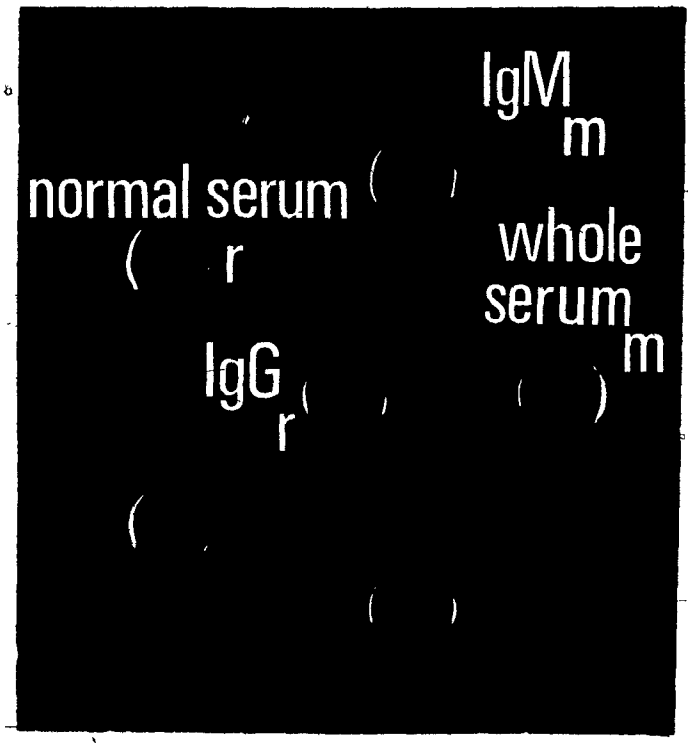



Figure 3A: ANTI-IgM PURITY – Ouchterlony gel diffusion.

A strong reaction is detected with IgM, but contaminants are seen with the whole serum.

m - mouse

r - rabbit

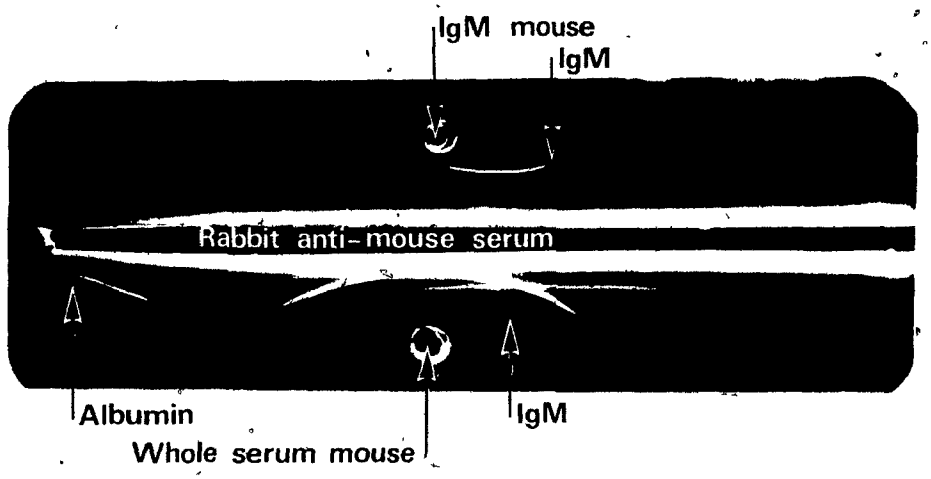



Figure 3B: ANTI-IgM PURITY - Immunoelectrophoresis.

The contaminants now can be partially identified. Probably there are antibodies against albumin. Some of the bands remain unidentified.

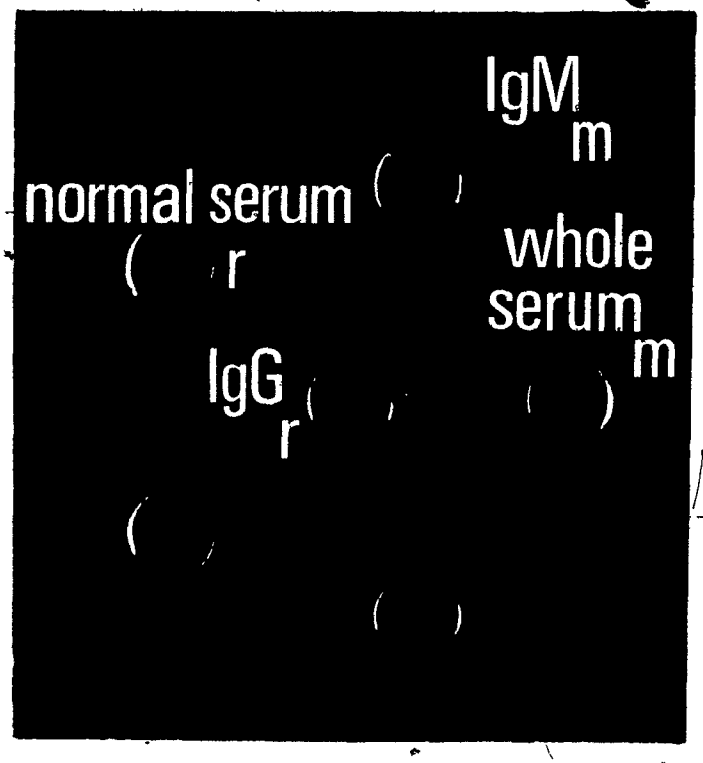



Figure 4: RADIOIODINATED ANTI-IgM PURITY - Ouchterlony gel diffusion. The impurities encountered previously in reaction with whole mouse serum are no longer detectable.

m - mouse

r - rabbit

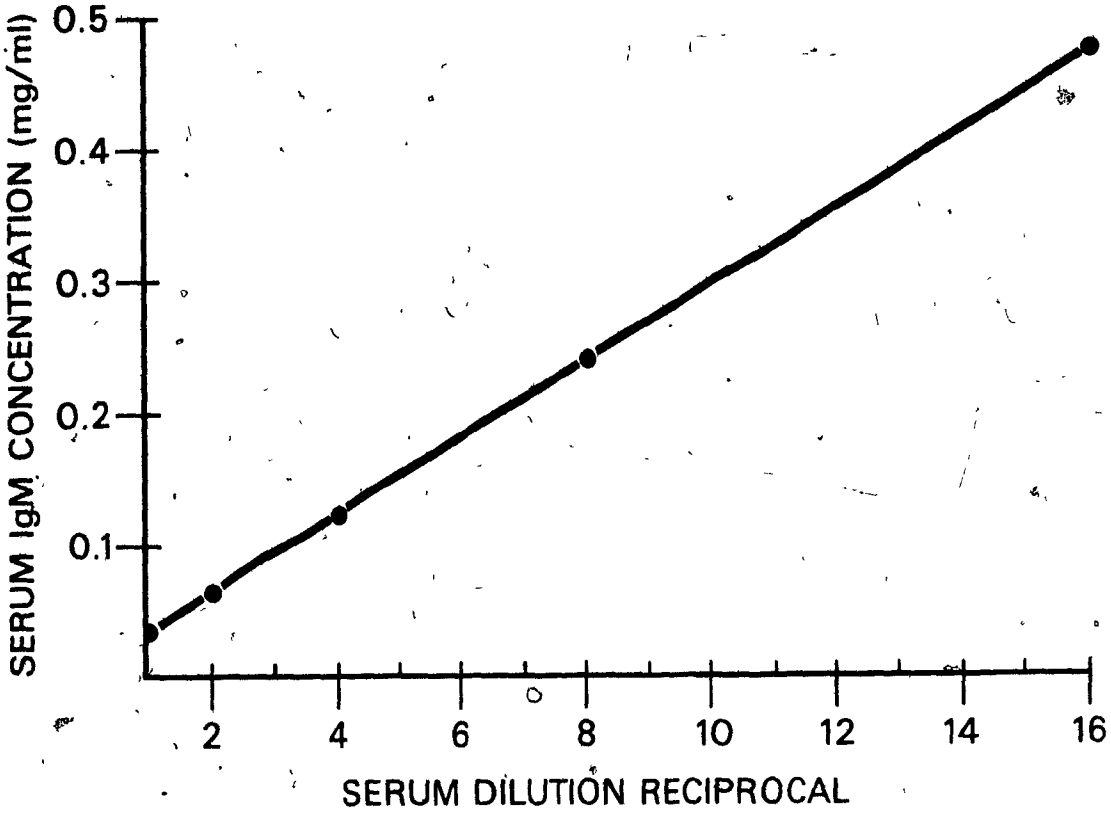



Figure 5: STANDARD CURVE - Relationship between serum concentration of IgM, and its detectability by the serial dilution technique. Note that the lowest level of detectability is 0.03 mg/ml.

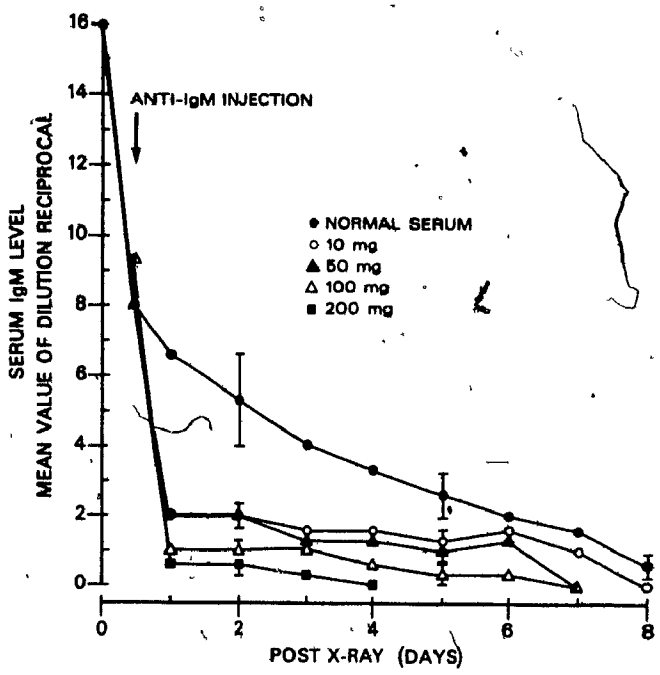
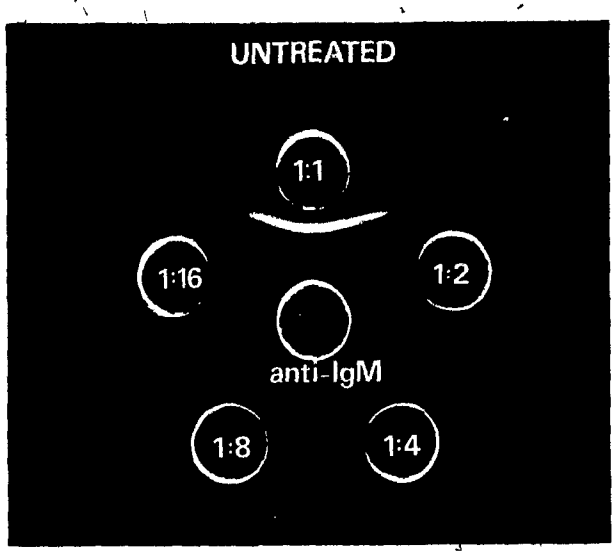



Figure 6A: ANTI-IgM EFFICACY - Mean values ± standard error of serum dilution reciprocals are recorded. Note that IgM $T_{1/2}$ is about 18 hours, and that 10 mg anti-IgM is almost as efficient as 200 mg.



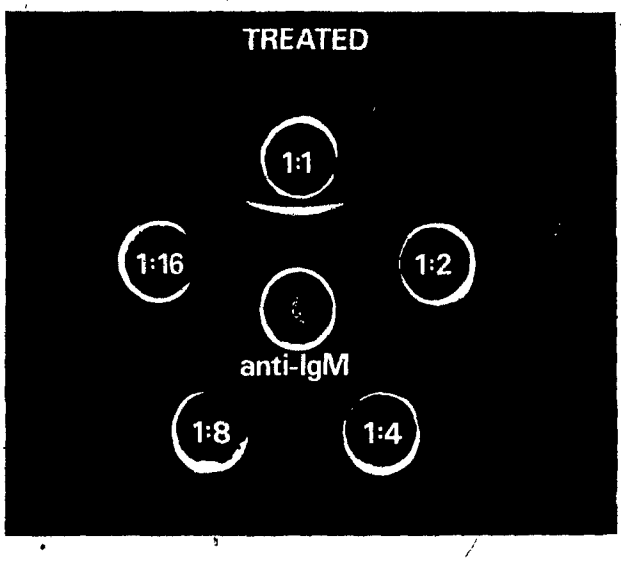




Figure 6B: ANTI-IgM EFFICACY -Ouchterlony gel diffusion of mouse treated with 200 mg, immediately before and four hours after anti-IgM injection.

A

B

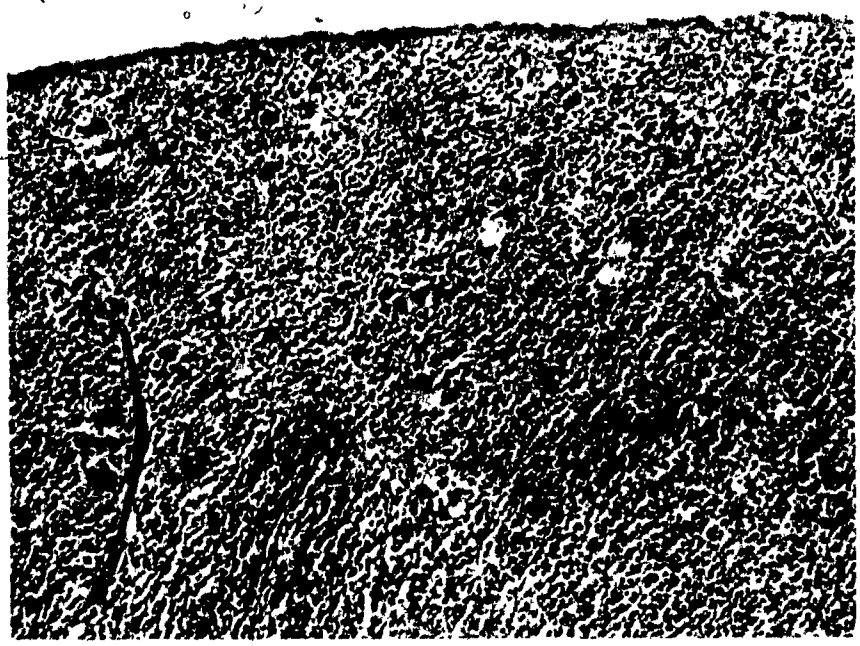


Figure 7: SPLENIC HISTOLOGY OF DBA/2 NEONATES (DAY 35 POST-BIRTH)

A. Control Group - Note the presence of germinal centres situated around blood vessels.

B. Treated Group - Here no germinal centres were present. Rather, there was probably an attempt to form a medullary cord.

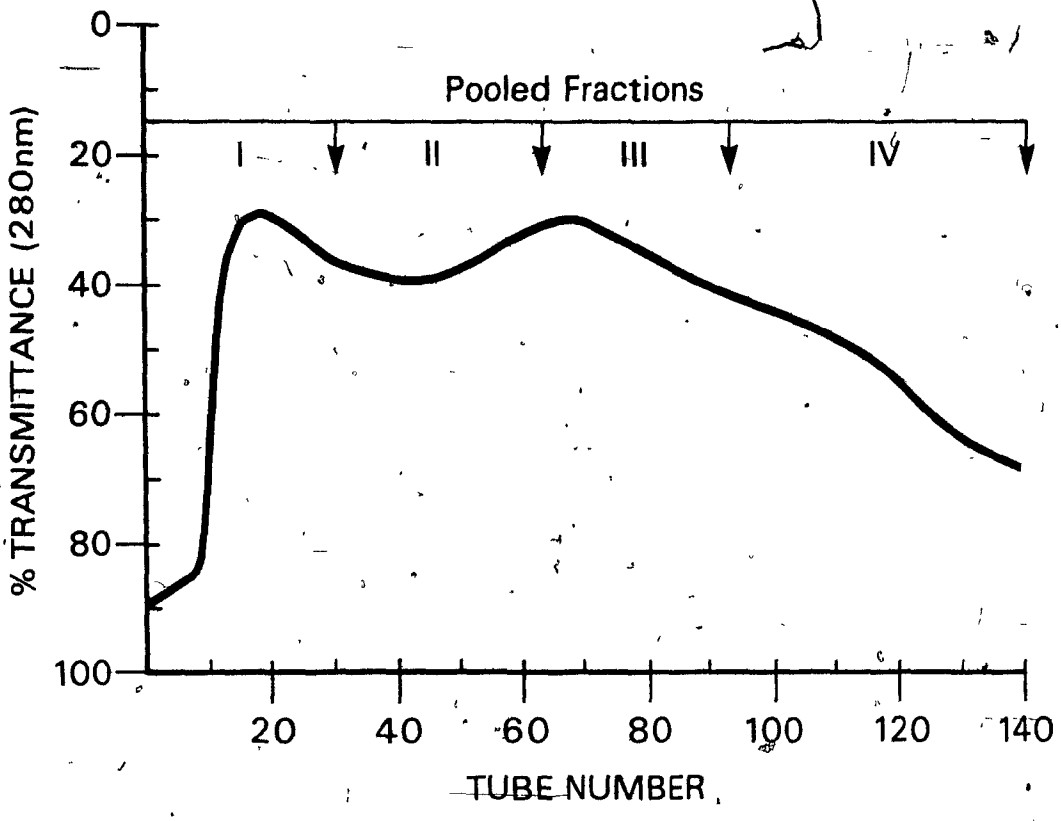



Figure 8: SEPHADEX G-200 FRACTIONATION - The tubes were pooled according to the fractions above, and then concentrated. Testing for TSA was then done by immunofluorescence.

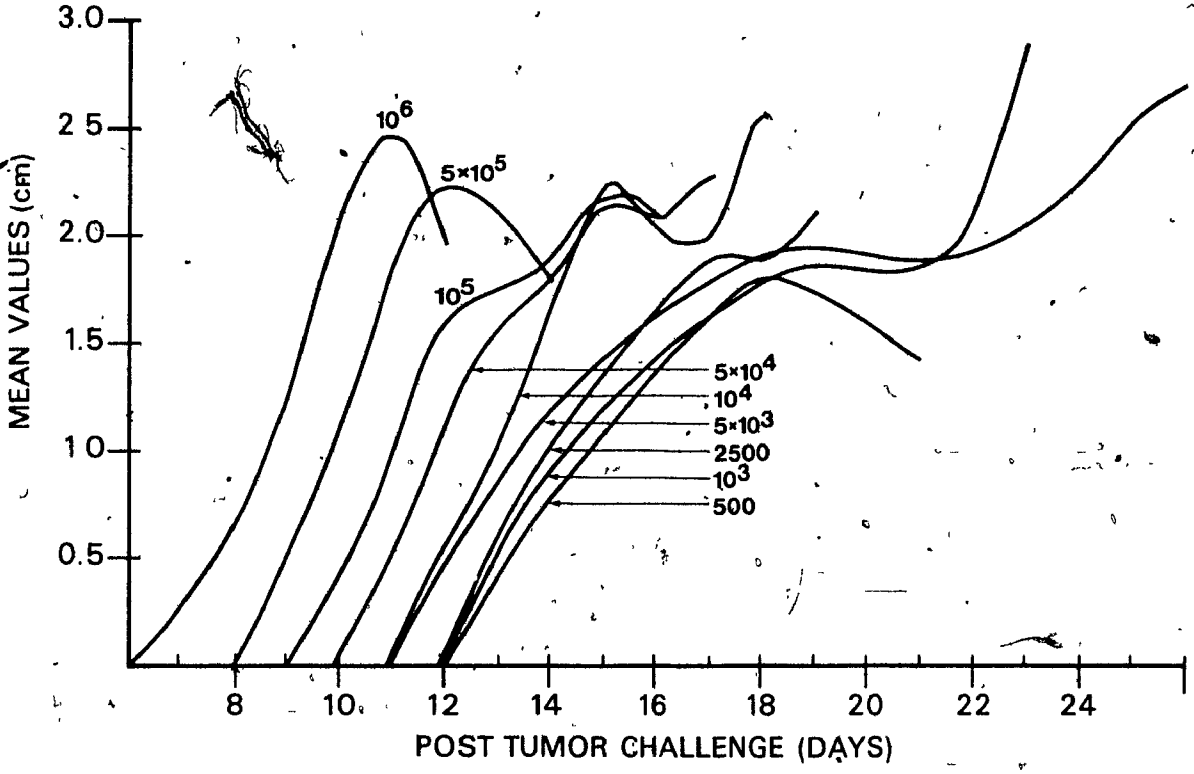



Figure 9: P-815-X2 DOSE-RESPONSE RELATIONSHIP - Note the consistency in slope and separation that is evident between 0.50 and 1.00 cm.

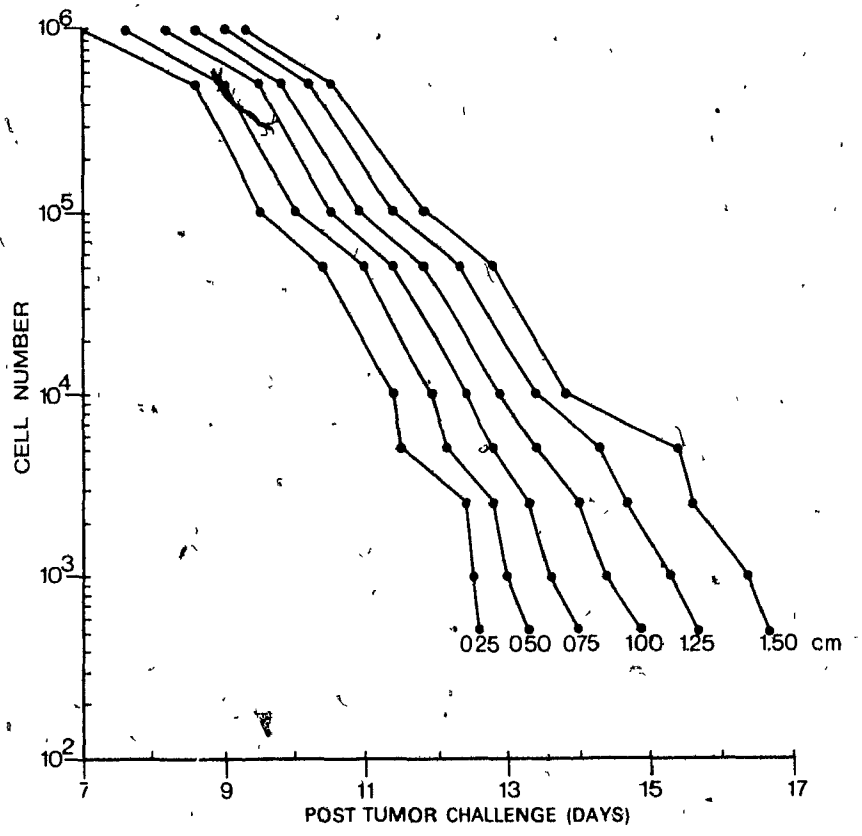



Figure 10: P-815-X2 STANDARD CURVE OF SIZE ISOBARS - This allowed tumor growth differences to be expressed as the number of tumor cell equivalents.

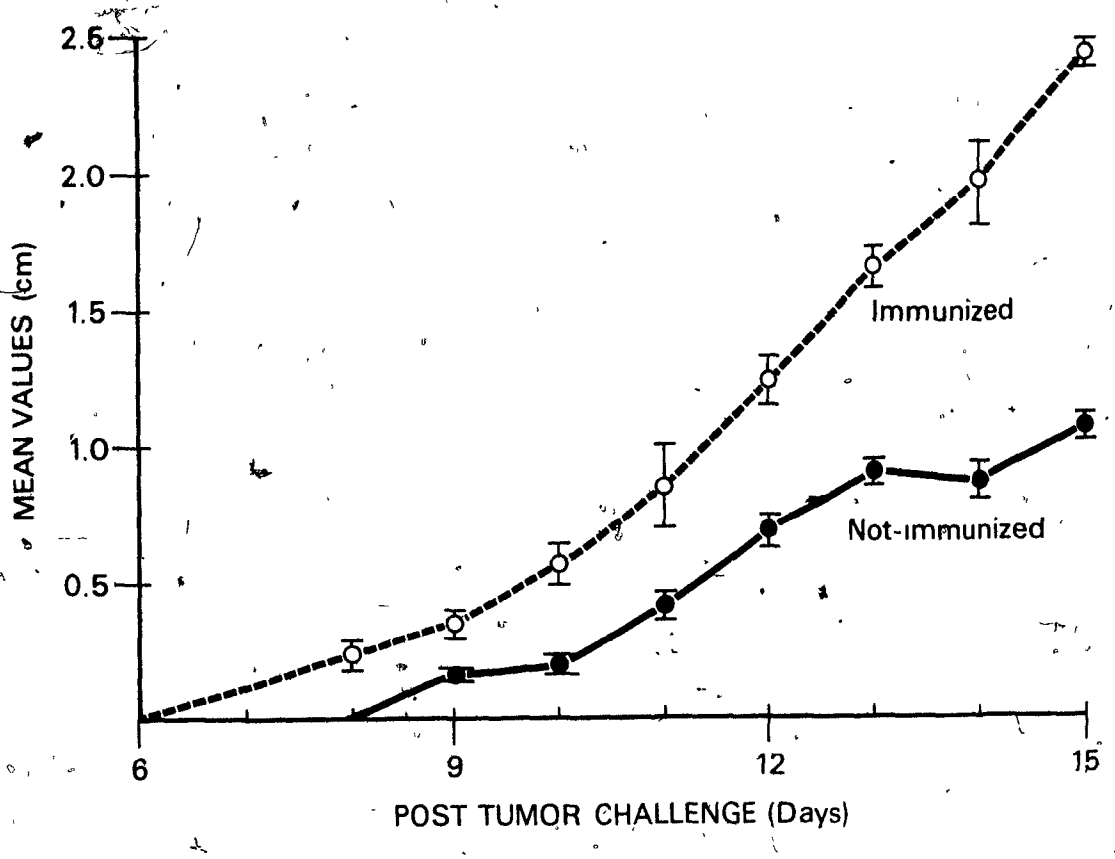



Figure 11: P-815-X2 ENHANCEMENT WITH TSA #1 - This corresponded to a challenge of 13,893 ± 8,586 cells for the non-immunized group, and 114,000 ± 33,246 cells for the immunized group.

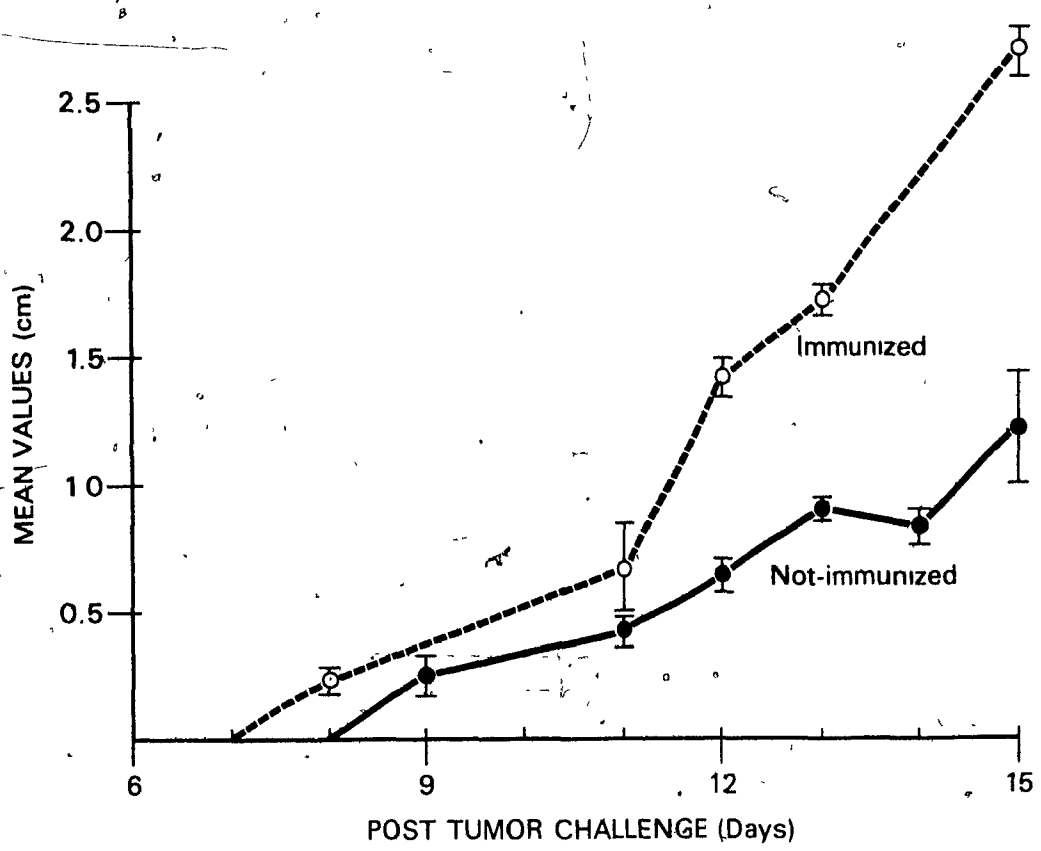



Figure 12: P-815-X2 ENHANCEMENT WITH TSA #2 - This corresponded to a challenge of 4,940 $\pm$ 2,125 cells for the non-immunized group, and 82,333 $\pm$ 16,343 cells for the immunized group.

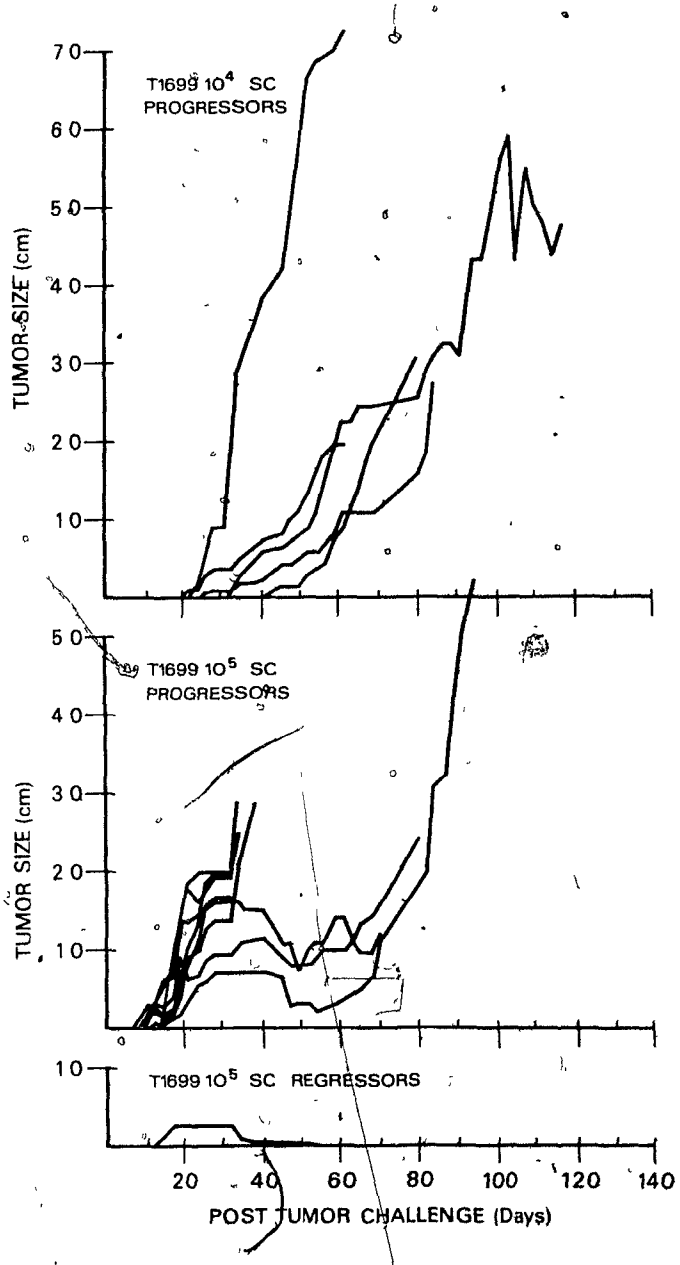



Figure 13:- T1699 DOSE-RESPONSE RELATIONSHIP

The values plotted represent individual tumor growth. Note that, at a challenge of $10^5$ cells SC in the milk line, three different growth patterns emerge.

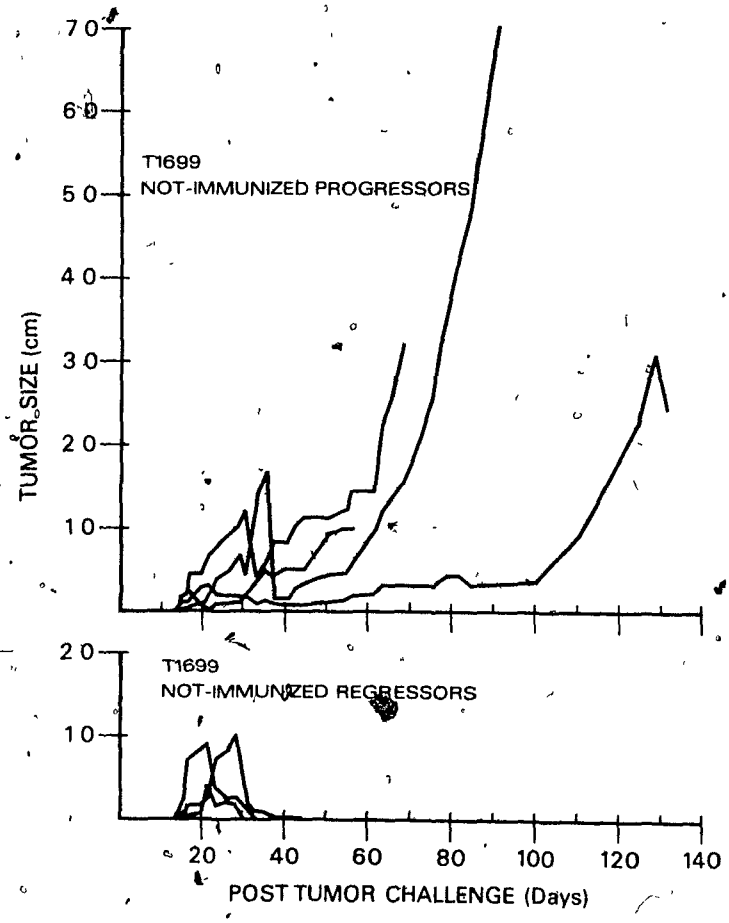



Figure 14A: T1699 ENHANCEMENT WITH TSA #2 - All three growth patterns are present within the non-immunized group.

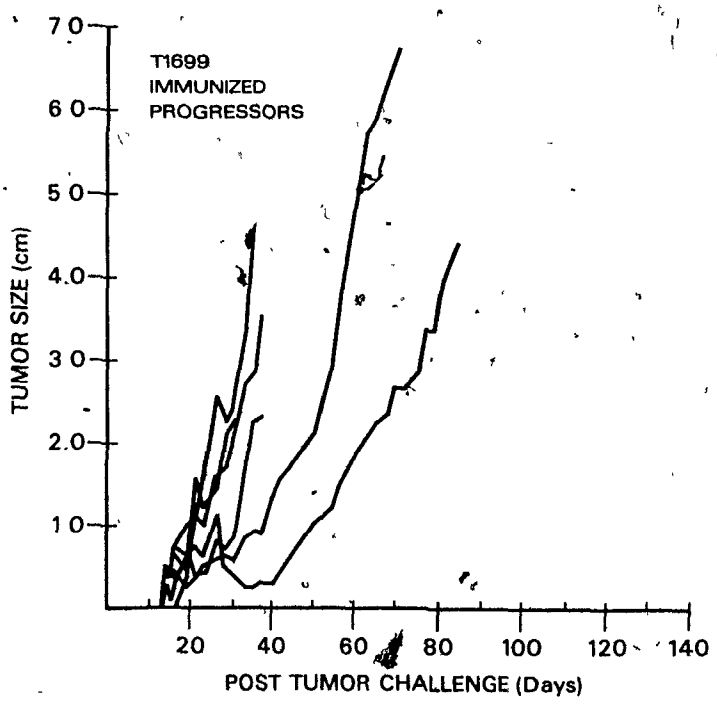
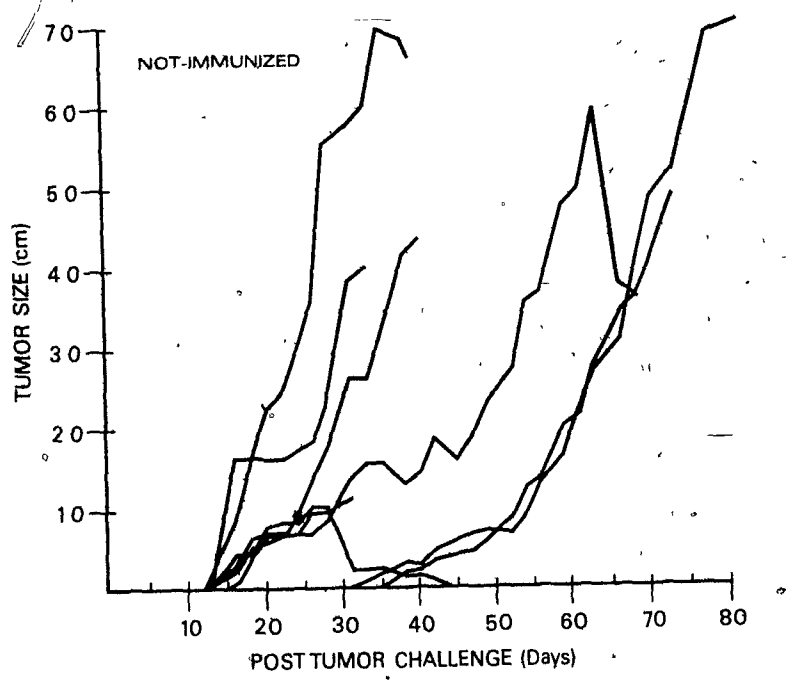



Figure 14B: T1699 ENHANCEMENT WITH TSA #2 - Note the subtle change in slope between the progressors in the control and treatment groups.

Figure 15: COMPARISON OF T1699 GROWTH PATTERNS IN CONTROL AND TREATMENT GROUPS

The immunizing material is indicated on each graph. All challenges were with T1699 $10^5$ viable cells SC in the right milk line.

Each line represents a single neoplasm.



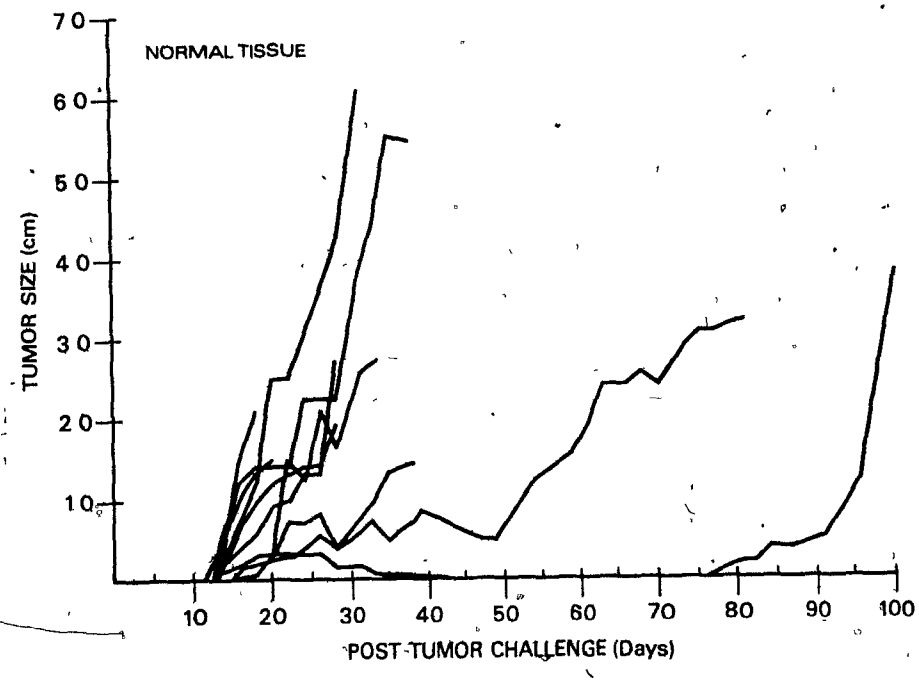
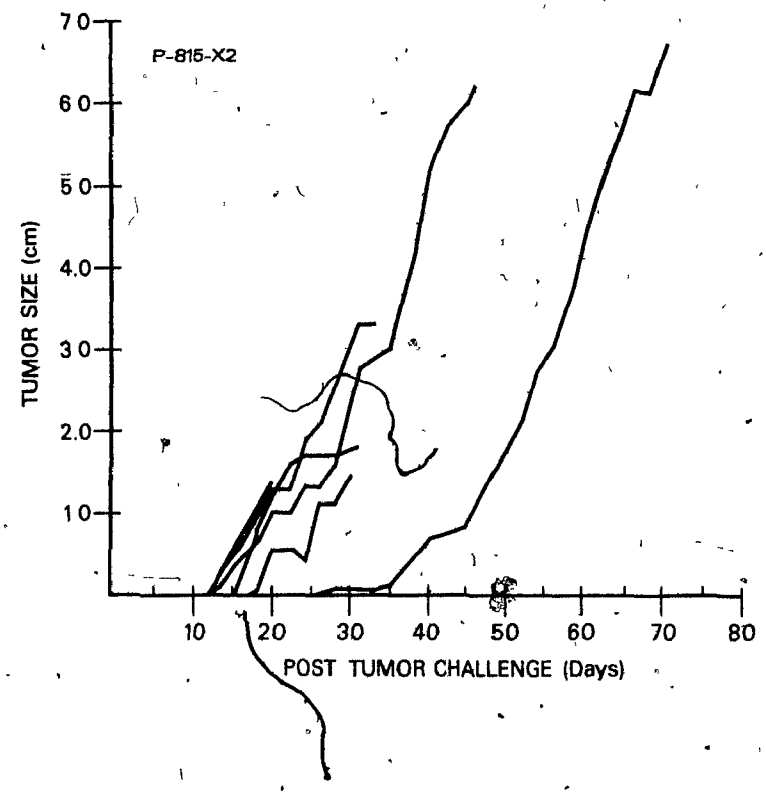




Figure 15: UNTREATED MALES



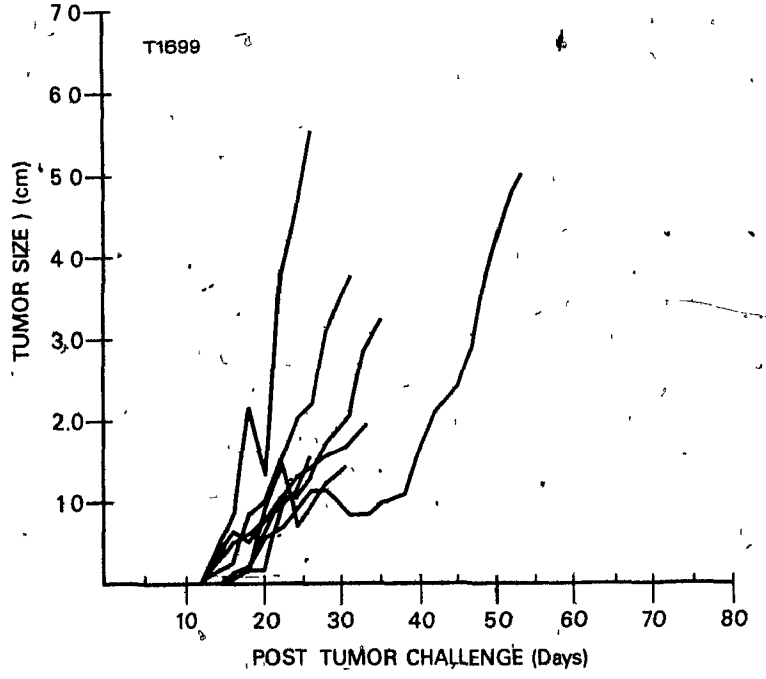
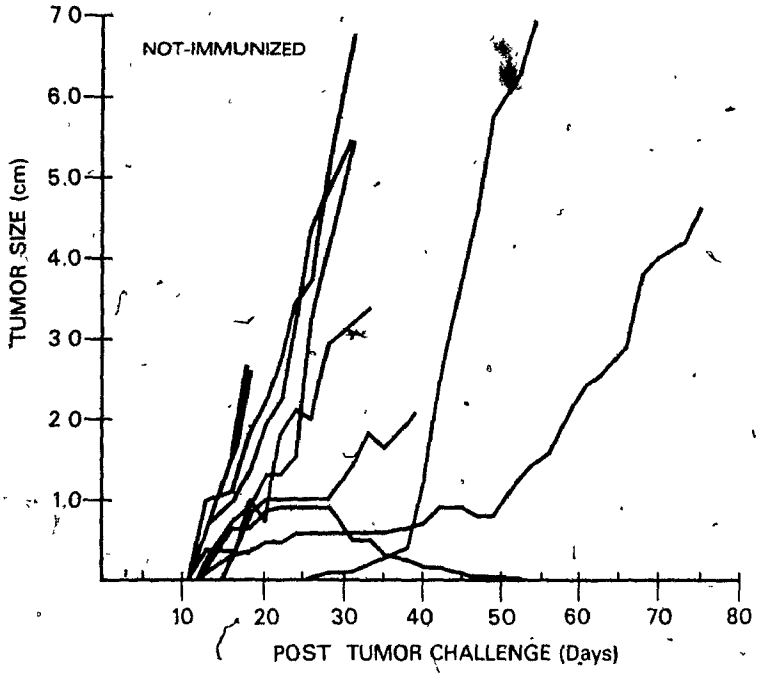




Figure 15: UNTREATED MALES



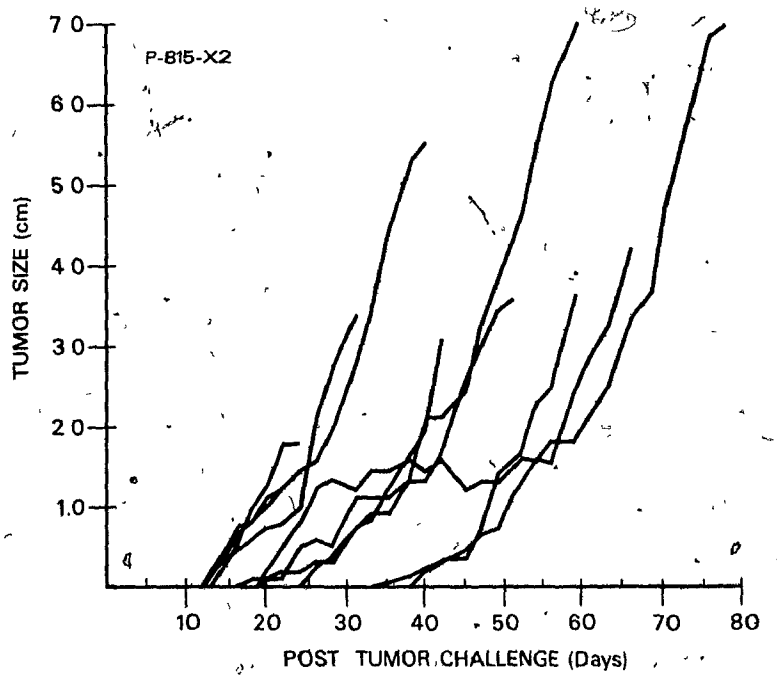




Figure 15: UNTREATED FEMALES

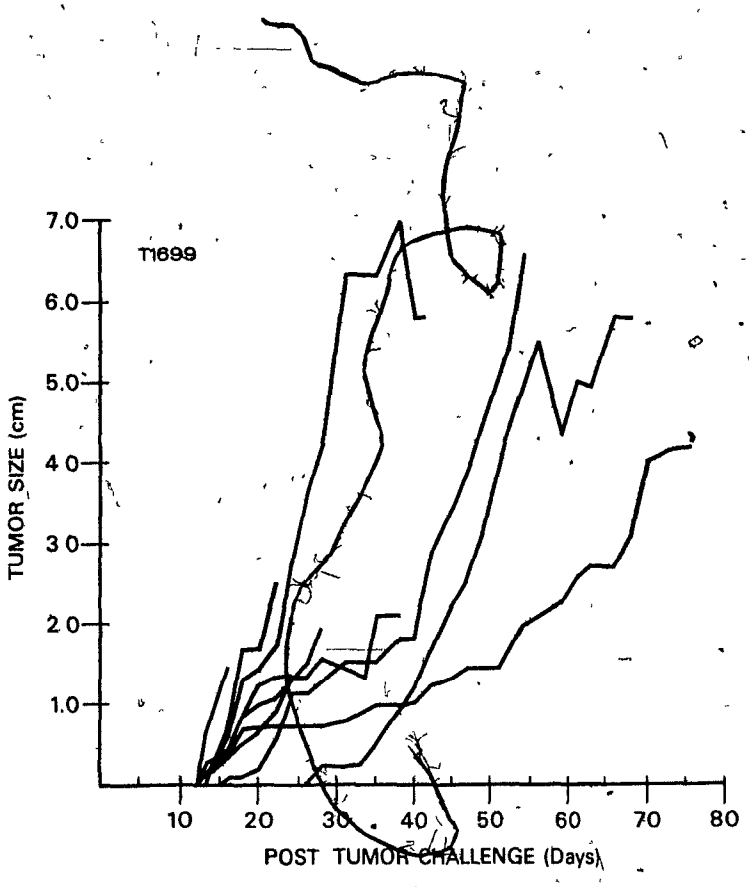
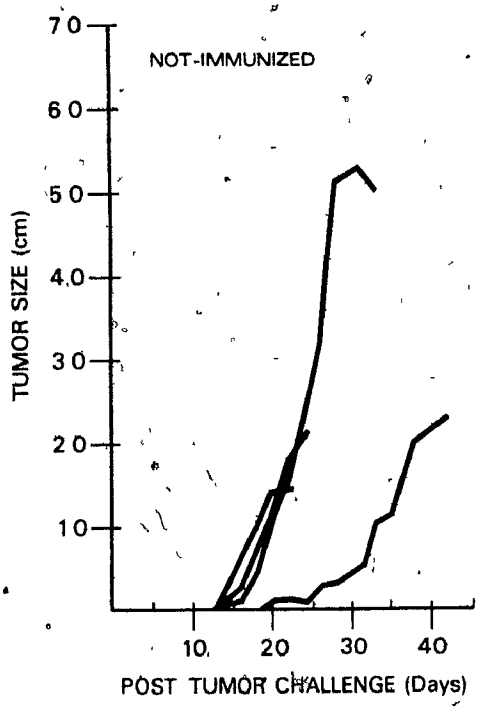



Figure 15: UNTREATED FEMALES



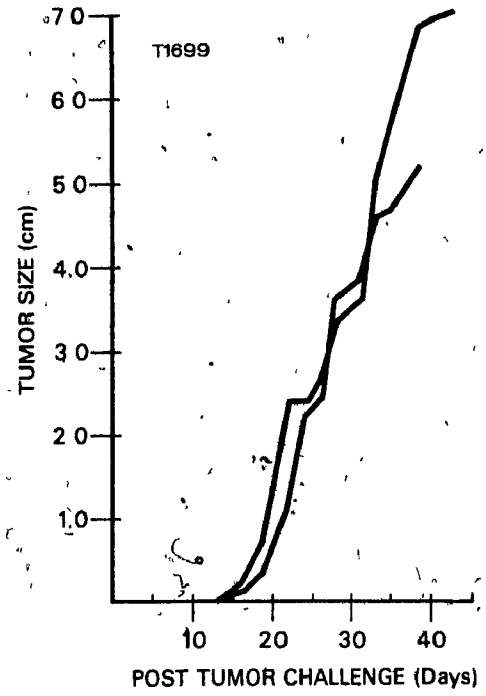




Figure 15: TREATED MALES

# TABLES

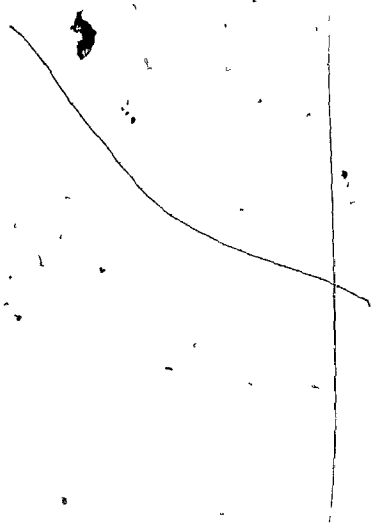


TABLE I: COMPOSITE PICTURE OF ANTI-IgM TREATED NEWBORNS ON DAY 35.

Note that they appear to have been effectively bursectomized when compared to controls.

t - test statistic

p - probability

BM - bone marrow

TABLE I

| Group | Non-Immunological: Body Weight (grams) | Non-Immunological: Spleen Weight (grams) | Non-Immunological: Total Cells $\times 10^6$ | Non-Immunological: Ratio Spleen/Body Weight | Immunological: Immunoglobulins per 100 cells: IgM | IgA | $IgG_1$ | $IgG_2$ | Immunofluorescence per 100 cells: Anti-θ | Anti-Ig | Hemolytic Plaques (Direct) per $10^6$ Spleen Cells | Autoradiography per 100 cells: Spleen | PM | Blood |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 21.1 | 0.158 | 87 | 0.0075 | 2 | 1 | 32 | 16 | 68 | 45 | 620 | | | |
| | 22.7 | 0.170 | 78 | 0.0075 | 4 | 2 | 32 | 16 | 70 | 48 | 488 | | | |
| | 19.8 | 0.162 | 72 | 0.0082 | 2 | 2 | 16 | 32 | 74 | 49 | 526 | | | |
| | 20.7 | 0.157 | 83 | 0.0076 | 2 | 4 | 16 | 16 | 64 | 39 | 560 | | | |
| | 20.9 | 0.145 | 68 | 0.0069 | 4 | 1 | 32 | 8 | 69 | 43 | 584 | | | |
| Untreated | 23.0 | | | | 4 | 2 | 16 | 32 | | | | 69.0 | 41.0 | 43.0 |
| | 22.1 | | | | 2 | 4 | 32 | 16 | | | | 63.7 | 38.3 | |
| | $\bar{x}$ 21.5 | 0.158 | 84.0 | 0.0075 | 2.85 | 2.29 | 25.14 | 19.43 | 69.0 | 44.8 | 555 | 66.4 | 39.7 | |
| | SD 1.2 | 0.008 | 8.2 | 0.0003 | 1.07 | 1.25 | 8.55 | 9.07 | 3.6 | 4.0 | 51 | 3.7 | 1.9 | |
| | SE 0.4 | 0.003 | 3.7 | 0.0000 | 0.40 | 0.47 | 3.23 | 3.42 | 1.6 | 1.8 | 22 | 2.7 | 1.4 | |
| Animal # | 7 | 5 | 5 | 5 | 7 | 7 | 7 | 7 | 5 | 5 | 5 | 2 | | |
| | 15.8 | 0.125 | 54 | 0.0079 | 0 | 0 | 1 | 1 | 62 | 23 | 23 | | | |
| | 17.5 | 0.105 | 52 | 0.0060 | 0 | 1 | 1 | 2 | 66 | 25 | 18 | | | |
| | 16.4 | 0.112 | 43 | 0.0068 | 0 | 1 | 2 | 2 | 58 | 22 | 9 | | | |
| Treated | 15.9 | 0.117 | 30 | 0.0074 | 0 | 0 | 1 | 0 | 70 | 26 | 14 | | | |
| | 17.1 | | | | 0 | 0 | 0 | 1 | | | | 1.3 | 0.3 | 1.5 |
| | 16.7 | | | | 0 | 0 | 1 | 1 | | | | 1.0 | 1.0 | |
| | $\bar{x}$ 16.6 | 0.115 | 45.0 | 0.00703 | 0 | 0.33 | 1.0 | 1.17 | 64.0 | 24.0 | 16.0 | 1.2 | 0.7 | |
| | SD 0.7 | 0.008 | 5.3 | 0.00077 | 0 | 0.52 | .63 | .75 | 5.2 | 1.8 | 5.9 | 0.2 | 0.5 | |
| | SE 0.3 | 0.003 | 2.7 | 0.00031 | 0 | 0.21 | .26 | .30 | 2.6 | 0.9 | 2.9 | 0.2 | 0.4 | |
| Animal # | 6 | 4 | 4 | 4 | 6 | 6 | 6 | 6 | 4 | 4 | 4 | 2 | | |
| t= | 8.095 | 7.070 | 7.202 | 1.667 | 6.001 | 3.293 | 4.336 | 4.523 | 1.499 | 8.509 | 18.54 | 17.46 | 19.85 | |
| p= | <0.0005 | <0.0005 | <0.0005 | <0.10 | <0.0005 | <0.05 | <0.0005 | <0.0005 | <.10 | <0.0005 | <0.0005 | <0.0025 | <0.0025 | |

TABLE 2

TSA ACTIVITY IN EXTRACTS OF T1699

| Preparatory Method | Non-fractionated | | | Fractionated | | | |
|---|---|---|---|---|---|---|---|
| | Stored at 4°C | Frozen and Thawed | Lyophilized | I | II | III | IV |
| TSA | ++++ | +++ | + | - | + | - | - |
| H-2 | ++++ | +++ | - | - | +++ | - | - |

This demonstrated the loss of activity when fractionation was employed.

H-2 antigen detectability was recorded to allow comparison.

Grading was on a scale of 0 to 4+.

TABLE 3

T1699 DOSE-RESPONSE RELATIONSHIP

| Challenge Dose (Number of cells) | Tumor Growth Characteristics | | | |
|---|---|---|---|---|
| | Takes | Progressors | Regressors | Latent Period (Days) |
| $10^1$ | - | - | - | - |
| $10^2$ | - | - | - | - |
| $10^3$ | - | - | - | - |
| $10^4$ | 5/10 | 5/5 | 0/5 | 27.2±3.9 |
| $10^5$ | 9/10 | 8/9 | 1/9 | 10.3±1.6 |
| | | | | $p < 0.0005$ |

Note that for a challenge dose of $10^5$ cells SC in the milk line that there is a high incidence of takes, the appearance of regressors, and a short latency period.

TABLE 4

T1699 GROWTH CHARACTERISTICS WITH TSA #2

| Experimental Group | Tumor Growth Characteristics | | | |
|---|---|---|---|---|
| | Takes | Progressors | Regressors | Latent Period (Days) |
| Non-immunized | 7/10 | 4/7 | 3/7 | 14.2±0.9 |
| Immunized | 6/10 | 6/6 | 0/6 | 12.1±0.8 |
| | | | | $p < 0.10$ |

Note the decrease in the latent period, and a lack of regressors in the immunized group.

TABLE 5

EXPERIMENTAL FORMAT

| Group | Sex | Immunization | Tumor Challenge | Number of Animals |
|---|---|---|---|---|
| Untreated | ♂ | - | T1699 | 12 |
| | | Normal tissue | T1699 | 12 |
| | | P-815-X2 | T1699 | 12 |
| | | T1699 | T1699 | 12 |
| | | - | P-815-X2 | 12 |
| | | T1699 | P-815-X2 | 12 |
| | | P-815-X2 | P-815-X2 | 12 |
| | ♀ | - | P-815-X2 | 12 |
| | | Normal tissue | P-815-X2 | 12 |
| | | T1699 | P-815-X2 | 12 |
| | | P-815-X2 | P-815-X2 | 12 |
| | | - | T1699 | 12 |
| | | P-815-X2 | T1699 | 12 |
| | | T1699 | T1699 | 12 |
| Treated | ♂ | - | T1699 | 7 |
| | | T1699 | T1699 | 7 |
| | ♀ | - | P-815-X2 | 7 |
| | | P-815-X2 | P-815-X2 | 7 |

This is a composite picture of the experimental design.

TABLE 6: EXPERIMENTAL RESULTS

A. This is a composite picture of the experimental results.

B. Calculated values allowing assessment of the degree of immunosuppression.

TABLE 6A

| Group | Experimental Design: Immunization | Tumor Challenge | No. of Animals | Results: Incidence | Latent Period (Mean ± SD) | Pattern: Progressors | Pro/Reg | Regressors | Regressors (Days) | Serum Immunoglobulin (Day 75): IgM | IgA (Mean ± SD) | $IgG_1$ | $IgG_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Untreated | - | T1699 | 12 | 8/12 | 17.6±9.4 | 6/8 | 1/8 | 1/8 | 30-40 | 3.17±1.03 | 2.25±1.14 | 21.3±9.8 | 17.3±7.5 |
| | Normal tissue | T1699 | 12 | 11/12 | 16.3±11.2 | 8/11 | 2/11 | 1/11 | 20-40 | 2.8±1.02 | 2.08±.97 | 20.7±8.7 | 15.3±8.7 |
| | P-815-X2 | T1699 | 12 | 6/12 | 15.8±5.5 | 5/6 | 1/6 | 0/6 | | 3.16±1.02 | 2.25±1.14 | 21.3±9.8 | 16.7±8.6 |
| | T1699 | T1699 | 12 | 7/12 | 12.9±1.5 | 6/7 | 1/7 | 0/7 | | 3.10±1.8 | 2.75±1.14 | 20.0±7.2 | 20.0±9.3 |
| | - | P-815-X2 | 12 | No growth | - | - | - | - | - | 2.83±1.03 | 2.50±1.16 | 18.0±10.8 | 15.3±8.7 |
| | T1699 | P-815-X2 | 12 | No growth | - | - | - | - | - | 2.83±1.03 | 2.17±.94 | 23.3±9.3 | 16.7±10.4 |
| | P-815-X2 | P-815-X2 | 12 | No growth | - | - | - | - | - | 2.67±0.98 | 2.17±1.19 | 21.3±7.9 | 16.0±8.4 |
| | - | P-815-X2 | 12 | No growth | - | - | - | - | - | 3.0±1.04 | 2.33±1.07 | 20.7±8.7 | 16.7±9.9 |
| | Normal tissue | P-815-X2 | 12 | No growth | - | - | - | - | - | 3.0±1.04 | 2.33±1.07 | 16.0±8.4 | 18.0±9.1 |
| | T1699 | P-815-X2 | 12 | No growth | - | - | - | - | - | 3.0±1.04 | 2.50±1.17 | 18.7±10.4 | 20.0±9.3 |
| | P-815-X2 | P-815-X2 | 12 | No growth | - | - | - | - | - | 3.25±1.14 | 2.33±1.3 | 18.7±8.9 | 18.7±8.6 |
| | - | T1699 | 12 | 10/12 | 13.2±4.2 | 7/10 | 2/10 | 1/10 | 28-40 | 2.91±1.16 | 2.0±1.04 | 20.0±9.3 | 18.7±8.6 |
| | P-815-X2 | T1699 | 12 | 10/12 | 17.6±7.2 | 9/10 | 1/10 | 0/10 | | 3.3±0.98 | 1.66±.89 | 17.3±7.5 | 18.7±10.4 |
| | T1699 | T1699 | 12 | 9/12 | 11.1±4.0 | 9/9 | 0/9 | 0/9 | | 3.00±1.04 | 1.8±.83 | 17.3±7.5 | 17.3±9.5 |
| Treated | - | T1699 | 7 | 4/7 | 14.8±3.5 | 4/4 | 0/4 | 0/4 | | 0±0 | .29±.49 | 1.42±.79 | 1.14±.90 |
| | T1699 | T1699 | 7 | 2/7 | 12.0±0 | 2/2 | 0/2 | 0/2 | | 0±0 | .43±.53 | 1.43±.53 | 1.14±.70 |
| | - | P-815-X2 | 7 | No growth | - | - | - | - | - | 0±0 | .29±.47 | 1.71±.49 | 1.43±.53 |
| | P-815-X2 | P-815-X2 | 7 | No growth | - | - | - | - | - | 0±0 | .29±.49 | 1.43±.53 | 1.00±.81 |

TABLE 6B

| Group | Serum Immunoglobulins ($\bar{x}$±SD) IgM | IgA | $IgG_1$ | $IgG_2$ |
|---|---|---|---|---|
| Untreated | 2.975 ± 1.147 | 2.309 ± 1.086 | 20.667 ± 8.887 | 12.333 ± 8.678 |
| Number of animals | 168 | 168 | 168 | 168 |
| Treated | 0 ± 0 | 0.321 ± 0.476 | 1.500 ± 0.577 | 1.179 ± 0.723 |
| Number of animals | 28 | 28 | 28 | 28 |
| Test Statistic | 13.656 | 9.486 | 11.352 | 9.796 |
| Degrees of Freedom | 194 | 194 | 194 | 194 |
| Probability | <0.0005 | <0.0005 | <0.0005 | <0.0005 |

# CLAIMS TO ORIGINALITY

PART I: Adaptation of Panspecific Immunosuppression to the Murine Strain DBA/2; with Documentation of IgM Specificity via Autoradiography.

PART II: Enhancement of a Solid Syngeneic Neoplasm, P-815-X2, in the SC Location.