INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

**ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600**

UMI[®]

**Th2 cytokine expression in atopic
children with otitis media with effusion**

Steven E. Sobol, MD

Department of Otolaryngology
McGill University, Montreal

July 2001

A thesis submitted to the Faculty of Graduate Studies and
Research in partial fulfillment of the requirements of the
degree of Master of Science

© Steven E. Sobol 2001

**National Library
of Canada**

**Acquisitions and
Bibliographic Services**

**395 Wellington Street
Ottawa ON K1A 0N4
Canada**

**Bibliothèque nationale
du Canada**

**Acquisitions et
services bibliographiques**

**395, rue Wellington
Ottawa ON K1A 0N4
Canada**

Your file Votre référence

Our file Notre référence

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-75345-X

Abstract

Background: Otitis media with effusion (OME) is more common in atopic children. Few studies have looked for the presence of inflammatory mediators in the middle ear effusions of this population.

Objectives: We hypothesize that atopic children with OME have a different inflammatory cell and cytokine profile than non-atopic children with the disease.

Methods: The atopic status of 26 children with OME was determined. Using immunocytochemistry, fluid specimens were assessed for T lymphocytes, eosinophils, neutrophils, mast cells, and basophils. The expression of IL-4, IL-5, and IFN- γ mRNA was assessed using in-situ hybridization.

Results: There is a higher percentage of eosinophils, T lymphocytes and cells expressing IL-4 and IL-5 mRNA in atopic children (n=8) compared to non-atopic controls (n=18) ($p<0.01$).

Conclusion: The predominance of eosinophils, T lymphocytes and Th2 mediators in the middle ear effusions of atopic children provides strong evidence that atopy plays a role in the pathogenesis of this condition.

Abregé

L'Otite moyenne avec épanchement (OMÉ) est plus courante chez les enfants atopiques. Peu d'études ont porté sur la présence de médiateurs chimiques d'allergie dans le cas d'OMÉ de cette population.

Nous proposons que les patients atopiques avec OMÉ auront un profil de cytokines et cellules inflammatoires différent des patients non-atopiques avec la maladie.

L'état atopique de 26 enfants avec OME a été déterminé. En utilisant l'immunocytochimie, des échantillons d'épanchement ont été évaluées pour la présence de neutrophiles, cellules T, éosinophiles, mastocytes, et basophiles. L'expression de l'ARNm de IL-4, IL-5, et IFN- γ a été considérée par l'hybridation in-situ.

Il y a un plus grand nombre d'éosinophiles, cellules T et de cellules qui exprime IL-4 and IL-5 chez les patients atopique (n=8) que chez les patients non-atopiques (n=18) ($p<0.01$).

La prédominance d'éosinophiles, cellules T et les médiateurs Th2 dans l'épanchements de l'oreille moyenne des patients atopiques supporte fortement le rôle joué par l'allergie dans la pathogénie de OMÉ.

Acknowledgements

I would like to acknowledge the following individuals and organizations for their support of this project:

- My supervisor and mentor Dr. Qutayba Hamid for his constant and unwavering support of this project.
- The Medical Research Council of Canada for their generous support of this project.
- The McGill University Head and Neck Cancer Research fund for their generous support of this project.
- Ms. Elsa Schotman for her technical assistance in the performance of immunocytochemistry and in-situ hybridization.
- Maria Makroyanni for her secretarial assistance.
- Ms. Genevieve Brooks-Legault for translation of the abstract
- I would like to thank my wife Jennifer for supporting me through the highs and lows of this project.

Contributions of authors

The work for this thesis was performed at the Meakins-Christie Laboratories, McGill University, from July, 2001-August, 2002.

My role in this study includes the following:

- Development of the research hypothesis and objectives.
- Development of the research design including the establishment of the middle ear effusion collection protocol (with help from Dr. Rame Taha).
- Obtainment of approval from the investigational review board of the Montreal Children's Hospital.
- Recruitment of all patients participating in this study.
- Skin prick testing of all patients participating in this study (under the guidance of Dr. Bruce D. Mazer).
- Performance of myringotomies and collection of middle ear fluid from all patients participating in this study (with help from Drs. Melvin D. Schloss, John J. Manoukian and Ted L. Tewfik).
- Performance of immunocytochemistry and in-situ hybridization (with help from Ms. Elsa Schotman)
- Counting of cells for each marker from every patient (with Dr. Qutayba Hamid).
- All data processing, statistical analysis, and writing of manuscripts was performed by myself with input from all of my coauthors.
- Presentation at the national meeting of the Canadian Society of Otolaryngology-Head and Neck Surgery, Vancouver, BC, May 26, 2001.

I am indebted to my co-authors who contributed to the practical work as follows:

- Dr. Rame Taha for his ingenuity and help in establishing the fluid processing protocol.
- Dr. Melvin D. Schloss for his role clinical supervisor of this project, and for his patience and help in the development of a bloodless technique for the acquisition of middle ear fluid.
- Drs. John J. Manoukian and Ted L. Tewfik for allowing me access to their patients.
- Dr. Bruce D. Mazer for teaching me the principles of allergy and the basics of skin prick testing.
- Dr. Qutayba Hamid for his direction and supervision of all stages of this project.

Publications arising from this work

Papers

1. Sobol SE, Taha R, Schloss MD, Mazer BD, Manoukian JJ, Tewfik TL, Hamid Q:
Th2 cytokine expression in atopic children with otitis media with effusion. J
Allergy Clin Immunol *Submitted for publication.*
2. Sobol SE, Taha R, Schloss MD, Mazer BD, Manoukian JJ, Tewfik TL, Hamid Q:
Increased expression of major basic protein, CD3 and BB1 in atopic children with
otitis media with effusion. J Otolaryngol *Submitted for publication.*
3. Sobol SE, Schloss MD, Hamid Q: The role of atopy in the pathogenesis of otitis
media with effusion. Laryngoscope *Submitted for publication.*

Abstracts

1. Sobol SE, Taha R, Schloss MD, Mazer BD, Hamid Q: Inflammatory cell and
cytokine profiles in middle ear effusions of atopic and non-atopic children. J
Otolaryngol 2001;30(suppl. 1):18.

Table of Contents

Abstract	1
Abregé	2
Acknowledgements	3
Contributions of authors	4
Publications arising from this work	6
List of figures and tables	8
Abbreviations used in this text	9
Chapter 1. General introduction	11
1.1 Otitis media with effusion	12
1.2 Clinical evidence of atopy in otitis media with effusion	14
1.3 Molecular evidence of atopy in otitis media with effusion	17
1.4 The role of atopy in upper airway disease	23
1.5 Management of atopic diseases of the upper airway	26
1.6 Rationale for this study	28
1.7 General hypothesis	29
1.8 Aims of this study	30
1.9 References	31
Chapter 2. Th2 cytokine expression in atopic children with otitis media with effusion	49
2.1 Abstract	50
2.2 Introduction	51
2.3 Methods	52
2.4 Results	56
2.5 Discussion	58
2.6 Acknowledgments	61
2.7 References	62
2.8 Tables	69
2.9 Figure legend	71
2.10 Figures	73
Chapter 3. Overall conclusions	77
3.1 The role of atopy in otitis media with effusion	78
3.2 Limitations of this study	81
3.3 Future studies	82

List of figures and tables

Chapter 1. General introduction

Figure 1. Cells and cytokines in allergic inflammation

Chapter 2. Th2 cytokine expression in atopic children with otitis media with effusion

Table 1. Clinical presentation of atopic and non-atopic children with otitis media with effusion

Table 2. Results of skin prick testing in atopic children with otitis media with effusion

Figure 1. Inflammatory cells in the middle ear effusions of atopic and non-atopic children with otitis media with effusion

Figure 2. Results of immunocytochemistry: CD3, MBP, and BB1 positive cells in the middle ear effusions of atopic children with otitis media with effusion

Figure 3. Cytokine expression in the middle ear effusions of atopic and non-atopic children with otitis media with effusion

Figure 4. Results of in-situ hybridization: IL-5, IL-4, and IFN- γ mRNA expression in the middle ear effusions of atopic children with otitis media with effusion

Abbreviations used in this text

BB1	basophil granule-specific monoclonal antibody
CD	cluster of differentiation
cDNA	complementary deoxyribonucleic acid
ECP	eosinophil cationic protein
ELISA	enzyme-linked immunosorbent assay
EN	eosinophil neurotoxin
EPO	eosinophil peroxidase
GM-CSF	granulocyte/macrophage colony stimulating factor
GM-CSFR	granulocyte/macrophage colony stimulating factor receptor
ICC	immunocytochemistry
IFN-γ	interferon gamma
Ig	immunoglobulin
IL	interleukin
ISH	in-situ hybridization
LT	leukotriene
MBP	major basic protein
MEE	middle ear effusion
mRNA	messenger ribonucleic acid
OME	otitis media with effusion
PAF	platelet activating factor
PG	prostaglandin
SEM	standard error of the mean

Th1	T helper lymphocyte type 1
Th2	T helper lymphocyte type 2
TNF-α	tumor necrosis factor alpha
VCAM-1	vascular cell adhesion molecule-1

Chapter 1. General introduction

1.1 Otitis media with effusion

1.2 Clinical evidence of atopy in otitis media with effusion

1.2.1 *Results of skin testing in patients with otitis media with effusion*

1.2.2 *Association between otitis media with effusion and other atopic diseases*

1.2.3 *Allergen-induced Eustachian tube dysfunction*

1.3 Molecular evidence of atopy in otitis media with effusion

1.3.1 *Role of the early allergic response in otitis media with effusion*

1.3.2 *Role of the late allergic response in otitis media with effusion*

1.3.2.1 *Eosinophils*

1.3.2.2 *T lymphocytes*

1.3.2.3 *Basophils*

1.3.2.4 *Th2 cytokines*

1.4 The role of atopy in upper airway disease

1.4.1 *Allergic rhinitis*

1.4.2 *Allergic chronic sinusitis*

1.5 Management of atopic diseases of the upper airway

1.6 Rationale for this study

1.7 General hypothesis

1.8 Aims of this study

1.9 References

1.1 Otitis media with effusion

Otitis media with effusion (OME) is a major pediatric health care issue, with an estimated prevalence of 15-20 % ¹, and annual costs in the billions of dollars ². OME is the most common indication for surgery in children, and can have significant sequelae, including hearing loss, maldevelopment of communication skills, as well as permanent middle ear mucosal damage ³. Although the exact etiology of OME is not known, it is thought to occur after a treated acute infection, in the setting of Eustachian tube dysfunction. However, 57-82 % of effusions do not have bacterial growth ⁴, and antibiotic therapy is no better than placebo in the resolution of OME ⁵⁻⁷. A viral etiology has been proposed by some authors, but viral isolates are only present in 16-19 % of cases ⁸.

The inflammatory cell and cytokine profile of asthma ⁹, allergic rhinitis ¹⁰, and chronic sinusitis ¹¹ is well documented in the literature. Mast cell degranulation, occurring in response to allergen binding of cross-linked IgE, is the predominant effector of early allergic responses. Eosinophils, basophils, T-helper cells, and the Th2 cytokines (IL-4, IL-5, IL-13) are responsible for the late phase allergic response. Given that the middle ear mucosa is contiguous with that of the nasal cavity and sinuses via the Eustachian tube, it has been hypothesized that similar inflammatory responses may occur in patients with otitis media with effusion.

Whereas the role of allergy mediators in the pathogenesis of chronic sinusitis and allergic rhinitis has been well defined, the role of such mediators in the pathogenesis of OME is unclear. This introduction reviews both clinical and molecular biological studies

that have both supported and refuted the hypothesis that atopy plays a role in the pathogenesis of OME.

1.2 Clinical evidence of atopy in otitis media with effusion

1.2.1 Results of skin testing in patients with otitis media with effusion

The role of allergy in the pathogenesis of recurrent otitis media and OME has been evaluated, and has been reported to be a risk factor in 0-88 % of patients ¹²⁻¹⁸. While some investigators have concluded that atopy is a significant risk factor for recurrent otitis media ¹⁹⁻²³, others have found no relationship between the two conditions ^{24,25}. The major problem with most of these studies is the lack of uniform diagnostic criteria for atopy. Moreover, in some studies the diagnosis of atopy was based on history rather than objective skin tests, provocation tests or serum IgE analysis.

The prevalence of positive skin tests in OME patients varies from 9-88 % between different studies ²⁶⁻³⁰. A study by Lecks et al found that 88 % of OME patients had positive skin scratch tests ³⁰. Using the skin prick technique, Caffarelli et al found no difference in the prevalence of allergen reactivity between OME patients (27 %) and controls (31 %) ²⁶. The relatively high number of OME patients who were found to be positive for atopy in earlier compared to later studies, may be partly explained by the use of the skin scratch test, which has a high false positive rate compared to the prick test.

1.2.2 Association between otitis media with effusion and other atopic diseases

The presence of atopic conditions including allergic rhinitis, eczema, and asthma has been evaluated in OME patients ^{12-15,18,31}. Several authors have noted an increased prevalence of OME in allergic children compared to non-allergic controls ²⁰,

32-34. Draper reported the presence of OME in 52 % of allergic rhinitis patients, compared to 24 % of non-allergic controls ³⁴. Other studies have found an increased prevalence of atopic conditions in OME patients compared to non-OME controls. For example, Caffarelli et al found an increased prevalence of eczema and allergic rhinitis in OME patients compared to controls ²⁶, which is consistent with data found in other studies ^{29,35-40}. In an epidemiological study looking at the role of allergies in the development of acute otitis media, Pukander et al concluded that infants with a history of allergy had significantly longer persistence of MEE compared to non-allergic infants ⁴¹.

1.2.3 Allergen-induced Eustachian tube dysfunction

The nasal mucosa is continuous with that of the middle ear by way of the Eustachian tube. In theory, it is possible that allergic inflammation of the nasal mucosa could also affect the Eustachian tube resulting in edema and obstruction, with the subsequent development of OME.

Eustachian tube obstruction has been demonstrated in allergic patients after exposure to both natural and induced allergen exposure ⁴²⁻⁴⁹. Clinical studies have shown that in atopic patients, exposure to allergen challenge results in a dose dependent decrease in Eustachian tube patency ^{42,48}. This response is consistently found regardless of the whether seasonal (ragweed) ⁴² or perennial (dust mite) allergen ⁴⁸ was used in the challenge. What is not clear from these studies, however, is the role of allergen-induced Eustachian tube obstruction on the subsequent development of OME.

Animal models have been used to evaluate the histological response of the Eustachian tube to allergen exposure in sensitized subjects ^{50,51}. As early as one hour

after exposure, increased numbers of mast cells and eosinophils were found in the distal Eustachian tube mucosa ⁵⁰. Using a dog model of OME and allergic rhinitis, Mogi et al concluded that allergen challenge contributes to the chronicity but not the actual development of OME in sensitized animals ⁵¹.

1.3 Molecular evidence of atopy in otitis media with effusion (figure 1)

1.3.1 Role of the early allergic response in otitis media with effusion

Allergy mediators play an important role in the pathogenesis of chronic upper airway disease such as allergic rhinitis and chronic sinusitis (see below). Although there are few immunocompetent cells in the normal middle ear mucosa ⁵², mast cells have been found in the tympanic cavity of guinea pigs ⁵³ and humans ⁵⁴, leading to speculation that the middle ear may be a target of the early allergic response.

The mast cell plays an important role in mediating the early phase allergic response. Mast cells store a number of pro-inflammatory mediators, including tryptase, histamine, TNF- α and IL-4 ^{55,56}. Allergen inspiration and crosslinkage of IgE result in the degranulation and release of these stores, as evidenced by the detection of elevated tryptase and histamine levels in nasal lavage fluid of allergic rhinitis patients ⁵⁷. Allergen activation also induces mast cell synthesis of leukotrienes, prostaglandins, PAF and bradykinin ⁵⁹⁻⁶⁰.

Several investigators have looked at the levels of total and specific IgE in MEE and serum of children with OME. Whereas some authors have concluded that OME is an IgE mediated disease ^{61,62}, others have not found increased IgE in MEE compared to serum samples ^{41,63-67}. A recent study by Labadie et al demonstrated the development of OME in sensitized rats when exposed to lipopolysaccharide ⁶⁸. Middle ear effusions in these rats could be prevented by the co-administration of the antihistamine diphenhydramine, leading the authors to conclude that OME may be an antigen-IgE mediated response. Bernstein et al found that levels of IgE are higher in MEE compared

to serum samples in 46 % of allergic children with OME ⁶¹. They concluded that a local IgE response may be responsible for the generation of MEE in susceptible patients.

On the other hand, Mogi et al looked at total and specific IgE in the serum and MEE of children with OME ^{41,63}. They concluded that the presence of IgE in MEE was the result of systemic allergic disease rather than the cause of the OME. When exposed to allergen challenge, the middle ear mucosa of sensitized primates did not demonstrate any IgE response. Since IgE plays a major role in the early phase allergic response, its role in OME, which is a chronic condition, is questionable.

1.3.2 Role of the late allergic response in otitis media with effusion

1.3.2.1 Eosinophils

Eosinophils play a particularly important role in the late phase allergic response through the release of mediators including ECP, EN, EPO, and MBP ^{69,70}, which cause extracellular matrix deposition, epithelial denudation and basement membrane disruption ⁷¹⁻⁷⁷. Eosinophils also secrete LTC₄ ⁷⁸, which is known to cause mucosal edema and mucus secretion, and PAF ^{69,70} which stimulates eosinophil and neutrophil chemotaxis, increases vascular permeability, promotes mucosal edema and inhibits apoptosis of B cells ⁷⁹. Eosinophils appear to be an important source of cytokines such as IL-3, IL-5 and GM-CSF, which have been implicated in hematopoiesis and may act in an autocrine fashion to promote eosinophil differentiation and survival ⁸⁰.

There have been a number of studies that have attempted to identify the role of allergic inflammatory cells in OME ⁸¹⁻⁸⁸. Early studies demonstrated that eosinophils were not common in the effusions and middle ear mucosa of OME patients ^{81,89,90}.

Most of these studies, however, were done before the advent of modern molecular biology techniques. Hurst et al recently demonstrated that 87.5 % of MEE and 80 % of middle ear mucosal biopsy specimens were positive for ECP ⁸⁷. He concluded that eosinophils play a major role in the pathogenesis of this condition. These studies, however, did not compare the cellular profiles of atopic and non-atopic OME patients. In order to overcome this deficiency, Hurst et al recently reported increased levels of ECP and tryptase in the supernatant of atopic OME patients compared to non-atopic controls ⁹¹. Wright et al recently demonstrated increased expression of IL-5 and MBP in the middle ear mucosa of patients with OME compared to normal controls ⁸².

1.3.2.2 T lymphocytes

T lymphocytes (CD3⁺ cells) are thought to play a central role in the initiation and regulation of the inflammatory response through the release of cytokines. The combination of cytokines secreted by the T lymphocyte determines what type of inflammatory reaction results. Initial studies of mouse T-helper cells (CD4⁺ T lymphocyte clones) revealed that they could be differentiated from Th0 cells into two basic functional subsets termed Th1 and Th2 ⁹². Th1 cells secrete IL-2, IFN- γ , and TNF- β , whereas Th2 cells secrete IL-4, IL-5, IL-6, and IL-10. Other cytokines including TNF- α , IL-3 and GM-CSF are secreted by both types of CD4⁺ T lymphocytes. In general, the Th1 cytokines are crucial for the onset of delayed type hypersensitivity reactions, cell mediated immunity, and clearance of intracellular pathogens, whereas Th2 cytokines are thought to play a major role in the late phase allergic response ^{93,94}.

The role of Th2 cells in allergic diseases including, allergic rhinitis, chronic sinusitis, and asthma has been well established in the literature ⁹⁵. There have been few studies that have looked for the presence of this cell in the middle ear of patients with OME. Wright et al demonstrated a significantly higher number of CD3 positive cells in the middle ear mucosa of atopic OME patients compared to controls ⁸². Given the pivotal role of T lymphocytes in the development of allergic disease, further studies are needed to evaluate the role of this cell in OME.

1.3.2.3 Basophils

Basophils are thought to play an important role in the late phase allergic response, although their exact role has yet to be clearly elucidated. Similar to the mast cell, basophils bind allergen via IgE cross linkage and release histamine upon activation ⁹⁶. Since the level of histamine, but not tryptase is found to be elevated during the late phase response, basophils are thought to be the major source of this mediator as opposed to secondary mast cell degranulation ⁹⁶⁻⁹⁸. The role of the basophil in OME has yet to be evaluated.

1.3.2.4 Th2 cytokines

As mentioned above, the Th2 cytokines are thought to play a major role in the initiation and regulation of the late phase allergic inflammatory response. IL-5 is important for eosinophil differentiation from myeloid progenitors in the bone marrow, and is important for the recruitment, activation, and survival of mature eosinophils at the site of inflammation ⁹⁹.

IL-4 is important for the differentiation of naïve T-helper cells (Th0) towards the Th2 phenotype ¹⁰⁰, and plays an important role in the local production of IgE in tissues of atopic patients ¹⁰¹. Through induction of endothelial VCAM-1, IL-4 is thought to promote eosinophil infiltration to the site of inflammation ¹⁰².

The recent literature has looked at the role of cytokines in MEE, both in animals and humans ¹⁰³⁻¹⁰⁶. Most studies have assessed for the presence of non-specific inflammatory mediators such as IL-1, IL-6, IL-8 and TNF- α . Others have looked for the presence of antibodies in the systemic circulation and in MEE specimens ⁴¹.

Wright et al recently demonstrated an increased number of cells expressing IL-5 mRNA in the middle ear mucosa of atopic OME patients compared to controls ⁸². The presence of IL-4 and IL-5 in the MEE of atopic and non-atopic OME patients has yet to be determined, and may play a critical role in the pathogenesis of this condition.

Figure 1

1.4 The role of atopy in upper airway disease

1.4.1 Allergic rhinitis

Allergic rhinitis is a chronic inflammatory disorder of the nasal mucosa, which occurs after exposure to perennial or seasonal allergens, including dust mite, animal dander, mold spores and pollen. Encounter with the allergen results in the classical manifestations of allergic rhinitis such as sneezing, nasal pruritis, rhinorrhea and congestion. This response is thought to occur secondary to glandular hypersecretion, increased vascular permeability, afferent nerve stimulation, and the infiltration of inflammatory cells at the site of the nasal mucosa ¹⁰⁷⁻¹¹⁰.

Allergen stimulation of the nasal mucosa results in an early phase reaction, which develops rapidly and lasts for 60 minutes. Mast cells, which are found in higher numbers within the nasal epithelium during allergy season, play an important role in mediating the immediate response to allergen by increasing their expression of the IgE receptor gene, indicating their increased ability to bind IgE ¹¹¹⁻¹¹³. Once activated, mast cells release histamine over a time course that correlates clinically with the early irritative and secretory manifestations of allergic rhinitis ¹¹⁴⁻¹¹⁶. Other mediators of the early phase response include the cysLT and PGD₂, which irritate afferent nerve ending, increase vascular permeability and glandular secretion ¹¹⁶⁻¹¹⁹.

The early phase response is followed by a late phase reaction, which begins 3-6 hours after allergen exposure, and subsides within 12-24 hours. Dual responses are observed in 40-50% of patients ¹²⁰. Therefore, a single allergen challenge can precipitate chronic inflammatory alterations in the nasal mucosa and can enhance subsequent mediator release on rechallenge ¹¹⁴.

The late phase allergic response manifests as a re-occurrence of sneezing, rhinorrhea and congestion. This response is thought to arise from the expression of cytokines and chemokines, which lead to the recruitment and activation of inflammatory cells, including T lymphocytes, eosinophils and basophils ^{97,121}.

Eosinophils play a particularly important role in the late phase reaction, through the release of mediators such as MBP and ECP, which have been shown to cause degranulation of other inflammatory cells as well as epithelial cell damage ^{72,73,75-77}.

Basophils are thought to play an important role in the late phase allergic response, although their exact role has yet to be clearly elucidated. Similar to the mast cell, basophils bind allergen via IgE cross linkage and release histamine upon activation ⁹⁶. Since the level of histamine, but not tryptase is found to be elevated during the late phase response, basophils are thought to be the major source of this mediator as opposed to secondary mast cell degranulation ⁹⁶⁻⁹⁸.

CD4⁺ T lymphocytes elevated in the nasal mucosa of allergic patients and are thought to play an important role in the pathogenesis of allergic rhinitis by the release of Th2 type cytokines including, IL-3, IL-4, IL-5, IL-13 and GM-CSF ^{10,122,123}. The role of the Th2 cytokines has already been described in the previous section. Co-localization studies have demonstrated that approximately 70-80% of IL-4, IL-5 and IL-13 mRNA positive cells were T lymphocyte-associated ^{122,124,125}.

1.4.2 Allergic Chronic Sinusitis

Allergic chronic sinusitis is an inflammatory condition of the paranasal sinuses, which is thought to result, in the majority of cases, from allergic stimulation of the nasal

and paranasal mucosa. Like allergic rhinitis, the inflammatory infiltrate in allergic chronic sinusitis is consistent with the late phase response ^{10,71}. The allergen-induced inflammation leads to hypertrophy of the sinus mucosa and polypoid changes, which leads to a narrowing of the sinus ostia and obstruction of mucous drainage.

The sinus mucosa of patients with allergic chronic sinusitis is characterized by a higher percentage of eosinophils, T lymphocytes and B cells, compared to normal controls ^{11,126}. Like allergic rhinitis, the pathophysiology of chronic sinusitis has been largely attributed to the effects of Th2 cytokines, including IL-3, IL-4, IL-5 and IL-13 and GM-CSF, which are found in a higher percentage of cells within the sinus mucosa allergic chronic sinusitis patients compared to normal controls ^{126,127}. Receptors for IL-4 and IL-5 are upregulated in the mucosa of patients with allergic chronic sinusitis, while the GM-CSF receptor was more predominantly expressed in non-allergic chronic sinusitis patients ¹²⁸.

1.5 Management of atopic diseases of the upper airway

There have been very few studies that have looked at the response of OME to anti-allergy therapy. Kawauchi et al found that azelastine hydrochloride was more effective than placebo in clearing MEE in children with OME and allergic rhinitis ⁴¹. They found a similar response in a Guinea pig model, where accelerated MEE clearance was noted when azelastine hydrochloride was used ¹²⁹. Hurst reported promising results in the management of specific IgE positive OME patients with anti-allergy therapy ¹³⁰.

The use of corticosteroids for the treatment of chronic inflammatory diseases such as allergic rhinitis and chronic sinusitis has been well established. Steroids reduce the irritative and hypersecretory manifestations of allergic rhinitis and restore the normal architecture of the nasal mucosa ¹³¹⁻¹³³.

Steroid administration is also seen to coincide with a reduction in the number of inflammatory cells and Th2 type cytokines within the nasal mucosa of patients with allergic rhinitis. Corticosteroids treatments have been found to reduce the number of basophils, eosinophils and the level of ECP release of patients with allergic rhinitis ^{97,112}. Although they do not appear to reduce mast cell or T lymphocyte number within the nasal mucosa, steroids are thought to reduce the activity of these inflammatory cells ^{97,134-136}.

Corticosteroids are thought to play a role in limiting cytokine and chemokine synthesis by inhibiting allergen-induced expression of mRNA encoding IL-4, IL-13, and eotaxin, a potent eosinophil chemotactic factor ^{112,122,124,135,137,138}. In allergic

chronic sinusitis, steroids have been seen to reduce the production of IL-4 and IL-13 ¹²⁷ as well as the number of cells expressing receptors for IL-4, IL-5 and GM-CSF ¹³⁹.

Immunotherapy is recommended for the small group of patients with atopic disease who fail to respond to standard pharmacotherapy. Durham et al. have recently shown that grass pollen immunotherapy is associated with a markedly reduced response to allergen challenge, including decreased infiltration of T lymphocytes and reduced expression of IL-4 mRNA in response to allergen challenge ¹⁴⁰. The beneficial effect of immunotherapy is thought to result from an inhibition of the late phase response by decreasing the infiltration of CD4+ T lymphocytes and eosinophils, and decreasing the release of histamine from basophils ¹⁴¹⁻¹⁴³. Although the exact mechanism of this effect is unclear, it appears to be mediated by the induction of Th1 cytokine expression, including IL-12 and IFN- γ , which leads to the subsequent downregulation of the Th2 response ^{141,144-146}.

1.6 Rationale for this study

The role of atopy in the pathogenesis of upper airway inflammatory diseases such as allergic rhinitis and chronic sinusitis has been clearly established in the literature. The role of atopy in the development of middle ear disease, however, remains unclear with evidence both supporting and refuting this hypothesis.

Recent studies have demonstrated that cells implicated in the allergic response are present in the middle ear effusions and mucosal specimens of atopic children with OME. There is a paucity of information, however, concerning the role of the Th2 cytokines in the pathogenesis of OME in atopic and non-atopic children.

Given that the middle ear mucosa is contiguous with that of the nasal cavity and sinuses via the Eustachian tube, it is reasonable to hypothesize that allergic inflammatory responses may play a role in the pathogenesis of OME.

1.7 General hypothesis

We hypothesize that the MEE of atopic children with OME is characterized by cellular and cytokine profiles consistent with the late-phase allergic response.

1.8 Aims of this study

1. To determine the atopic status of children with OME by skin testing for common perennial and seasonal allergens.
2. To investigate the cellular profile of MEE from atopic and non-atopic children using immunocytochemistry with markers for eosinophils (MBP), T lymphocytes (CD3), basophils (BB1), mast cells (tryptase) and neutrophils (elastase).
3. To investigate the expression of mRNA for IL-4, IL-5, and IFN- γ in MEE of atopic and non-atopic children using in-situ hybridization.

1.9 References

1. Zielhuis GA, Rach GH, van den Broek P. Predisposing factors for otitis media with effusion in young children. *Adv Otorhinolaryngol* 1988; 40:65-9.
2. Cypress BK. Patterns of ambulatory care in pediatrics: The National Ambulatory Medical Care Survey. *Vital Health Stat* 13 1983; 75:1-60.
3. Teele DW, Klein JO, Rosner BA. Otitis media with effusion during the first three years of life and development of speech and language. *Pediatrics* 1984; 74:282-7.
4. Watson P, Voss L, Barber C, Aickin R, Bremner D, Lennon D. The microbiology of chronic otitis media with effusion in a group of Auckland children. *N Z Med J* 1996; 109:182-4.
5. Teele DW, Pelton SI, Klein JO. Bacteriology of acute otitis media unresponsive to initial antimicrobial therapy. *J Pediatr* 1981; 98:537-9.
6. Williams RL, Chalmers TC, Stange KC, Chalmers FT, Bowlin SJ. Use of antibiotics in preventing recurrent acute otitis media and in treating otitis media with effusion. A meta-analytic attempt to resolve the brouhaha. *Jama* 1993; 270:1344-51.
7. Alho OP, Oja H, Koivu M, Sorri M. Chronic otitis media with effusion in infancy. How frequent is it? How does it develop? *Arch Otolaryngol Head Neck Surg* 1995; 121:432-6.
8. Shaw CB, Obermyer N, Wetmore SJ, Spirou GA, Farr RW. Incidence of adenovirus and respiratory syncytial virus in chronic otitis media with effusion using the polymerase chain reaction. *Otolaryngol Head Neck Surg* 1995; 113:234-41.

9. Robinson DS, Hamid Q, Ying S, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992; 326:298-304.
10. Durham SR, Ying S, Varney VA, et al. Cytokine messenger RNA expression for IL-3, IL-4, IL-5, and granulocyte/macrophage-colony-stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. *J Immunol* 1992; 148:2390-4.
11. Hamilos DL, Leung DY, Wood R, et al. Evidence for distinct cytokine expression in allergic versus nonallergic chronic sinusitis. *J Allergy Clin Immunol* 1995; 96:537-44.
12. Hall LJ, Asuncion J, Lukat M. Allergy skin testing under general anesthesia with treatment response in ninety-two patients with chronic serous otitis media. *Am J Otol* 1980; 2:150-7.
13. Sorensen CH, Holm-Jensen S. Middle ear effusion and risk factors. *J Otolaryngol* 1982; 11:46-51.
14. McMahan JT, Calenoff E, Croft DJ, Barenholtz L, Weber LD. Chronic otitis media with effusion and allergy: modified RAST analysis of 119 cases. *Otolaryngol Head Neck Surg* 1981; 89:427-31.
15. Bernstein JM. The role of IgE-mediated hypersensitivity in the development of otitis media with effusion: a review. *Otolaryngol Head Neck Surg* 1993; 109:611-20.
16. Bernstein JM, Lee J, Conboy K, Ellis E, Li P. The role of IgE mediated hypersensitivity in recurrent otitis media with effusion. *Am J Otol* 1983; 5:66-9.

17. Bernstein JM, Lee J, Conboy K, Ellis E, Li P. Further observations on the role of IgE-mediated hypersensitivity in recurrent otitis media with effusion. *Otolaryngol Head Neck Surg* 1985; 93:611-5.
18. Mogi G, Tomonaga K, Watanabe T, Chaen T. The role of type I allergy in secretory otitis media and mast cells in the middle ear mucosa. *Acta Otolaryngol Suppl* 1992; 493:155-63.
19. Ojala K, Sipila P, Sorri M, Karma P. Role of atopic allergy in chronic otitis media. Evaluation based on serum IgE and nasal/aural cytologic findings in patients with operated chronic ears. *Acta Otolaryngol* 1982; 93:55-60.
20. Kraemer MJ, Richardson MA, Weiss NS, et al. Risk factors for persistent middle-ear effusions. Otitis media, catarrh, cigarette smoke exposure, and atopy. *Jama* 1983; 249:1022-5.
21. Alho OP, Koivu M, Sorri M, Rantakallio P. Risk factors for recurrent acute otitis media and respiratory infection in infancy. *Int J Pediatr Otorhinolaryngol* 1990; 19:151-61.
22. Tainio VM, Savilahti E, Salmenpera L, Arjomaa P, Siimes MA, Perheentupa J. Risk factors for infantile recurrent otitis media: atopy but not type of feeding. *Pediatr Res* 1988; 23:509-12.
23. Borge P. Atopy and secretory otitis media. Immunological studies and responses to topical corticosteroid therapy. *J Laryngol Otol* 1983; 97:117-29.
24. Kvaerner KJ, Tambs K, Harris JR, Mair IW, Magnus P. Otitis media: relationship to tonsillitis, sinusitis and atopic diseases. *Int J Pediatr Otorhinolaryngol* 1996; 35:127-41.

25. Sipila M, Karma P, Pukander J, Timonen M, Kataja M. The Bayesian approach to the evaluation of risk factors in acute and recurrent acute otitis media. *Acta Otolaryngol* 1988; 106:94-101.
26. Caffarelli C, Savini E, Giordano S, Gianlupi G, Cavagni G. Atopy in children with otitis media with effusion. *Clin Exp Allergy* 1998; 28:591-6.
27. Barbee RA, Kaltenborn W, Lebowitz MD, Burrows B. Longitudinal changes in allergen skin test reactivity in a community population sample. *J Allergy Clin Immunol* 1987; 79:16-24.
28. Gerritsen J, Koeter GH, de Monchy JG, Knol K. Allergy in subjects with asthma from childhood to adulthood. *J Allergy Clin Immunol* 1990; 85:116-25.
29. Kim YY, Cho SH, Kim WK, et al. Prevalence of childhood asthma based on questionnaires and methacholine bronchial provocation test in Korea. *Clin Exp Allergy* 1997; 27:761-8.
30. Lecks HI, Kravis LP, Wood DW. Serous otitis media: reflections on pathogenesis and treatment. With a comment on the use of intranasal dexamethasone (turbinaire). 1967. *Clin Pediatr (Phila)* 1991; 30:174-7; discussion 178-82.
31. Bernstein JM, Tsutsumi H, Ogra PL. The middle ear mucosal immune system in otitis media with effusion. *Am J Otolaryngol* 1985; 6:162-8.
32. Schutte PK, Beales DL, Dalton R. Secretory otitis media--a retrospective general practice survey. *J Laryngol Otol* 1981; 95:17-22.
33. Tomonaga K, Kurono Y, Mogi G. The role of nasal allergy in otitis media with effusion. A clinical study. *Acta Otolaryngol Suppl* 1988; 458:41-7.

34. Draper WL. Secretory otitis media in children: a study of 540 children. *Laryngoscope* 1967; 77:636-53.
35. Leung R, Bishop J, Robertson CF. Prevalence of asthma and wheeze in Hong Kong schoolchildren: an international comparative study. *Eur Respir J* 1994; 7:2046-9.
36. Speight AN, Lee DA, Hey EN. Underdiagnosis and undertreatment of asthma in childhood. *Br Med J (Clin Res Ed)* 1983; 286:1253-6.
37. Clifford RD, Radford M, Howell JB, Holgate ST. Prevalence of respiratory symptoms among 7 and 11 year old schoolchildren and association with asthma. *Arch Dis Child* 1989; 64:1118-25.
38. Hill RA, Standen PJ, Tattersfield AE. Asthma, wheezing, and school absence in primary schools. *Arch Dis Child* 1989; 64:246-51.
39. Broder I, Higgins MW, Mathews KP, Keller JB. Epidemiology of asthma and allergic rhinitis in a total community, Tecumseh, Michigan. IV. Natural history. *J Allergy Clin Immunol* 1974; 54:100-10.
40. Viner AS, Jackman N. Retrospective survey of 1271 patients diagnosed as perennial rhinitis. *Clin Allergy* 1976; 6:251-9.
41. Mogi G, Suzuki M. The role of IgE-mediated immunity in otitis media: fact or fiction? *Ann N Y Acad Sci* 1997; 830:61-9.
42. Ackerman MN, Friedman RA, Doyle WJ, Bluestone CD, Fireman P. Antigen-induced eustachian tube obstruction: an intranasal provocative challenge test. *J Allergy Clin Immunol* 1984; 73:604-9.

43. Fireman P. Nasal provocation testing: an objective assessment for nasal and eustachian tube obstruction. *J Allergy Clin Immunol* 1988; 81:953-60.
44. Fireman P. Otitis media and eustachian tube dysfunction: connection to allergic rhinitis. *J Allergy Clin Immunol* 1997; 99:S787-97.
45. Fireman P. Allergy induced eustachian tube and middle ear pathophysiology. *N Engl Reg Allergy Proc* 1986; 7:246-52.
46. Osur SL, Volovitz B, Dickson S, Enck DC, Bernstein JM. Eustachian tube dysfunction in children with ragweed hayfever during natural pollen exposure. *Allergy Proc* 1989; 10:133-9.
47. Crifo S, Cittadini S, De Seta E, Andriani G. Eustachian tube permeability during the nasal provocation test. *Rhinology* 1977; 15:81-5.
48. Skoner DP, Doyle WJ, Chamovitz AH, Fireman P. Eustachian tube obstruction after intranasal challenge with house dust mite. *Arch Otolaryngol Head Neck Surg* 1986; 112:840-2.
49. Bernstein JM. Role of allergy in eustachian tube blockage and otitis media with effusion: a review. *Otolaryngol Head Neck Surg* 1996; 114:562-8.
50. Tomonaga K, Chaen T, Kurono Y, Mogi G. Type I allergic reactions of the middle ear and eustachian tube: an experimental study. *Auris Nasus Larynx* 1990; 17:121-31.
51. Mogi G, Chaen T, Tomonaga K. Influence of nasal allergic reactions on the clearance of middle ear effusion. *Arch Otolaryngol Head Neck Surg* 1990; 116:331-4.

52. Ichimiya I, Kawauchi H, Mogi G. Analysis of immunocompetent cells in the middle ear mucosa. *Arch Otolaryngol Head Neck Surg* 1990; 116:324-30.
53. Watanabe T, Kawauchi H, Fujiyoshi T, Mogi G. Distribution of mast cells in the tubotympanum of guinea pigs. *Ann Otol Rhinol Laryngol* 1991; 100:407-12.
54. Widemar L, Hellstrom S, Stenfors LE, Bloom GD. An overlooked site of tissue mast cells--the human tympanic membrane. Implications for middle ear affections. *Acta Otolaryngol* 1986; 102:391-5.
55. Ohkawara Y, Yamauchi K, Tanno Y, et al. Human lung mast cells and pulmonary macrophages produce tumor necrosis factor-alpha in sensitized lung tissue after IgE receptor triggering. *Am J Respir Cell Mol Biol* 1992; 7:385-92.
56. Bradding P, Feather IH, Howarth PH, et al. Interleukin 4 is localized to and released by human mast cells. *J Exp Med* 1992; 176:1381-6.
57. Wilson SJ, Lau L, Howarth PH. Inflammatory mediators in naturally occurring rhinitis. *Clin Exp Allergy* 1998; 28:220-7.
58. Tedeschi A, Milazzo N, Miadonna A. Nasal eosinophilia induced by PAF-acether is accompanied by the release of eosinophil cationic protein. *Eur Respir J* 1994; 7:1445-51.
59. Erjefalt I, Persson CG. Allergen, bradykinin, and capsaicin increase outward but not inward macromolecular permeability of guinea-pig tracheobronchial mucosa. *Clin Exp Allergy* 1991; 21:217-24.
60. Knani J, Campbell A, Enander I, Peterson CG, Michel FB, Bousquet J. Indirect evidence of nasal inflammation assessed by titration of inflammatory mediators

and enumeration of cells in nasal secretions of patients with chronic rhinitis. *J Allergy Clin Immunol* 1992; 90:880-9.

61. Bernstein JM, Park BH. Further observations on immunoregulation in children with chronic otitis media with effusion. *Auris Nasus Larynx* 1985; 12 Suppl 1:S58-62.
62. Phillips MJ, Knight NJ, Manning H, Abbott AL, Tripp WG. IgE and secretory otitis media. *Lancet* 1974; 2:1176-8.
63. Mogi G, Honjo S, Maeda S, Yoshida T, Watanabe N. Immunoglobulin E (IgE) in middle ear effusions. *Ann Otol Rhinol Laryngol* 1974; 83:393-8.
64. Palva T, Lehtinen T, Halmepuro L. Immunoglobulin E in mucoid secretory otitis media. *ORL J Otorhinolaryngol Relat Spec* 1985; 47:220-3.
65. Lewis DM, Schram JL, Birck HG, Lim DJ. Antibody activity in otitis media with effusion. *Ann Otol Rhinol Laryngol* 1979; 88:392-6.
66. Lewis DM, Schram JL, Lim DJ, Birck HG, Gleich G. Immunoglobulin E in chronic middle ear effusions: comparison of RIST, PRIST, and RIA techniques. *Ann Otol Rhinol Laryngol* 1978; 87:197-201.
67. Boedts D, De Groote G, Van Vuchelen J. Atopic allergy and otitis media with effusion. *Acta Otolaryngol Suppl* 1984; 414:108-14.
68. Labadie RF, Jewett BS, Hart CF, Prazma J, Pillsbury HC, 3rd. Allergy increases susceptibility to otitis media with effusion in a rat model. Second place--Resident Clinical Science Award 1998. *Otolaryngol Head Neck Surg* 1999; 121:687-92.
69. Gleich GJ, Adolphson CR. The eosinophilic leukocyte: structure and function. *Adv Immunol* 1986; 39:177-253.

70. Weller PF. The immunobiology of eosinophils. *N Engl J Med* 1991; 324:1110-8.
71. Harlin SL, Ansel DG, Lane SR, Myers J, Kephart GM, Gleich GJ. A clinical and pathologic study of chronic sinusitis: the role of the eosinophil. *J Allergy Clin Immunol* 1988; 81:867-75.
72. Frew AJ, Kay AB. The relationship between infiltrating CD4+ lymphocytes, activated eosinophils, and the magnitude of the allergen-induced late phase cutaneous reaction in man. *J Immunol* 1988; 141:4158-64.
73. Hisamatsu K, Ganbo T, Nakazawa T, et al. Cytotoxicity of human eosinophil granule major basic protein to human nasal sinus mucosa in vitro. *J Allergy Clin Immunol* 1990; 86:52-63.
74. Holgate ST, Bradding P, Sampson AP. Leukotriene antagonists and synthesis inhibitors: new directions in asthma therapy. *J Allergy Clin Immunol* 1996; 98:1-13.
75. Moy JN, Gleich GJ, Thomas LL. Noncytotoxic activation of neutrophils by eosinophil granule major basic protein. Effect on superoxide anion generation and lysosomal enzyme release. *J Immunol* 1990; 145:2626-32.
76. Oddera S, Silvestri M, Balbo A, et al. Airway eosinophilic inflammation, epithelial damage, and bronchial hyperresponsiveness in patients with mild-moderate, stable asthma. *Allergy* 1996; 51:100-7.
77. Thomas LL, Zheutlin LM, Gleich GJ. Pharmacological control of human basophil histamine release stimulated by eosinophil granule major basic protein. *Immunology* 1989; 66:611-5.

78. Weller PF, Lee CW, Foster DW, Corey EJ, Austen KF, Lewis RA. Generation and metabolism of 5-lipoxygenase pathway leukotrienes by human eosinophils: predominant production of leukotriene C4. *Proc Natl Acad Sci U S A* 1983; 80:7626-30.
79. Toledano BJ, Bastien Y, Noya F, Baruchel S, Mazer B. Platelet-activating factor abrogates apoptosis induced by cross-linking of the surface IgM receptor in a human B lymphoblastoid cell line. *J Immunol* 1997; 158:3705-15.
80. Moqbel R, Levi-Schaffer F, Kay AB. Cytokine generation by eosinophils. *J Allergy Clin Immunol* 1994; 94:1183-8.
81. Bernstein JM, Reisman R. The role of acute hypersensitivity in secretory otitis media. *Trans Am Acad Ophthalmol Otolaryngol* 1974; 78:ORL120-7.
82. Wright ED, Miotto D, Giguere C, Hamid Q. Increased expression of major basic protein (MBP) and interleukin-5(IL-5) in middle ear biopsy specimens from atopic patients with persistent otitis media with effusion. *Otolaryngol Head Neck Surg* 2000; 123:533-8.
83. Hurst DS, Amin K, Seveus L, Venge P. Mast cells and tryptase in the middle ear of children with otitis media with effusion. *Int J Pediatr Otorhinolaryngol* 1999; 49 Suppl 1:S315-9.
84. Amin K, Hurst DS, Roomans GM, Venge P, Seveus L. Eosinophils and neutrophils in biopsies from the middle ear of atopic children with otitis media with effusion. *Inflamm Res* 1999; 48:626-31.

85. Hurst DS, Amin K, Seveus L, Venge P. Evidence of mast cell activity in the middle ears of children with otitis media with effusion. *Laryngoscope* 1999; 109:471-7.
86. Hurst DS, Fredens K. Eosinophil cationic protein in mucosal biopsies from patients with allergy and otitis media with effusion. *Otolaryngol Head Neck Surg* 1997; 117:42-8.
87. Hurst DS, Venge P. Levels of eosinophil cationic protein and myeloperoxidase from chronic middle ear effusion in patients with allergy and/or acute infection. *Otolaryngol Head Neck Surg* 1996; 114:531-44.
88. Hurst DS, Venge P. The presence of eosinophil cationic protein in middle ear effusion. *Otolaryngol Head Neck Surg* 1993; 108:711-22.
89. Lim DJ, Birck H. Ultrastructural pathology of the middle ear mucosa in serous otitis media. *Ann Otol Rhinol Laryngol* 1971; 80:838-53.
90. Sipila P, Karma P. Inflammatory cells in mucoid effusion of secretory otitis media. *Acta Otolaryngol* 1982; 94:467-72.
91. Hurst DS, Venge P. Evidence of eosinophil, neutrophil, and mast-cell mediators in the effusion of OME patients with and without atopy. *Allergy* 2000; 55:435-41.
92. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989; 7:145-73.
93. Liew FY, Millott S, Li Y, Lechuk R, Chan WL, Ziltener H. Macrophage activation by interferon-gamma from host-protective T cells is inhibited by

- interleukin (IL)3 and IL4 produced by disease-promoting T cells in leishmaniasis. *Eur J Immunol* 1989; 19:1227-32.
94. Urban JF, Jr., Katona IM, Paul WE, Finkelman FD. Interleukin 4 is important in protective immunity to a gastrointestinal nematode infection in mice. *Proc Natl Acad Sci U S A* 1991; 88:5513-7.
 95. Christodoulopoulos P, Cameron L, Durham S, Hamid Q. Molecular pathology of allergic disease. II: Upper airway disease. *J Allergy Clin Immunol* 2000; 105:211-23.
 96. Iliopoulos O, Baroody FM, Naclerio RM, Bochner BS, Kagey-Sobotka A, Lichtenstein LM. Histamine-containing cells obtained from the nose hours after antigen challenge have functional and phenotypic characteristics of basophils. *J Immunol* 1992; 148:2223-8.
 97. Bascom R, Wachs M, Naclerio RM, Pipkorn U, Galli SJ, Lichtenstein LM. Basophil influx occurs after nasal antigen challenge: effects of topical corticosteroid pretreatment. *J Allergy Clin Immunol* 1988; 81:580-9.
 98. Naclerio RM, Baroody FM, Kagey-Sobotka A, Lichtenstein LM. Basophils and eosinophils in allergic rhinitis. *J Allergy Clin Immunol* 1994; 94:1303-9.
 99. Kotsimbos AT, Hamid Q. IL-5 and IL-5 receptor in asthma. *Mem Inst Oswaldo Cruz* 1997; 92 Suppl 2:75-91.
 100. Paul WE, Seder RA. Lymphocyte responses and cytokines. *Cell* 1994; 76:241-51.
 101. Del Prete G, Maggi E, Parronchi P, et al. IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cell clones and their supernatants. *J Immunol* 1988; 140:4193-8.

102. Schleimer RP, Sterbinsky SA, Kaiser J, et al. IL-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium. Association with expression of VCAM-1. *J Immunol* 1992; 148:1086-92.
103. Ondrey FG, Juhn SK, Adams GL. Early-response cytokine expression in adult middle ear effusions. *Otolaryngol Head Neck Surg* 1998; 119:342-5.
104. Nassif PS, Simpson SQ, Izzo AA, Nicklaus PJ. Interleukin-8 concentration predicts the neutrophil count in middle ear effusion. *Laryngoscope* 1997; 107:1223-7.
105. Nassif PS, Simpson SQ, Izzo AA, Nicklaus PJ. Epidermal growth factor and transforming growth factor-alpha in middle ear effusion. *Otolaryngol Head Neck Surg* 1998; 119:564-8.
106. Storgaard M, Larsen K, Blegvad S, et al. Interleukin-8 and chemotactic activity of middle ear effusions. *J Infect Dis* 1997; 175:474-7.
107. Sanico AM, Atsuta S, Proud D, Togias A. Dose-dependent effects of capsaicin nasal challenge: in vivo evidence of human airway neurogenic inflammation. *J Allergy Clin Immunol* 1997; 100:632-41.
108. McDonald DM. Neurogenic inflammation in the respiratory tract: actions of sensory nerve mediators on blood vessels and epithelium of the airway mucosa. *Am Rev Respir Dis* 1987; 136:S65-72.
109. Sommerhoff CP, Caughey GH, Finkbeiner WE, Lazarus SC, Basbaum CB, Nadel JA. Mast cell chymase. A potent secretagogue for airway gland serous cells. *J Immunol* 1989; 142:2450-6.

110. Persson CG, Svensson C, Greiff L, et al. The use of the nose to study the inflammatory response of the respiratory tract. *Thorax* 1992; 47:993-1000.
111. Pawankar R, Okuda M, Yssel H, Okumura K, Ra C. Nasal mast cells in perennial allergic rhinitis exhibit increased expression of the Fc epsilonRI, CD40L, IL-4, and IL-13, and can induce IgE synthesis in B cells. *J Clin Invest* 1997; 99:1492-9.
112. Lozewicz S, Wang J, Duddle J, et al. Topical glucocorticoids inhibit activation by allergen in the upper respiratory tract. *J Allergy Clin Immunol* 1992; 89:951-7.
113. Bradding P, Feather IH, Wilson S, et al. Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects. The mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. *J Immunol* 1993; 151:3853-65.
114. Iliopoulos O, Proud D, Adkinson NF, Jr., et al. Relationship between the early, late, and rechallenge reaction to nasal challenge with antigen: observations on the role of inflammatory mediators and cells. *J Allergy Clin Immunol* 1990; 86:851-61.
115. Naclerio RM. Pathophysiology of perennial allergic rhinitis. *Allergy* 1997; 52:7-13.
116. Okuda M, Watase T, Mezawa A, Liu CM. The role of leukotriene D4 in allergic rhinitis. *Ann Allergy* 1988; 60:537-40.
117. Doyle WJ, Boehm S, Skoner DP. Physiologic responses to intranasal dose-response challenges with histamine, methacholine, bradykinin, and prostaglandin in adult volunteers with and without nasal allergy. *J Allergy Clin Immunol* 1990; 86:924-35.

118. Naclerio RM. Effects of antihistamines on inflammatory mediators. *Ann Allergy* 1993; 71:292-5.
119. Juliusson S, Pipkorn U, Karlsson G, Enerback L. Mast cells and eosinophils in the allergic mucosal response to allergen challenge: changes in distribution and signs of activation in relation to symptoms. *J Allergy Clin Immunol* 1992; 90:898-909.
120. Pelikan Z. Late and delayed responses of the nasal mucosa to allergen challenge. *Ann Allergy* 1978; 41:37-47.
121. Bentley AM, Meng Q, Robinson DS, Hamid Q, Kay AB, Durham SR. Increases in activated T lymphocytes, eosinophils, and cytokine mRNA expression for interleukin-5 and granulocyte/macrophage colony-stimulating factor in bronchial biopsies after allergen inhalation challenge in atopic asthmatics. *Am J Respir Cell Mol Biol* 1993; 8:35-42.
122. Ghaffar O, Laberge S, Jacobson MR, et al. IL-13 mRNA and immunoreactivity in allergen-induced rhinitis: comparison with IL-4 expression and modulation by topical glucocorticoid therapy. *Am J Respir Cell Mol Biol* 1997; 17:17-24.
123. Varney VA, Jacobson MR, Sudderick RM, et al. Immunohistology of the nasal mucosa following allergen-induced rhinitis. Identification of activated T lymphocytes, eosinophils, and neutrophils. *Am Rev Respir Dis* 1992; 146:170-6.
124. Ying S, Durham SR, Jacobson MR, et al. T lymphocytes and mast cells express messenger RNA for interleukin-4 in the nasal mucosa in allergen-induced rhinitis. *Immunology* 1994; 82:200-6.

125. Ying S, Durham SR, Barkans J, et al. T cells are the principal source of interleukin-5 mRNA in allergen-induced rhinitis. *Am J Respir Cell Mol Biol* 1993; 9:356-60.
126. Hamilos DL, Leung DY, Wood R, et al. Chronic hyperplastic sinusitis: association of tissue eosinophilia with mRNA expression of granulocyte-macrophage colony-stimulating factor and interleukin-3. *J Allergy Clin Immunol* 1993; 92:39-48.
127. al Ghamdi K, Ghaffar O, Small P, Frenkiel S, Hamid Q. IL-4 and IL-13 expression in chronic sinusitis: relationship with cellular infiltrate and effect of topical corticosteroid treatment. *J Otolaryngol* 1997; 26:160-6.
128. Wright ED, Frenkiel S, Al-Ghamdi K, et al. Interleukin-4, interleukin-5, and granulocyte-macrophage colony-stimulating factor receptor expression in chronic sinusitis and response to topical steroids. *Otolaryngol Head Neck Surg* 1998; 118:490-5.
129. Suzuki M, Kerakawauchi H, Kawauchi H, Mogi G. Efficacy of an antiallergic drug on otitis media with effusion in association with allergic rhinitis. An experimental study. *Ann Otol Rhinol Laryngol* 1999; 108:554-8.
130. Hurst DS. Allergy management of refractory serous otitis media. *Otolaryngol Head Neck Surg* 1990; 102:664-9.
131. Scadding GK, Lund VJ, Jacques LA, Richards DH. A placebo-controlled study of fluticasone propionate aqueous nasal spray and beclomethasone dipropionate in perennial rhinitis: efficacy in allergic and non-allergic perennial rhinitis. *Clin Exp Allergy* 1995; 25:737-43.

132. Mygind N. Glucocorticosteroids and rhinitis. *Allergy* 1993; 48:476-90.
133. Orgel HA, Meltzer EO, Bierman CW, et al. Intranasal flucortin butyl in patients with perennial rhinitis: a 12-month efficacy and safety study including nasal biopsy. *J Allergy Clin Immunol* 1991; 88:257-64.
134. Rak S, Jacobson MR, Sudderick RM, et al. Influence of prolonged treatment with topical corticosteroid (fluticasone propionate) on early and late phase nasal responses and cellular infiltration in the nasal mucosa after allergen challenge. *Clin Exp Allergy* 1994; 24:930-9.
135. Meltzer EO. Nasal cytological changes following pharmacological intervention. *Allergy* 1995; 50:15-20.
136. Okuda M, Sakaguchi K, Ohtsuka H. Intranasal beclomethasone: mode of action in nasal allergy. *Ann Allergy* 1983; 50:116-20.
137. Christodoulopoulos P, Wright E, Frenkiel S, Luster A, Hamid Q. Monocyte chemotactic proteins in allergen-induced inflammation in the nasal mucosa: effect of topical corticosteroids. *J Allergy Clin Immunol* 1999; 103:1036-44.
138. Minshall EM, Cameron L, Lavigne F, et al. Eotaxin mRNA and protein expression in chronic sinusitis and allergen-induced nasal responses in seasonal allergic rhinitis. *Am J Respir Cell Mol Biol* 1997; 17:683-90.
139. Kondo H, Nachtigal D, Frenkiel S, Schotman E, Hamid Q. Effect of steroids on nasal inflammatory cells and cytokine profile. *Laryngoscope* 1999; 109:91-7.
140. Durham SR, Walker SM, Varga EM, et al. Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med* 1999; 341:468-75.

141. Durham SR, Ying S, Varney VA, et al. Grass pollen immunotherapy inhibits allergen-induced infiltration of CD4+ T lymphocytes and eosinophils in the nasal mucosa and increases the number of cells expressing messenger RNA for interferon-gamma. *J Allergy Clin Immunol* 1996; 97:1356-65.
142. Mayer M, Doenicke A, Nebauer AE, Rosenberger C, Lorenz W, Peter K. [Pharmacodynamics and clinical adverse effects of mivacurium. The effect of oral premedication with H1/H2 antagonists]. *Anaesthesist* 1993; 42:592-6.
143. Kuna P, Alam R, Kuzminska B, Rozniecki J. The effect of preseasonal immunotherapy on the production of histamine-releasing factor (HRF) by mononuclear cells from patients with seasonal asthma: results of a double-blind, placebo-controlled, randomized study. *J Allergy Clin Immunol* 1989; 83:816-24.
144. Hamid QA, Schotman E, Jacobson MR, Walker SM, Durham SR. Increases in IL-12 messenger RNA+ cells accompany inhibition of allergen-induced late skin responses after successful grass pollen immunotherapy. *J Allergy Clin Immunol* 1997; 99:254-60.
145. Gajewski TF, Fitch FW. Anti-proliferative effect of IFN-gamma in immune regulation. IV. Murine CTL clones produce IL-3 and GM-CSF, the activity of which is masked by the inhibitory action of secreted IFN-gamma. *J Immunol* 1990; 144:548-56.
146. Rousset F, Robert J, Andary M, et al. Shifts in interleukin-4 and interferon-gamma production by T cells of patients with elevated serum IgE levels and the modulatory effects of these lymphokines on spontaneous IgE synthesis. *J Allergy Clin Immunol* 1991; 87:58-69.

Chapter 2. Th2 cytokine expression in atopic children with otitis media with effusion

2.1 Abstract

2.2 Introduction

2.3 Methods

2.3.1 *Patient selection*

2.3.2 *Determination of atopy*

2.3.3 *Middle ear effusion collection methods*

2.3.4 *Immunocytochemistry*

2.3.5 *In-situ hybridization*

2.3.6 *Quantification*

2.3.7 *Statistical analysis*

2.4 Results

2.4.1 *Clinical data*

2.4.2 *Cellular composition of middle ear effusion specimens*

2.4.3 *Cytokine profile of middle ear effusion specimens*

2.5 Discussion

2.6 Acknowledgments

2.7 References

2.8 Tables

2.8.1 *Table 1*

2.8.2 *Table 2*

2.9 Figure legend

2.10 Figures

2.1 Abstract

Background: Otitis media with effusion (OME) is more common in atopic children. Few studies have looked for the presence of inflammatory mediators in the middle ear effusions of this population.

Objectives: We hypothesize that atopic children with OME have a different inflammatory cell and cytokine profile than non-atopic children with the disease.

Methods: Twenty-six patients with OME undergoing myringotomy and ventilation tube placement were recruited at the McGill University Hospital Center. The atopic status was determined for each patient using standard skin testing. Using immunocytochemistry, fluid specimens were assessed for T lymphocytes (CD3), eosinophils (MBP), neutrophils (elastase), mast cells (tryptase) and basophils (BB1). Using in-situ hybridization the expression of IL-4, IL-5, and IFN- γ mRNA was assessed.

Results: There is a higher percentage of eosinophils and T lymphocytes in atopic OME patients (n=8) compared to the non-atopic group (n=18) ($p<0.01$). There is a higher percentage of neutrophils in non-atopic OME patients compared to the atopic group ($p<0.01$). In examining cytokine profiles, there is a higher percentage of cells expressing IL-4 and IL-5 mRNA in atopic OME patients compared to the non-atopic group ($p<0.01$).

Conclusions: The predominance of eosinophils, T lymphocytes and Th2 mediators in the middle ear effusions of atopic patients provides strong evidence that allergy plays a role in the pathogenesis of this condition and emphasizes the need to introduce anti-allergy therapy into the treatment algorithm of OME.

2.2 Introduction

Otitis media with effusion (OME) is a major pediatric health care issue, with an estimated prevalence of 15-20 % ¹, and annual costs in the billions of dollars ². OME is the most common indication for surgery in children, and can have significant sequelae, including hearing loss, maldevelopment of communication skills, as well as permanent middle ear mucosal damage ³. Although the exact etiology of OME is not known, it is thought to occur after a treated acute infection, in the setting of Eustachian tube dysfunction. However, 57-82 % of effusions do not have bacterial growth ⁴, and antibiotic therapy is no better than placebo in the resolution of OME ⁵⁻⁷. Some authors have proposed a viral etiology, but viral isolates are only present in 16-19 % of cases ⁸.

The inflammatory cell and cytokine profile of asthma ⁹, allergic rhinitis ¹⁰, and chronic sinusitis ¹¹ is well documented in the literature. Mast cell degranulation, occurring in response to allergen binding of cross-linked IgE, is the predominant effector of early allergic responses. Eosinophils, basophils, T-helper cells, and the Th2 cytokines (IL-4, IL-5) are responsible for the late phase allergic response. Given that the middle ear is contiguous with the upper airway, it is reasonable to hypothesize that allergy mediators play a role in the development of chronic middle ear effusions in atopic children.

We hypothesize that there is a higher number of eosinophils, basophils, mast cells and T lymphocytes and increased expression of Th2 cytokines such as IL-4, and IL-5 in the middle ear fluid specimens of atopic children with OME compared non-atopic controls.

2.3 Methods

2.3.1 Patient selection

Patients were recruited from the Department of Otolaryngology at the McGill University Hospital Center over a six-month period. Twenty-six patients with OME undergoing myringotomy and ventilation tube placement were included in the study. The criteria for myringotomy and ventilation tube placement in these patients was made based on a history of persistent MEE for greater than three months. Exclusion criteria included the presence of a known immunodeficiency disorder, craniofacial malformation, current middle ear infection or use of topical or systemic steroids within six weeks prior to surgery.

2.3.2 Determination of atopy

A history and physical examination was performed, assessing for general medical problems and risk factors for allergy. Eligible patients reviewed a detailed consent form approved by the investigational review board of the Montreal Children's Hospital. At the time of preoperative testing or during general anesthesia, skin testing for perennial and seasonal allergens was done, using a standard panel including alternaria, aspergillus, cladosporium, grass, penicillium, ragweed, trees, cat, cockroach, dog, and dust mites *D. Farinae*, and *D. Pteronyssinus*. Histamine (1 mg/ml) was used as a positive control and saline as a negative control. A positive skin reaction was judged as a wheal great or equal to 3mm greater than the negative control. Children were considered atopic if they had a positive skin test, and non-atopic if all skin tests were negative, regardless of clinical history.

2.3.3 Middle ear effusion collection methods

At the time of myringotomy, middle ear fluid was collected in a Juhn Tym-Taps (Xomed Treace Products, Jacksonville, FL). One milliliter (ml) of sterile phosphate-buffered saline (PBS) was added to the sample. After transportation to the Meakins-Christie Laboratories, the sample was resuspended and transferred to a siliconized centrifuge tube, and centrifuged at 1200 g for 10 minutes. The supernatant was stored at – 80 °c until assayed. One ml of mucolexx (Baxter, McGraw Park, IL) or erythrocyte lysing buffer (Sigma, St. Louis, MO) was added to the pellet for viscous or bloody samples, respectively. The pellet was resuspended and centrifuged, washed three times with sterile PBS and fixed. The pellet was cytocentrifuged at 750 g for 2 minutes onto frosted slides, and differential cell counts were made by Quick Diff (Baxter) staining and microscopic examination. Slides were also prepared for immunocytochemistry and in-situ hybridization. For ICC, the cytopins were briefly fixed in a solution of acetone:methanol (40:60), air dried, and stored at – 80 °c until further use. For ISH the cytopins were air-dried and fixed in 4 % paraformaldehyde for 30 minutes, washed twice for five minutes with PBS, kept at 37 c overnight, and stored at – 80 °c until further use.

2.3.4 Immunocytochemistry

Immunocytochemistry was performed using the alkaline phosphatase anti-alkaline phosphatase (APAAP) method, as previously described ¹². Monoclonal antibodies including anti-CD3, MBP, BB1, tryptase and elastase were used to detect T lymphocytes, eosinophils, basophils, mast cells and neutrophils, respectively. Slides were developed

using Fast Red substrate for alkaline phosphatase. A negative control slide was included in each ICC experiment.

2.3.5 In-situ hybridization

Radiolabelled complementary riboprobes for IL-4, IL-5, and IFN- γ were used to identify the presence of mRNA. The cDNA of each of these individual cytokines was inserted into pGEM vectors, grown in E.Coli, and linearized with the appropriate enzymes. Before ISH, in vitro transcription to generate sense and antisense probes was performed in the presence of ^{35}S -uridine triphosphate and the appropriate polymerases as previously reported ¹³. Cytospins were permeabilized, treated with acetic anhydride and triethanolamine to reduce non-specific binding, and pre-hybridized in formamide at 42 °c. ^{35}S -labelled antisense probes were used (10^6cpm/section), followed by high-stringency post-hybridization washings. Unhybridized single-stranded RNA was removed by treating the preparation with a solution of RNase. After hybridization, the slides were immersed in emulsion fluid and exposed for 10-14 days. The autoradiographs were developed, fixed and counterstained with hematoxylin. To ensure ISH specificity, sections and cytopins were hybridized with the sense probe or pre-treated with an RNase solution.

2.3.6 Quantification

Specimens were coded and the number of positive cells for protein and mRNA were counted 'blind' using an Olympus microscope with an eyepiece graticule at 200x

magnification. At least 6 fields were counted for each patient at each time point. Results were expressed as the mean percentage of positive cells $\pm$ the SEM.

2.3.7 Statistical analysis

Cell counts were compared between atopic and non-atopic patients using a Mann Whitney U test, with $p < 0.01$ regarded as statistically significant. At least two high power fields were counted for each marker, and the mean value of these slides was reported. To avoid observer bias, slides was coded before analysis and read in a blinded fashion.

2.4 Results

2.4.1 Clinical data

Tables 1 and 2 demonstrate the clinical data of atopic and non-atopic children with OME. In atopic OME patients (n=8), the mean age was 4.75 (2-7) years compared to 3.56 (1-8) years in the non-atopic group (n=18). Forty-six percent of patients greater than 3 years of age had at least one positive skin test, compared to 17 % of children less than 3 years.

Two patients (25 %) in the atopic group and 4 (22 %) non-atopic controls had a history of asthma. Two patients (25 %) in the atopic group and 5 (28 %) non-atopic controls had a history of rhinitis. One patient (13 %) in the atopic group and 1 (6 %) of the non-atopic controls had a history of eczema. One patient (13 %) in the atopic group and 5 (29 %) non-atopic controls had a family history of allergy.

Atopic patients were most commonly reactive to dog dander (62.5 %). Fifty percent of atopic patients were reactive to dust mite, 25 % to cat dander, and 13 % to tree mix and ragweed. No patients were reactive to cockroach, grass or molds.

2.4.2 Cellular composition of middle ear effusion specimens (figures 1,2)

The percentage of eosinophils and T lymphocytes is significantly higher in the MEE of atopic patients (7.7 ± 1.4 ; 20.0 ± 3.2) compared to non-atopic patients (0.9 ± 0.2 ; 5.5 ± 0.6) ($p < 0.01$). On the other hand, a significantly higher percentage of neutrophils are found in the MEE of non-atopic patients (43.0 ± 2.4), compared to atopic patients (15.3 ± 3.1) ($p < 0.01$). There is no significant difference in the percentage of mast cells found in MEE of atopic patients (7.6 ± 2.2) compared to non-atopic patients (3.4 ± 0.6)

($p=0.029$). There is no significant difference in the percentage of basophils found in MEE of atopic patients (6.0 ± 2.0) compared to non-atopic patients (1.8 ± 0.4) ($p=0.015$).

2.4.3 Cytokine profile of middle ear effusion specimens (figures 3,4)

The percentage of IL-4 and IL-5 mRNA positive cells is significantly higher in the MEE of atopic patients (6.1 ± 0.5 ; 7.5 ± 0.6), compared to non-atopic patients (1.7 ± 0.3 ; 2.6 ± 0.4) ($p<0.01$). On the other hand, a significantly higher percentage of IFN- γ positive cells are found in the MEE of non-atopic patients (6.0 ± 0.4), compared to atopic patients (2.0 ± 0.5) ($p<0.01$).

2.5 Discussion

In our study population, we found a 30 % incidence of atopy by routine skin testing. Seven of the eight atopic children were reactive to perennial allergens. The role of atopy in the pathogenesis of recurrent otitis media and OME has been evaluated, and has been reported to be a risk factor in 0-88 % of patients ¹⁴⁻²⁰. In theory, allergic inflammation can cause recurrent otitis media or OME indirectly by causing a transient blockage of the Eustachian tube ^{21, 22}, or directly by middle ear mucosal inflammation. While some investigators have concluded that atopy is a significant risk factor for recurrent otitis media ²³⁻²⁸, others have found no relationship between the two conditions ^{29,30}. The major problem with most of these studies is the lack of uniform diagnostic criteria for atopy.

Atopic children in our study have significantly higher percentages of T lymphocytes, eosinophils, IL-4 and IL-5 mRNA positive cells compared non-atopic children. Although not statistically significant, the percentage of mast cells and basophils was also higher in the atopic effusions compared to controls. These results are consistent with the late phase allergic response seen in other areas of the respiratory tract, which is implicated in the pathogenesis of asthma, allergic rhinitis, and chronic sinusitis. Eosinophils play a particularly important role in the late phase allergic response through the release of mediators including, ECP, MBP, and LTC₄, which causes extracellular matrix deposition, epithelial denudation and basement membrane disruption ^{12,31-36}. Basophils are thought to play an important role in the late phase allergic response, although their exact role has yet to be clearly elucidated. Similar to the mast cell, basophils bind allergen via IgE cross linkage and release histamine upon activation ³⁷.

Since the level of histamine, but not tryptase is found to be elevated during the late phase response, basophils are thought to be the major source of this mediator as opposed to secondary mast cell degranulation ³⁷⁻³⁹.

IL-5 is important for eosinophil production, recruitment, activation, and survival ⁴⁰. IL-4 is important for the differentiation of naïve T-helper cells (TH0) towards the TH2 phenotype, and plays an important role in the local production of IgE in tissues of atopic patients ⁴¹. Through induction of endothelial VCAM-1, IL-4 is thought to promote eosinophil infiltration to the site of inflammation.

The recent literature has looked at the role of cytokines in middle ear effusions, both in animals and humans ⁴²⁻⁴⁵. Most studies have assessed for the presence of non-specific inflammatory mediators such as IL-1, IL-6, IL-8 and TNF- α . Others have looked for the presence of IgE antibodies in the systemic circulation and in middle ear fluid specimens ⁴⁶.

There have been a number of studies that have attempted to identify the role of allergic mediators in OME. Hurst et al showed that eosinophils were present in the fluid of atopic patients with OME ⁴⁷⁻⁵². Wright et al recently demonstrated increased expression of IL-5 and major basic protein (eosinophils) in the middle ear mucosa of patients with OME compared to normal controls ⁵³. Although these studies provide preliminary evidence for the role of allergy mediators in OME, they do not look at non-atopic patients with OME, and are limited by the necessity to do middle ear mucosal biopsies in order to measure appreciable levels of cytokines.

In summary, we have found that 30 % of children with OME in our study were atopic by skin testing. Seven of the eight atopic children were reactive to perennial

allergens. Middle ear effusions of atopic children with OME are characterized by higher percentages of eosinophils, T lymphocytes and Th2 positive cells compared to non-atopic controls. This inflammatory profile is consistent with that seen in other upper airway diseases whose pathogenesis is related to the late phase allergic response. It is likely that in atopic patients MEE develops after exposure to allergen, resulting in a sustained late phase allergic response.

Neutrophils and IFN- γ positive cells were present in significantly higher percentages in non-atopic patients, providing evidence that MEE in these patients are likely the result of an infectious process. It is likely that MEE in these patients develop after an acute infection in the presence of Eustachian tube dysfunction.

Demonstration of allergy mediators provides evidence for the need to introduce anti-allergy therapy in to the treatment algorithm of OME. This may include avoidance therapy and the use of specific pharmacotherapy for certain patients in order to prevent the complications of OME. These results would also provide the rationale for doing studies to further elucidate the role of allergy in the pathogenesis of OME.

2.6 Acknowledgments

The authors thank Elsa Schotman for her technical assistance, the Medical Research Council of Canada and the McGill Head and Neck Cancer Research Fund for financial support of this study.

2.7 References

1. Zielhuis GA, Rach GH, van den Broek P. Predisposing factors for otitis media with effusion in young children. *Adv Otorhinolaryngol* 1988; 40:65-9.
2. Cypress BK. Patterns of ambulatory care in pediatrics: The National Ambulatory Medical Care Survey. *Vital Health Stat* 13 1983; 75:1-60.
3. Teele DW, Klein JO, Rosner BA. Otitis media with effusion during the first three years of life and development of speech and language. *Pediatrics* 1984; 74:282-7.
4. Watson P, Voss L, Barber C, Aickin R, Bremner D, Lennon D. The microbiology of chronic otitis media with effusion in a group of Auckland children. *N Z Med J* 1996; 109:182-4.
5. Teele DW, Pelton SI, Klein JO. Bacteriology of acute otitis media unresponsive to initial antimicrobial therapy. *J Pediatr* 1981; 98:537-9.
6. Williams RL, Chalmers TC, Stange KC, Chalmers FT, Bowlin SJ. Use of antibiotics in preventing recurrent acute otitis media and in treating otitis media with effusion. A meta-analytic attempt to resolve the brouhaha. *Jama* 1993; 270:1344-51.
7. Alho OP, Oja H, Koivu M, Sorri M. Chronic otitis media with effusion in infancy. How frequent is it? How does it develop? *Arch Otolaryngol Head Neck Surg* 1995; 121:432-6.
8. Shaw CB, Obermyer N, Wetmore SJ, Spirou GA, Farr RW. Incidence of adenovirus and respiratory syncytial virus in chronic otitis media with effusion using the polymerase chain reaction. *Otolaryngol Head Neck Surg* 1995; 113:234-41.

9. Robinson DS, Hamid Q, Ying S, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992; 326:298-304.
10. Durham SR, Ying S, Varney VA, et al. Cytokine messenger RNA expression for IL-3, IL-4, IL-5, and granulocyte/macrophage-colony-stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. *J Immunol* 1992; 148:2390-4.
11. Hamilos DL, Leung DY, Wood R, et al. Evidence for distinct cytokine expression in allergic versus nonallergic chronic sinusitis. *J Allergy Clin Immunol* 1995; 96:537-44.
12. Frew AJ, Kay AB. The relationship between infiltrating CD4+ lymphocytes, activated eosinophils, and the magnitude of the allergen-induced late phase cutaneous reaction in man. *J Immunol* 1988; 141:4158-64.
13. Hamid Q, Wharton J, Terenghi G, et al. Localization of atrial natriuretic peptide mRNA and immunoreactivity in the rat heart and human atrial appendage. *Proc Natl Acad Sci U S A* 1987; 84:6760-4.
14. Hall LJ, Asuncion J, Lukat M. Allergy skin testing under general anesthesia with treatment response in ninety-two patients with chronic serous otitis media. *Am J Otol* 1980; 2:150-7.
15. Sorensen CH, Holm-Jensen S. Middle ear effusion and risk factors. *J Otolaryngol* 1982; 11:46-51.
16. McMahan JT, Calenoff E, Croft DJ, Barenholtz L, Weber LD. Chronic otitis media with effusion and allergy: modified RAST analysis of 119 cases. *Otolaryngol Head Neck Surg* 1981; 89:427-31.

17. Bernstein JM. The role of IgE-mediated hypersensitivity in the development of otitis media with effusion: a review. *Otolaryngol Head Neck Surg* 1993; 109:611-20.
18. Bernstein JM, Lee J, Conboy K, Ellis E, Li P. The role of IgE mediated hypersensitivity in recurrent otitis media with effusion. *Am J Otol* 1983; 5:66-9.
19. Bernstein JM, Lee J, Conboy K, Ellis E, Li P. Further observations on the role of IgE-mediated hypersensitivity in recurrent otitis media with effusion. *Otolaryngol Head Neck Surg* 1985; 93:611-5.
20. Mogi G, Tomonaga K, Watanabe T, Chaen T. The role of type I allergy in secretory otitis media and mast cells in the middle ear mucosa. *Acta Otolaryngol Suppl* 1992; 493:155-63.
21. Ackerman MN, Friedman RA, Doyle WJ, Bluestone CD, Fireman P. Antigen-induced eustachian tube obstruction: an intranasal provocative challenge test. *J Allergy Clin Immunol* 1984; 73:604-9.
22. Fireman P. Nasal provocation testing: an objective assessment for nasal and eustachian tube obstruction. *J Allergy Clin Immunol* 1988; 81:953-60.
23. Ojala K, Sipila P, Sorri M, Karma P. Role of atopic allergy in chronic otitis media. Evaluation based on serum IgE and nasal/aural cytologic findings in patients with operated chronic ears. *Acta Otolaryngol* 1982; 93:55-60.
24. Kraemer MJ, Richardson MA, Weiss NS, et al. Risk factors for persistent middle-ear effusions. Otitis media, catarrh, cigarette smoke exposure, and atopy. *Jama* 1983; 249:1022-5.

25. Alho OP, Koivu M, Sorri M, Rantakallio P. Risk factors for recurrent acute otitis media and respiratory infection in infancy. *Int J Pediatr Otorhinolaryngol* 1990; 19:151-61.
26. Tainio VM, Savilahti E, Salmenpera L, Arjomaa P, Siimes MA, Perheentupa J. Risk factors for infantile recurrent otitis media: atopy but not type of feeding. *Pediatr Res* 1988; 23:509-12.
27. Borge P. Atopy and secretory otitis media. Immunological studies and responses to topical corticosteroid therapy. *J Laryngol Otol* 1983; 97:117-29.
28. Caffarelli C, Savini E, Giordano S, Gianlupi G, Cavagni G. Atopy in children with otitis media with effusion. *Clin Exp Allergy* 1998; 28:591-6.
29. Kvaerner KJ, Tambs K, Harris JR, Mair IW, Magnus P. Otitis media: relationship to tonsillitis, sinusitis and atopic diseases. *Int J Pediatr Otorhinolaryngol* 1996; 35:127-41.
30. Sipila M, Karma P, Pukander J, Timonen M, Kataja M. The Bayesian approach to the evaluation of risk factors in acute and recurrent acute otitis media. *Acta Otolaryngol* 1988; 106:94-101.
31. Harlin SL, Ansel DG, Lane SR, Myers J, Kephart GM, Gleich GJ. A clinical and pathologic study of chronic sinusitis: the role of the eosinophil. *J Allergy Clin Immunol* 1988; 81:867-75.
32. Hisamatsu K, Ganbo T, Nakazawa T, et al. Cytotoxicity of human eosinophil granule major basic protein to human nasal sinus mucosa in vitro. *J Allergy Clin Immunol* 1990; 86:52-63.

33. Holgate ST, Bradding P, Sampson AP. Leukotriene antagonists and synthesis inhibitors: new directions in asthma therapy. *J Allergy Clin Immunol* 1996; 98:1-13.
34. Moy JN, Gleich GJ, Thomas LL. Noncytotoxic activation of neutrophils by eosinophil granule major basic protein. Effect on superoxide anion generation and lysosomal enzyme release. *J Immunol* 1990; 145:2626-32.
35. Oddera S, Silvestri M, Balbo A, et al. Airway eosinophilic inflammation, epithelial damage, and bronchial hyperresponsiveness in patients with mild-moderate, stable asthma. *Allergy* 1996; 51:100-7.
36. Thomas LL, Zheutlin LM, Gleich GJ. Pharmacological control of human basophil histamine release stimulated by eosinophil granule major basic protein. *Immunology* 1989; 66:611-5.
37. Iliopoulos O, Baroody FM, Naclerio RM, Bochner BS, Kagey-Sobotka A, Lichtenstein LM. Histamine-containing cells obtained from the nose hours after antigen challenge have functional and phenotypic characteristics of basophils. *J Immunol* 1992; 148:2223-8.
38. Bascom R, Wachs M, Naclerio RM, Pipkorn U, Galli SJ, Lichtenstein LM. Basophil influx occurs after nasal antigen challenge: effects of topical corticosteroid pretreatment. *J Allergy Clin Immunol* 1988; 81:580-9.
39. Naclerio RM, Baroody FM, Kagey-Sobotka A, Lichtenstein LM. Basophils and eosinophils in allergic rhinitis. *J Allergy Clin Immunol* 1994; 94:1303-9.
40. Kotsimbos AT, Hamid Q. IL-5 and IL-5 receptor in asthma. *Mem Inst Oswaldo Cruz* 1997; 92 Suppl 2:75-91.

41. Christodoulopoulos P, Cameron L, Durham S, Hamid Q. Molecular pathology of allergic disease. II: Upper airway disease. *J Allergy Clin Immunol* 2000; 105:211-23.
42. Ondrey FG, Juhn SK, Adams GL. Early-response cytokine expression in adult middle ear effusions. *Otolaryngol Head Neck Surg* 1998; 119:342-5.
43. Nassif PS, Simpson SQ, Izzo AA, Nicklaus PJ. Interleukin-8 concentration predicts the neutrophil count in middle ear effusion. *Laryngoscope* 1997; 107:1223-7.
44. Nassif PS, Simpson SQ, Izzo AA, Nicklaus PJ. Epidermal growth factor and transforming growth factor-alpha in middle ear effusion. *Otolaryngol Head Neck Surg* 1998; 119:564-8.
45. Storgaard M, Larsen K, Blegvad S, et al. Interleukin-8 and chemotactic activity of middle ear effusions. *J Infect Dis* 1997; 175:474-7.
46. Mogi G, Suzuki M. The role of IgE-mediated immunity in otitis media: fact or fiction? *Ann N Y Acad Sci* 1997; 830:61-9.
47. Hurst DS, Amin K, Seveus L, Venge P. Mast cells and tryptase in the middle ear of children with otitis media with effusion. *Int J Pediatr Otorhinolaryngol* 1999; 49 Suppl 1:S315-9.
48. Amin K, Hurst DS, Roomans GM, Venge P, Seveus L. Eosinophils and neutrophils in biopsies from the middle ear of atopic children with otitis media with effusion. *Inflamm Res* 1999; 48:626-31.

49. Hurst DS, Amin K, Seveus L, Venge P. Evidence of mast cell activity in the middle ears of children with otitis media with effusion. *Laryngoscope* 1999; 109:471-7.
50. Hurst DS, Fredens K. Eosinophil cationic protein in mucosal biopsies from patients with allergy and otitis media with effusion. *Otolaryngol Head Neck Surg* 1997; 117:42-8.
51. Hurst DS, Venge P. Levels of eosinophil cationic protein and myeloperoxidase from chronic middle ear effusion in patients with allergy and/or acute infection. *Otolaryngol Head Neck Surg* 1996; 114:531-44.
52. Hurst DS, Venge P. The presence of eosinophil cationic protein in middle ear effusion. *Otolaryngol Head Neck Surg* 1993; 108:711-22.
53. Wright ED, Hurst D, Miotto D, Giguere C, Hamid Q. Increased expression of major basic protein (MBP) and interleukin-5(IL-5) in middle ear biopsy specimens from atopic patients with persistent otitis media with effusion. *Otolaryngol Head Neck Surg* 2000; 123:533-8.

2.8 Tables

2.8.1 Table 1: Clinical presentation of atopic and non-atopic children with otitis media
with effusion

	<u>Atopic OME patients</u>	<u>Non-atopic OME patients</u>
Age	4.75 (2-7 yrs)	3.56 (1-8 yrs)
Sex (M:F)	7:1	12:6
Asthma	2 (25%)	4 (22%)
Rhinitis	2 (25%)	5 (28%)
Eczema	1 (13%)	1 (6%)
Family history of allergy	1 (13%)	5 (29%)

2.8.2 Table 2: Results of skin prick testing in atopic children with otitis media with effusion

Dog	5
Dust Mite	4
Cat	2
Tree mix	1
Ragweed	1
Cockroach	0
Grass	0
Molds	0

2.9 Figure legend

Figure 1. *Inflammatory cells in the middle ear effusions of atopic and non-atopic children with otitis media with effusion*

This figure demonstrates the mean percentage of positive cells $\pm$ the standard error of the mean present in each patient group. The percentage of eosinophils and T lymphocytes is significantly higher in the MEE of atopic patients compared to non-atopic patients ($p < 0.01$). On the other hand, a significantly higher percentage of neutrophils are found in the MEE of non-atopic patients compared to atopic patients ($p < 0.01$). There is no significant difference in the percentage of basophils and mast cells found in MEE of atopic patients compared to non-atopic patients.

Figure 2. *Results of immunocytochemistry: MBP, CD3, and BB1 positive cells in the middle ear effusions of atopic children with otitis media with effusion*

Development of the slide using Fast Red substrate for alkaline phosphatase allows for the identification of (A) T lymphocytes (CD3), (B) eosinophils (MBP) and (C) basophils (BB1) in atopic patients with OME.

Figure 3. *Th1 and Th2 cytokine mRNA expression in the middle ear effusions of atopic and non-atopic children with otitis media with effusion*

This figure demonstrates mean percentage of cells expressing cytokine mRNA $\pm$ the standard error of the mean in each patient group. The percentage of IL-4 and IL-5 mRNA positive cells is significantly higher in the MEE of atopic patients compared to non-atopic patients ($p < 0.01$). On the other hand, a significantly higher percentage of IFN- γ

mRNA positive cells are found in the MEE of non-atopic patients compared to atopic patients ($p < 0.01$).

Figure 4. Results of in-situ hybridization: IL-5, IL-4, and IFN- γ mRNA expression in the middle ear effusions of atopic children with otitis media with effusion

Development of the slide by autoradiography allows for the demonstration of cells containing (A) IL-5, (B) IL-4, and (C) IFN- γ mRNA positive cells in the middle ear effusions of atopic OME patients.

2.10 Figures

Figure 1

A

B

C

Figure 3

A

B

C

Chapter 3. Overall conclusions

3.1 The role of atopy in otitis media with effusion

3.1.1 *Clinical*

3.1.2 *Cellular and cytokine profiles*

3.1.3 *Recommendations*

3.2 Limitations of this study

3.3 Future studies

3.1 The role of atopy in otitis media with effusion

3.1.1 Clinical

Thirty percent of patients with OME in our study were atopic by skin testing, having an average age of 4.75 years. Seven of the eight atopic patients were reactive to perennial allergens. Non-atopic patients represented 70 % of our patient group, having an average age of 3.56 years. These clinical findings are consistent with those found in other studies. Given that the sample size in this study is only 26, it is difficult to draw conclusions regarding the epidemiological significance of our data. It seems apparent, however, that older children with OME have a predisposition for allergies, given that nearly half of them are skin test positive. Moreover, the nearly uniform reactivity to perennial allergens in OME patients is consistent with that seen in asthmatic patients. Taken together, this clinical data supports the hypothesis that OME is an atopic disease, although further studies are necessary in order to properly resolve this question.

3.1.2 Cellular and cytokine profiles

Middle ear effusions of atopic patients with OME are characterized by higher percentages of eosinophils, T lymphocytes and Th2 mRNA positive cells compared to non-atopic controls. This inflammatory profile is consistent with that seen in other upper airway diseases whose pathogenesis is related to the late phase allergic response. It is likely that in atopic patients middle ear effusions develop after exposure to allergen, which may reach the middle ear cleft through the Eustachian tube. The allergen may then be processed by dendritic cells located in the middle ear mucosa, and presented to Th2 cells, which subsequently produce and secrete Th2 mediators, including IL-4 and IL-5.

IL-5 may then promote the production, recruitment, activation, and survival of eosinophils within the middle ear cleft. IL-4 may perpetuate the Th2 response within the middle ear by promoting the differentiation of naïve T-helper cells (TH0) towards the TH2 phenotype. Through induction of endothelial VCAM-1, IL-4 may also contribute to the eosinophilic infiltrates found in the middle ear of atopic OME patients.

Neutrophils and IFN- γ mRNA positive cells were present in significantly higher percentages in non-atopic patients, providing evidence that middle ear effusions in these patients are likely the result of an infectious process. It is likely that middle ear effusions in these patients develop after an acute infection in the presence of Eustachian tube dysfunction.

3.1.3 Recommendations

Demonstration of allergy mediators provides evidence for the need to introduce anti-allergy management in to the treatment algorithm of OME. Based on the results of this study we recommend the following.

- 1) Children with OME over three years of age should be referred to a pediatric allergist for an evaluation, which should include skin prick testing.
- 2) Children with OME less than three years of age should be referred to a pediatric allergist if they are at moderate to high risk of being atopic (strong family history, history of asthma, eczema, rhinitis), with consideration for skin prick testing in selected cases.
- 3) Therapy for atopic OME patients should include specific avoidance of the reactive allergen if possible.

- 4) The role of pharmacotherapy, including systemic or nasal corticosteroids, and antihistamines needs to be evaluated by randomized controlled trials.
- 5) The role of immunotherapy in older children with refractory OME needs to be evaluated by randomized controlled trials.
- 6) Myringotomy and ventilation tube placement is the treatment of choice in selected OME patients having middle ear effusions for greater than three months with hearing loss of greater than 15 decibels.
- 7) Observation of selected patients with OME may be considered, given that the long-term effects of middle ear effusions on speech and language development is controversial.

3.2 Limitations of this study

The limitations of this study lie in its inability to answer several questions related to the pathogenesis of OME. For example, one question that needs to be answered is whether or not the middle ear mucosa is the sole target of allergic inflammation in OME patients, or is the inflammatory process continuous along the Eustachian tube and nasopharyngeal mucosa ? Another question that needs to be addressed is the role of the adenoid in the pathogenesis of OME in atopic patients. The role of these two anatomical structures in the pathogenesis of OME needs to be established before any conclusions can be made about the role of allergies.

Another limitation of our study is the inability to determine whether or not the cells and cytokines present in atopic middle ear effusions represent inflammation within the middle ear mucosa, or if they emanate from the circulation (i.e. a systemic allergic response). Unfortunately, it is not possible to overcome this limitation without taking biopsy specimens of middle ear mucosa from OME patients. Even if middle ear effusions in atopic patients are the result of a systemic response, the goal of management should include treating the underlying allergies contributing to OME.

The control group in our study was comprised of non-atopic children with OME. It was not possible to have a control group of normal children without OME, given that the middle ear cleft does not normally have fluid. Therefore, another limitation of our study is that we cannot determine what cells and cytokines are present in the middle ear cleft of children without OME. Unfortunately, it is not possible to overcome this limitation without taking biopsy specimens of middle ear mucosa from both OME patients and normal children.

3.3 Future studies

Future studies are necessary in order to further elucidate the role of allergies in the pathogenesis of OME, and to assess the role of anti-allergy therapy in the treatment of OME patients.

We are currently in the process of designing a study, which will evaluate the cellular and cytokine profiles of the nasopharyngeal mucosa, adenoid, and middle ear effusions of atopic and non-atopic patients with OME. The goal of this study will be to answer questions concerning the role of the adenoid and Eustachian tube in the pathogenesis of OME in both atopic and non-atopic children.

The role of other inflammatory cells and cytokines, including B cells, macrophages, and IL-13 should be evaluated in future studies in order to determine their role in the pathogenesis of OME.

Randomized clinical trials are needed in order to assess the efficacy of anti-allergy therapies for the treatment of OME. In specific, the role of avoidance, immunotherapy and specific pharmacotherapy needs to be evaluated.