The Middle Ear: The Inflammatory Response in Children with Otitis Media with Effusion and the Impact of Atopy

Clinical and Histochemical Studies

BY

DAVID S. HURST
Dissertation for the Degree of Doctor of Philosophy (Faculty of Medicine) in Medical Sciences presented at Uppsala University in 2000

ABSTRACT


Otitis media with effusion (OME) is the major form of chronic relapsing inflammatory disease of the middle ear, constitutes the most common diagnosis for children under 15 years old and is the major cause of auditory dysfunction in pre-school children. OME is a disease more commonly found in allergic children. These studies sought to investigate the inflammatory response in the middle ear of patients and test the hypothesis that an allergic-like response might occur in the ear. Atopy was diagnosed by standard in vitro tests. Immunochemical techniques used to study classic allergic rhinitis and asthma were extrapolated to the evaluation of OME children whose effusion persisted beyond 2 months. Not only eosinophil cationic protein (ECP), tryptase, CD3-positive and IL-5 producing cells, but also myeloperoxidase (MPO) was found in middle ear fluid and/or mucosa in the majority of patients with OME and atopy.

Initially, levels of ECP, MPO, and tryptase were measured in effusions from 97 random OME patients whose atopic status was determined by in vitro testing to 12 inhalants and 5 foods. The response of eosinophils, neutrophils and mast cells in the middle ear was distinctly different between atopic and non-atopic patients (p<0.001) with higher levels of the cell markers in the atopic group of patients. This suggested that 1) perhaps OME was predominantly a disease of atopics and that 2) they differed in their response from non-atopics.

Tryptase was measured in middle ear effusions from 38 patients with OME, 94.7% of whom were atopic by in vitro testing. Tryptase was elevated only in the effusion of atopic patients as compared to 5 controls (p<0.01). Biopsies stained histochemically for tryptase showed evidence of mast cells in the mucosa and submucosa from 6 of 8 OME ears but absent in 4 normals.

Middle ear biopsies, embedded in a plastic resin to improve the structural preservation, from 5 patients with OME and 5 normals were evaluated for the presence of eosinophils and neutrophils with monoclonal antibodies against 4 specific granule proteins. Eosinophils and neutrophils were present in the mucosa and mucus in significantly higher numbers than in the control group.

In an effort to determine whether the middle ear itself might be involved in allergic disease, evidence that some of the cells, mediators and cytokines associated specifically with a Th-2 response were sought for in the middle ear mucosa of these children. Middle ear biopsies from 7 atopic patients with OME and 4 controls demonstrated the presence of activated eosinophils, CD-3+ T cells and IL-5 mRNA cells only in the mucosa from atopic OME children.

Conclusion: Effusion and mucosal biopsies containing ECP, tryptase, and/or IL-5 mRNA cells, CD3+ T cells, eosinophils, and mast cells indicate that many of the mediators and cells essential to the production of a Th-2 immune mediated response are present in ears with chronic effusion. The increased levels of MPO in atopic patients further suggest that the general inflammatory response to putative inciting agents such as bacterial and viral products may be altered in atopy. These studies support the hypothesis that the exaggerated inflammation within the middle ear associated with most cases of OME is possibly the result of an atopic response within the middle ear itself.

Key words: Allergy, atopy, eosinophil cationic protein, IL-5, tryptase, otitis media with effusion, in vitro testing.

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to:
  My Parents
  for Life
  and teaching me the value
  of charity and truth,

to:
  My teachers and patients
  for showing the way,

and to:
  Joshua, Heather, Daniel
  and especially Melissa
  for love.
This thesis is based on the following papers, which are referred to in the text by their Roman numerals:


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Abbreviations:

AA1 ........................ Anti-tryptase antibody 1
ECP    ....................... Eosinophil Cationic Protein
ET  ........................ Eustachian tube
ICAM ........................ Intercellular Adhesion Molecule
IDT ........................ Intradermal skin testing
IL-5 ........................ Interleukin 5
MEE ........................ Middle ear effusion
MBP ........................ Major basic protein
MPO ........................ Myeloperoxidase
M&T        ........................ Myringotomy and tubes
OM  ................................ Otitis media
OME .......................... Otitis media with effusion
PUR-OME ...................... Purulent otitis media with effusion
RAOM ........................ Recurrent Acute Otitis Media
R/PUR  ............................ RAOM in PUR-OME group
SOM     ............................ Secretory Otitis Media
SPT ................................. Skin Prick Test
VCAM .............................. Vascular Adhesion Molecule
VGEF................................ Vascular Endothelial Growth Factor
+AE      ............................. Atopic with effusion
-AE  .......................... Nonatopic with effusion
+AE/NR  ........................... Non-related atopy with effusion

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Prologue:

Twenty-five years as an otolaryngologist in a solo, rural practice is a humbling experience. All of one’s failures return and serve as constant reminders of the fallibility of one’s medical care for chronic middle ear disease. It was my frustration with the lack of resolution of these patients’ ear disease, the recurrence of their effusion, the need to repeatedly re-insert tympanostomy tubes and the observation that many patients had multiple allergic symptoms, including rhinitis and asthma, that led me to question whether allergy was contributing to their ear disease.

Introduction

Otitis media with effusion (OME) is the major form of chronic relapsing inflammatory disease of the middle ear. Chronic OME is associated with hearing loss, delayed speech development and may cause permanent middle ear damage with mucosal changes.[162] It is a disease of immense social and financial impact among families of young children, accounting for over 16 million office visits a year at an annual cost of over $3.5 billion for antibiotics in the United States alone.[1] Ten percent of patients with acute otitis media still have OME 12 weeks after diagnosis, even after appropriate therapy.[58] The prevalence of persistent OME ranges 2.2% to over 10%[165] with as many as 88% of confirmed cases being undiagnosed prior to screening in China[165] to 50% in Sweden.[9] Despite extensive investigation over several decades, a simple explanation for the cause of OME has not emerged. Until predisposing and triggering factors have been clearly identified, therapeutic and preventative strategies for this condition must be based on interrupting the immunopathologic mechanisms involved. Therefore identification of factors involved in the chronicity of otitis media is an essential step in the treatment and ultimate prevention of this chronic disease.

Terminology and Definitions

The term *otitis media*, like its archaic predecessors *otic cattahar* and *atopic coryza*, is non-specific, confusing, and often serves to hinder our understanding and communication about the disorder. The term describes a symptom—not a disease. It is used to categorize a broad spectrum of middle-ear pathology, which may involve recurrent acute infections occurring sporadically or extend to conditions of prolonged, refractory changes in a middle ear harboring fluids of varying viscosity. Confusion occurs when practitioners mistakenly equate inflammation with infection. “Children” include predominately those aged 3 to
18, “allergy” refers to “symptomatology” of asthma, rhinitis or otitis vs atopy or “sensitivity” as reflected by elevated IgE antibodies to various allergens without necessarily having symptoms present.[45] and OME refers to “chronic middle ear effusion of at least 2 months duration”.

The classification of OM was agreed upon in 1979 at the Second International Symposium on Otitis Media with Effusion. Otitis Media is defined as an inflammation in the middle ear. Acute OM is an infection in the middle ear with sudden onset and short duration. The clinical diagnosis includes both acute ear-related symptoms and signs of fluid in the middle ear. The Swedish consensus conference in 1991 defined secretory otitis media (SOM) to mean OME without acute signs and symptoms.[91] An episode of acute OM that begins after a symptom-free interval of 30 days has been designated by both conferences as recurrent acute otitis media (RAOM). Chronic suppurative OM refers to a chronic discharge through a perforation of the tympanic membrane. OME is similarly defined by the Current Guidelines from the U.S. Agency for Health Care Policy and Research as “fluid in the middle ear without signs or symptoms of infection; OME is not to be confused with acute otitis media (inflammation of the middle ear with signs of infection).”[152]

The most distinct clinical subsets of ear disease are patients with RAOM and refractory OME. Middle-ear effusion (MEE) may be defined as the presence of fluid within the middle-ear space. The fluid itself may be further characterized as serous (generally a thin transudate), secretory (mucoid secretions consisting of mucopolysaccharides from goblet cells and glands in a metaplastic or hyperplastic middle ear mucosa), purulent (from an active infection), or a combination of the above. Donaldson[40] characterized four patterns of OM history temporally: (1) the isolated pattern of a separate episode of acute OM successfully treated by conventional methods, (2) a recurrent pattern in which each episode is a distinct new entity separated by variable periods of normalcy, (3) a persistent pattern in which an effusion persists after conventional therapy in the absence of further acute episodes, and (4) a relapsing pattern in which MEE persists even between episodes of acute OM. Each patient’s otitis will exhibit three distinct variables: frequency, viscosity, and duration of effusion.[67] Our research focused on those patients who developed and maintained persistent effusion for more than two months and who subsequently required the placement of tympanostomy tubes.

**Importance and management of the disease**

Chronic middle-ear disease is not a benign condition. A review of 20 studies, primarily of children who have had chronic OME, including 9767 intubated ears of 4719 children in nine countries, indicates a 20% to 40% incidence of
sequelae during one to 12 years of follow-up. Among these collective patients, TM pathology included diffuse atrophy (9%), diffuse tympanosclerosis (9%), retraction (15%), chronic perforation (1.3%), middle-ear atelectasis (7.3%), adhesions (2.4%), ossicular disruption (0.9%), deep retraction pocket (3.5%), and cholesteatoma (0.6%).[56]

The most devastating aspect of persistent fluid in the middle ear is the concomitant hearing loss. Clinicians and parents often view OME as “no pain - no problem”. However, OME is a major cause of auditory dysfunction in pre-school and grade school aged children.[163] The intellectual and linguistic sequelae of middle ear disease has been thoroughly documented. Otitis in the first 3 years of life in 207 children resulted in significantly lower scores in mathematics, reading and articulation skills.[161] Infants with recurrent OM suffer from expressive language deficits.[170] Even unilateral effusion for as short as 3 months will produce delays or regression in speech development in toddlers. Children with hearing loss secondary to OME constitute the largest group of people with a reversible learning disorder in the world. Most disturbing is the fact that under managed care systems referral to the otolaryngologist in the United States has been delayed to such an degree that 47% have had their effusion present an average of 22 months. Patients presented at a University Hospital with a high rate of hearing loss(92%), speech delay (17%) and a high complication rate (10% perforations) at the time of initial referral for M&T.[65]

A more diffuse inflammatory disease process involving the mucosa beyond the middle ear underlies the development and persistence of OME. The mastoid air cell system is not a separate space and should not be disregarded in the treatment of SOM. Persistent low grade infection results in arrest of mastoid development. Small mastoid cavities seen on X-ray suggest the involvement of the whole middle ear cleft in OM.[167] During SOM mastoid growth is slowed, but mastoid development can be restored if aeration by ventilation tube therapy is instituted early.[90]

Perhaps the worst complication of chronic SOM is the need for a mastoidectomy. This surgical procedure is required following repeated or prolonged discharge from a ventilation tube or perforation that is unresponsive to medical management.[143] Palva found an incidence of 1.4% of ears with SOM required mastoidectomy. Others have reported a frequency of up to 7.5%.[83] Mastoid surgery is usually successful in providing a dry ear in 50% to 70% of cases. Unfortunately, radical mastoidectomy results in permanent hearing loss.
Treatment of OM is as varied as the disease. It has been suggested that a different mechanism underlies the pathogenesis of RAOM vs. persistent OME. Prior attacks of acute OM predispose to serous otitis (a older reference to OME), yet this is not always the case. Chronic inflammation has been shown to be a direct continuation of an acute episode in only half the cases of OME, and it is not necessarily the result of inadequate therapy.\[4\]

The disparity of “practice guidelines” accepted in European countries, as opposed to those in use in England or in the United States implicitly attests to a conflict in opinion as to the etiology of ET dysfunction. Thus, the appropriate care of OME remains in dispute. Since the 1950’s, the underlying assumption behind medical therapy for OME is that chronic otitis media is the result of an acute infection that has been inadequately treated. Antibiotics are usually prescribed for acute infections, yet are found to be only 22% more effective than placebo.\[135\] The current algorithm in the United States is still to treat children 1 to 3 years old who present with an acute infection with antibiotics until they resolve,\[152\],\[41\] although by definition OME is fluid with no signs of infection. Only “if the effusion is still present at 4-6 months with bilateral hearing loss greater than 20dB then the patient is a candidate for myringotomy and the placement of tympanostomy tubes (M&T).” These guidelines are promulgated despite the fact that meta analysis of 28 studies regarding the use of antibiotics for chronic OM concludes that antibiotics are no more effective than placebo in achieving resolution of the fluid.\[175\] It must be recognized that antibiotics have reduced the morbid complications of mastoiditis associated with acute OM. Unfortunately, the result of 30 years of increasing use of second and third generation antibiotics has only been the development of resistant strains of bacteria with no alteration in the frequency or severity of OME.\[125\] In fact, Poole notes that “medical treatment failures probably already surpass ET dysfunction as the most common reason for tympanostomy tube insertion.”

Management of ears with persistent fluid presents a further dilemma because it may take several months for medical therapy to be effective in eliminating the MEE. Removal of the fluid itself should not be deferred so long as to allow the child to experience delayed speech and language. Once it has been determined that the patient is not responding to medical management surgical intervention, despite its inherent complications, must be pursued.

Pathogenesis and Pathophysiology
Children with OME have in some studies demonstrated an association with several risk factors including parents or siblings with allergy, attendance at day care center, exposure to cigarette smoke, recurrent upper respiratory infections,
short duration of breast feeding and seasonal changes. Allergy has been proposed as a cause of chronic inflammation in the middle ear for half a century. Others have failed to show that a history of allergy is a risk factor. Furthermore, several of these and other factors are seemingly required to be present in the same child at the same time in order for the disease to persist. Boys are at slightly more risk than girls.

According to Pukander, acute OM is associated with a respiratory infection in 76% of cases. Culture studies of MEE demonstrate a viral etiology in 46% and bacterial agent in up to 76% of acute cases. An acute infection is generally treated with antibiotics within 24 to 48 hours of onset. After 2 weeks of antibiotics 57% of ears are sterile. Among the 35% of pre-school children who experience otitis media (OM), 50% maintain the effusion 14 days after initial treatment. Of the group who develop effusion, 70% persist at 2 weeks, 40% at 1 month, and 20% maintain effusion beyond 2 months. Episodes of acute otitis media are more frequent and the duration of OME is longer in infants with respiratory tract allergy as compared with either allergic children with only dermatologic manifestations of allergy or nonatopics.

The role of viruses as a primary cause of acute OM has been controversial. Respiratory virus infection of the nasopharynx has been a suggested etiology of OME, yet a virus occurs as the only pathogen in isolates of MEE in 0% to 13% of cases. Chonmaitree studied 84 children with MEE and found 39% had positive viral cultures of their effusion and/or nasal lavage at the time of their acute episode. Only 15% of his patients had no pathogen (bacteria or virus) in the effusion. Recent studies showed virus RNA, in particular human rhinovirus RNA, to be present in 30% of the effusions of children with OME. Other studies showed the presence of endotoxin in the majority of effusions and the presence of various bacteria as detected by PCR in as many as 85% of the cases.

Respiratory syncytial virus enhances synthesis of proinflammatory cytokines (IL-1b, TNFa, IL-6) and cell adhesion molecules (ICAM-1, ELAM-1, VCAM-1) in the middle ear of infected individuals. Ohashi found VCAM-1 to be significantly more elevated in the ears of atopics. Viral sensitization may contribute to the initial inflammatory process leading to OME. RSV, a common virus in the middle ear and nasopharynx, induces a state of IgE-mediated allergy in the nasopharynx, wherein those with elevated mast cells in the adenoid bed are more prone to OME. It has been suggested that both a respiratory virus infection and the presence of bacteria in the nasopharynx are required for the development of acute otitis. Garofalo examined the effusion from 20 children with acute otitis. He found that tryptase levels were elevated in 79%.
Samples that were negative for viral culture did not contain detectable levels of tryptase. He suggested that viral pathogens were “an essential trigger or priming factor for mast cell degranulation.” Neither virus nor bacteria alone appear to be capable of causing OM as frequently as the two combined.

Middle ear disease has many parallels to rhino-sinusitis and bronchial asthma. The research techniques familiar to rhinologists and pulmonologists should be applicable to the study of OME. Mechanisms purported to lead to OME must consider the fact that middle ear mucosa is merely an extension of the mucosa of the upper respiratory tract. Pseudostratified, ciliated columnar epithelial cells line the nasopharynx and ET with extension into the middle ear and mastoid. This mucosa harbors stem cells and goblet cells. Secretions are cleared from the middle ear by ciliary action directed toward the ET and nasopharynx. Middle ear clearance may be impaired by loss of this ciliary activity. The lining of the middle ear can change from normal mucosa to secreting mucosa with increased goblet and columnar cells in a short period of time; its reversal, however, may take weeks or months. The macroscopic appearance of the middle ear mucosa as viewed through a fine endoscope in active OME is described as thickened, edematous and hyperemic.[158] The ET orifice is stenosed with edema in 33% or plugged with effusion in 25% of ears with active OME. Histologically the mucosa of the middle ear is described as metaplastic. The normally low cuboidal epithelium changes to a high cylindrical epithelium rich in cytoplasm and mucus. The mucus-secreting glands do not develop until late as a secondary sign of chronic otitis.[59] The middle ear, like a nasal sinus or pulmonary segment, has a narrow opening. The ET structurally resembles a bronchial tube leading to an alveolus. Extrinsic or intrinsic obstruction of either the tube or its orifice leads to atelectasis, effusion and subsequent infection.

During the past 20 years we have come to understand that OME results from either extrinsic dysfunction of the Eustachian tube or from intrinsic disease within the middle ear itself. Certainly children with cleft palate have an incidence approaching 100% of developing OME. Otitis prone children have a significantly poorer active tubal function than controls.[157] Takahashi compared the passive tubal opening pressure, positive and negative middle ear pressure, equalizing functions and dye clearance functions in 27 children in convalescent stage of OME following placement of tympanostomy ventilation tubes in a group of 76 children with active OME. He included 34 patient controls. The negative middle ear pressure equalizing function was impaired in 97.2% of ears in the active group and was still abnormal in 93.9% of ears in the convalescent OME group. The ET clearance was impaired in 64% and 36% in the corresponding groups. Takahashi concluded that poor ability to equalize negative pressure was a fundamental defect of ET function in OME patients and
that the impaired clearance function in the active stage of OME was not a primary cause of OME. Yet he found that there was no organic obstruction or stenosis of the ET in patients with OME, finding an abnormally high ET opening pressure in only 11% of the active OME group and in none of the convalescent group. Sade further dispelled the myth that immature morphology of the ET is the major cause of ET dysfunction in infants. Comparing the pharyngeal portion of the ET in ten temporal bones from children with OM with those of 33 normals, he found ET size to be statistically similar in the two groups. This was similar to his earlier results regarding the isthmic region.

The ET has been shown to be involved functionally and morphologically in Type I reactions of the nose. Friedman used a double-blind protocol to show that a provocative intranasal pollen challenge of atopic individuals produced allergic rhinitis followed by ET obstruction. Placebo patients did not obstruct. He demonstrated that allergic reactions in the nose and nasopharynx inhibit even transient dilations of the Eustachian tube during swallowing. Double-blind protocols also show that provocative intranasal challenges with allergens or histamine produce severe functional obstruction of the Eustachian tube. The mucosa of the nasopharynx and ET was found to be hyperresponsive to histamine in patients with allergic rhinitis and predisposed them to OME. Allergic inflammation thus appears to be a contributing factor in the development of OME secondary to ET obstruction.

The incidence of atopic diseases is found to be higher among children (3 to 12 years old) with serous OM compared with a nonselected control group. OME may occur 5 times more frequently among atopic infants. Studies among children referred to otolaryngologists with chronic OME and examined for allergy before placement of ventilation tubes indicate that 40 to 50% of these children have allergic rhinitis confirmed with positive results of allergy skin tests or increased serum IgE to specific allergens. Although a recognized risk factor for developing OME, the relationship between allergy and different forms of otitis remains controversial, though it has been proposed as a cause of chronic inflammation in the middle ear for half a century. Allergy is thought to be one of the etiological factors in OME because OME occurs frequently in atopic patients and because favorable responses to allergy management have been reported. Bluestone outlined four hypothetical mechanisms in which allergy could be responsible for the production of OME. These included: (1) the middle ear functioning directly as a “shock organ,” (2) Eustachian tube (ET) dysfunction due to intrinsic mechanical obstruction from inflammatory swelling of the ET itself, (3) inflammatory obstruction of the nose, or (4) aspiration of bacteria-laden allergic secretions from the nasopharynx into the middle ear.
Secretory otitis media, first described by Politzer in 1869, received little mention in the literature until 1931 when Proetz noted a relationship between patients with allergic rhinitis and chronic OM. Koch’s study of 222 patients was the first to include observations of eosinophilia in otorrhea. Observations as to its incidence, etiology, pathology and therapy were reported with increased frequency through the 1950’s and 1960’s as the use of Armstrong’s ventilation tube, antibiotics and research in immunology flourished.

A detailed history will often discover that a large percentage of OME patients have additional symptoms of systemic allergy including asthma, rhinitis, headache, cough, eczema and food hypersensitivity; yet, this is not always the case. Many patients exhibit symptoms of only a single target organ be it rhinitis, asthma, eczema, or otitis. Considerable discrepancies between sensitization and symptomatic allergic disease are observed, particularly in children. The percentages of patients deemed atopic depend upon the investigator’s defining criteria. Tomonaga evaluated 256 OME patients, of which 72% were proven to have allergy by positive IDT. This is much higher than the prevalence of only 26.7% found by Caffarelli when using skin prick testing(SPT). Becker examined 35 infants with OME but no history of allergic rhinitis. Twelve (34%) of these children proved to have atopy by skin testing to dust and pollens, ten of the twelve (84%) were positive by RAST in serum and 50% had positive RAST in their middle ear effusion. He found no RAST positive children with negative IDT. Becker concluded that history alone does not preclude atopy and stated that “these results suggest an allergic genesis of recurrent middle ear effusions....in 20% to 30% of our cases.”

Table I

<table>
<thead>
<tr>
<th>YEAR</th>
<th>AUTHOR</th>
<th># PTS</th>
<th>% of Positive Tests</th>
<th>% Improved with allergy therapy</th>
</tr>
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<tbody>
<tr>
<td>’42</td>
<td>Dohlman[38]</td>
<td>178</td>
<td>56%</td>
<td></td>
</tr>
<tr>
<td>’42</td>
<td>Mao[99]</td>
<td>252</td>
<td>29%</td>
<td>of pathologically deaf children</td>
</tr>
<tr>
<td>’49</td>
<td>Jordan[79]</td>
<td>123</td>
<td>74%</td>
<td>98%</td>
</tr>
<tr>
<td>’58</td>
<td>Solow[148]</td>
<td>50</td>
<td>72%</td>
<td></td>
</tr>
<tr>
<td>’61</td>
<td>Lecks[93]</td>
<td>82</td>
<td>88%</td>
<td></td>
</tr>
<tr>
<td>’65</td>
<td>Fernandez[49]</td>
<td>113</td>
<td>55%</td>
<td>95%</td>
</tr>
<tr>
<td>’65</td>
<td>Whitcomb[174]</td>
<td>38</td>
<td>100%</td>
<td>87%</td>
</tr>
<tr>
<td>’67</td>
<td>Draper[44]</td>
<td>340</td>
<td>53%</td>
<td>91%</td>
</tr>
<tr>
<td>’80</td>
<td>Hall[60]</td>
<td>92</td>
<td>100%</td>
<td>82%</td>
</tr>
<tr>
<td>’81</td>
<td>McMahan[101]*</td>
<td>119</td>
<td>93%</td>
<td>86%</td>
</tr>
<tr>
<td>’86</td>
<td>Sanz[140]*</td>
<td>20</td>
<td>30%</td>
<td></td>
</tr>
<tr>
<td>’88</td>
<td>Tomonaga[164]</td>
<td>259</td>
<td>72%</td>
<td>of OME cases</td>
</tr>
</tbody>
</table>
During the past 60 years only twenty investigators have reported the use of strict objective tests such as RAST or skin testing to evaluate the atopic status of their entire patient populations.\[38\],\[99\],\[79\],\[148\],\[93\],\[49\],\[174\],\[44\],\[60\],\[101\],\[140\],\[164\],\[66\],\[11\],\[110\],\[32\],\[68\],\[144\],\[127\],\[27\] (Table I). Among these, only the studies by Tomonaga and Draper were designed to look at the epidemiology of OME among the general population. Several of these authors also reported the efficacy of various means of allergy therapy. Although these are not controlled clinical trials, they do serve as anecdotal evidence that allergy management can be effective. Shubich showed that patients with OME and allergies who had allergy treatment had half as many infections while their tubes were in place and required half as many replacements of tympanostomy tubes as the group with non-treated allergy.\[144\] It is this type of empirical observation that prompts further evaluation of the possible immunologic responses present in the middle ear which might help explain if such results are more than coincidental.

In addition to the above mentioned possible pathogenic mechanisms of OME, more recently, increased vascular permeability and endothelial cell growth as promoted by the vascular endothelial growth factor (VEGF),\[80\] has been suggested and also the increased number of adenoid mast cells.\[166\]

The current understanding of the immunologic process involved in atopy recognizes that an atopic response involves activation of the TH-2 cells by viral, bacterial, or environmental antigens with resultant up regulation and production of IL-4 and 5 and an expression of IgE on mast cells and eosinophils with a resultant release of tryptase, ECP and other mediators of inflammation.\[26\] This combination of events has as yet not been described in OME. The presence of

<table>
<thead>
<tr>
<th>Year</th>
<th>Study</th>
<th>Samples</th>
<th>SPT +</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>'90</td>
<td>Hurst[66]++</td>
<td>20</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>'94</td>
<td>Nsouli[110]*</td>
<td>104</td>
<td>78%</td>
<td>86%</td>
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<tr>
<td>'94</td>
<td>Corey[32]*</td>
<td>89</td>
<td>61%</td>
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</tr>
<tr>
<td>'96</td>
<td>Hurst[68]</td>
<td>73</td>
<td>87%</td>
<td></td>
</tr>
<tr>
<td>'96</td>
<td>Shubich[144]</td>
<td>40</td>
<td>100%</td>
<td>70% treatment group 45% controls</td>
</tr>
<tr>
<td>'98</td>
<td>Psifidis[127]</td>
<td>148</td>
<td>59%</td>
<td>78% prospective treatment</td>
</tr>
<tr>
<td>'98</td>
<td>Caffarelli[27]</td>
<td>172 Patients (26.7% SPT +) and 200 controls (30.5% SPT+) 37% OME Patients had other allergic symptoms 21% Controls had other allergic symptoms (p&lt;0.001)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table I: Studies of OME Patients with Allergy Confirmed by Skin Testing or In vitro Testing
++ patients not included in 1996 study, * = in vitro testing
mast cells and eosinophils is therefore essential, although not pathognomonic, for a disease process to be considered the result of atopy. Mast cell degranulation produces a biphasic mediator release with a histamine peak in the early phase followed by a later phase with tryptase release.[33] Allergen induced IgE dependent chemotactic factors stimulate the further release of mast cell mediators and cytokines which also contribute to the recruitment of eosinophils to the allergic inflammatory site.[129]

In the initial stages of serous otitis mast cells are found in the lamina propria and the pars flacida of diseased human middle ears.[95],[63] Mast cells release inflammatory mediators producing vasodilatation and mucosal edema, as well as neutrophil chemotaxis. Both heparin and tryptase contribute to fibrosis and bone resorption.[11] This may have added significance in understanding the pathophysiology of chronic scarring in the middle ear as well as in the bone destruction observed in cholesteatomas. This is another reason why it is important to discern if allergy is merely coincidental or actually a contributing factor to patients acquiring OME. Modern methods of immunofluorescent staining techniques using monoclonal or polyclonal antibodies have made the identification of specific cells more precise. Histological studies using these methods have indicated eosinophil reaction in allergic inflammation involving tissue damage of skin, kidney, lung and nasal mucosa.[52],[34],[122],[36] Inflammation involved in otitis can similarly be characterized from biopsies using immunohistochemical stains that are more sensitive and specific than conventional histologic methods. These new methods were used in Studies II, III and IV.

**Middle Ear Fluid and Mucosa**

The cellular content of OME fluids attained increased interest in the 1970’s when the disease became recognized world-wide. Palva described a series of 137 ears with serous effusion and noted that lymphocytes and neutrophils predominated, followed by monocytes and phagocytes. Bryan and Bryan described “glue ears” as having various stimulated lymphocytes with or without neutrophils.[25] They suggested that in chronic effusion there is a delayed-type hypersensitivity reaction and that hypersensitivity factors may play a major role in this disease. Palva used monoclonal antibodies to study 54 serous and 103 mucoid effusions.[115] He revealed both groups to have analogous findings. The serous effusions had granulocytes as the dominating cell in 67% of ears while 24% had monocytes and lymphocytes dominating. In the mucoid samples the percentages were 56% granulocytes and 28% monocytes. Most importantly for this study, he found that the relative number of T lymphocytes, based on a count of 100 successive lymphocytes, was 62% in serous effusions and 58% in mucoid secretions, while granulocytes were the dominating cell in both effusions. This
led to the use of the term “otitis media with effusion” to encompass both serous and mucoid ears which are essentially identical in their cellular content.

The pathological changes associated with OME have been described in detail.[47],[59] Mononuclear cell infiltrates have a preponderance of T helper cells grouped in the vicinity of follicles observed in the middle ear mucosa itself.[117] These follicles resemble those seen in mucosal associated lymphoid tissue (MALT) of the nasopharynx, adenoids, and bronchial mucosa. Eosinophils and allergy are also integral to the histopathophysiology of OME.[118],[112] As early as 1973 Miglets challenged the middle ear with ragweed and found polymorphonuclear leukocytes in the MEE with changes involving both eosinophils and neutrophils in the mucosa suggesting Type I reactions. He supported the allergic theory of OME and labeled the middle ear an allergic “shock organ”.\[103\] Guinea pigs challenged with antigen in their middle ear responded with a noticeable infiltration of eosinophils and mast cells with edema in the mucous membrane in the nose, nasopharynx, and in the membrane lining the tympanic cavity.\[104\]

Rakover\[130\] reviewed several animal and human studies and demonstrated proteins in MEE which compare to human mast cell proteins, suggesting that “mast cells play an important role in the etiology of SOM.” Degranulation of mast cells from the pars flaccida have been proposed as a source of experimental SOM\[6\] and Hellstrom demonstrated that MEE formation began in the region of the pars flaccida where he also found a significant number of mast cells.\[63\] Berger noted a high level of histamines in MEE of humans.\[12\]

Besides cellular analysis of effusion contents, several authors have performed mucosal biopsy studies of various stages of OME.\[82\] Biopsy specimens of the promontory mucosa were obtained during insertion of a tympanostomy tube. Kahonen, et al found that the granulocytes seen so frequently in the effusions were only rarely observed in the propria which was infiltrated mainly by mononuclear cells. In both serous and chronic otitis media the submucosal cell population represented cell mediated immune reactions. The mucosal cellular immune response as a whole had no signs of immune deficiency. As better immunohistochemical staining techniques have been developed in the past decade, interest in mucosal morphology has continued. Palva studied cell subpopulations in chronic OME and found that this disease is not characterized by a systemic reaction but is “a local process and it may be assumed that the fluid cytology and the biopsy cell make-up give an accurate picture of the ongoing immune biological events.”\[117\] Furthermore, he noted that, the fluid cytology reflected the cell pattern of the biopsy specimens.” He observed the
local immune response to involve both secretory immunoglobulins and cell-mediated mechanisms.

**Rationale for Experiments (I-IV)**

Studies of allergic responses of the respiratory system have been dominated by research on rhinitis and asthma because of the ease of access to the nose and lung. Rhinitis and asthma result from allergic responses that on a cellular level could serve as models for immune-mediated response of human middle ear cleft respiratory epithelium. The problem is that the middle ear is an extension of the upper respiratory tract that has not been as amenable to examination and testing. By definition, atopy involves a Type I, IgE mediated hypersensitivity reaction in which activated mast cells and eosinophils participate in a Th2 driven inflammatory reaction. [33] Thus an appreciation of the function of the mast cell and eosinophil is fundamental to understanding modern immunology and constitutes an important rationale of this thesis. In order to understand the inflammatory processes that allow OME to persist it is essential to characterize the cellular constituents and their degree of activity in the diseased middle ear. The basic inflammatory response in the upper airway has been defined as either allergic, involving a Th-2 response or infectious, involving a Th-1 response.[33] Conventional histology does not readily detect degranulated or activated eosinophils, neutrophils, or mast cells and has led to various conclusions. Mediators specific to the cells of inflammation have now been identified which indicate active degranulation even though the cells themselves currently have often been unidentifiable by light microscopy.

Specific mediators may be used as indirect evidence of the pathogenesis of the response of upper airway cells to a variety of stimuli,[35] as is the case for eosinophil cationic protein (ECP) from eosinophils,[98] myeloperoxidase from neutrophils,[88] or tryptase from mast cells in rhinitis.[108] Middle ear inflammation can similarly be studied histochemically by examining the contents of the effusion (I,II,IV), as in studies of asthmatics using bronchoalveolar lavage fluid. The contents of MEE indirectly provide quantitative information regarding the inflammatory response of the mastoid mucosa and middle ear cleft.

**Origin of Hypothesis**

It was my frustration with repeated failed attempts to manage a group of patients who had chronic OME that led to a sentinel study[66] which preceded the work in this thesis. An original group of 20 patients were refractory in that (1) on extrusion of their tubes they experienced the reaccumulation of their effusion which necessitated tube replacement and/or 2) they had persistent otorhea from
their tubes. I realized that many of these patients had otolaryngologic symptoms of allergy, which led to the consideration of allergy as an etiology for their ear disease. RAST testing, skin testing and food elimination diets confirmed atopy in all 20 patients. Among those choosing allergy treatment, 65% maintained normal hearing, normal tympanograms, and the elimination of recurrent infections for three years using immunotherapy. The remaining 35% resolved on appropriate food elimination diets. None of the control's symptoms resolved. Refractory OME appeared to respond to classic diagnostic and management techniques. The fascinating result was that only by treating these patients’ allergy was their OME seemingly brought under control. As a clinician it was obvious that these patients were suddenly able to control their ear disease after years of failed attempts using conventional means. There had to be a scientific explanation beyond chance alone. Although crude, these results led me to search for some marker of allergy in the middle ear of OME patients.

Early in the 1980’s ECP in nasal and pulmonary effusions had just begun to be used as an indicator of atopy in investigations of rhinitis and asthma. I thought that the same techniques might be used to evaluate the hypothesis that allergy also played a role in the etiology of OME. In a pilot study, random samples of middle ear fluid from eight ears with OME were found to contain ECP. Both serum and ear ECP levels from patients with refractory OME exceeded reference norms for serum ECP. Effusion ECP levels were found to be unrelated to serum ECP or IgE levels. This was the first report in the world literature of the presence of ECP in middle ear fluid and provided a new tool to investigate the inflammatory processes involved in cases of chronic otitis.

Having found that ECP was indeed present in middle ear fluid we designed a preliminary study of effusion from patients whose atopic status was determined by IDT and RAST. It was found that an effusion ECP > 10µg/l correlated with atopy, as determined by the parameters of IDT, RAST and IgE with a positive predictive value of 92.1 % and a diagnostic sensitivity of 87.5%. These findings suggested that ECP measurements in middle ear fluids might be used as an indicator of an allergic reaction in the middle ear (except in the presence of pus).
We recognized that the technique of sampling MEE is random and that the concentration of a mediator, its site of origin (be it the middle ear, mastoid mucosa or ET mucosa) and whether that mediator really participates in the inflammatory reaction are unknown. It is also necessary to examine biopsies of the middle ear mucosa, comparing them to normals, to be certain that those changes involving mast cells and eosinophils are in fact occurring in the mucosa of the target organ and not a mere passive reflection of systemic but unrelated atopy. This was confirmed by the first recorded biopsy study to demonstrate eosinophils degranulating in the middle ear.[69] The degree of degranulation in mucosal biopsies was compared to effusion ECP and correlated significantly (p=0.01).

The issue of dilution of effusion samples at the time of collection (see Methods) was addressed in an earlier paper.[72] Fourteen samples containing lithium chloride[97] showed that effusion volumes in these studies were quite similar, ranging from 0.21 to 0.43 ml (mean 0.32 ml ± 2 S.D). Mean ECP within diseased ears was elevated, regardless of viscosity, and became even higher as the fluid became more viscous (thin, 35.8µg/L; mucoid, 84.8µg/L; pus, 264µg/L). This allowed the groups to be compared as a whole to avoid any concentrating effect of viscosity. The statistically significant differences observed between group means were thus unlikely to be explained by variation of volumes and dilution of samples.
Having thus suggested ECP as a marker of allergy in middle effusion,[72] we then examined the relation of OME to allergy. Atopy was established by IDT and RAST, and correlated to clinical history.[68] Among 89 individuals (117 ears) 87% had atopy which correlated to their ear disease. In these individuals the effusion levels of ECP was found to be almost as high as those found in bacterial infection, indicating a potent stimulus of eosinophil degranulation in these ears. Based on the assumption that the presence of ECP (in the absence of pus) is a consequence of an allergic mechanism, the results supported the hypothesis that allergic inflammation involving the degranulation of eosinophils within the middle ear mucosa was significant in the development and maintenance of OME and suggested that clinically OME is a physical sign of allergy.

We realized that while these studies lent further support to an association between OME and allergy, the evidence remained circumstantial. We had postulated earlier that if the ear was involved in allergic disease then specific IgE for at least some antigens should be demonstrable in middle ear effusion at levels different from that seen in serum.[68] In atopic children with OME, we reported that there appeared to be no relation between serum and effusion level of IgE for specific antibodies(p<0.001) as measured by ELISA testing. This supported the hypothesis that isolated local synthesis of specific IgE occurred within the middle ear *per se* and was in concordance with the report by Johnson of the independent production of cytokines associated with a Th2 response in OME ears.[78] These results were in keeping with previous reports of local production of various inflammatory mediators in allergic mucosa in rhinitis patients. As our thinking evolved, we postulated that consideration of allergy as an etiologic factor in OME would be even more strongly substantiated if it could be shown that the middle ear behaved like any other target organ of allergy. Although a logical assumption considering the histologic origin of middle ear mucosa, this would require the finding of immunocompetent cells in the mucosal inflammatory infiltrate and the demonstration of the expression of those components essential to a Th-2 mediated response in the middle ear mucosa itself, specifically: activated T-cells, cells producing Th-2 cytokines such as IL-5, as well as activated eosinophils and mast cells.

Several critical questions require answers: “Why is it that 5-10% of patients with acute otitis media progress to chronic OME[160] despite adequate antimicrobial therapy?” “Why do some children with no obvious viral or bacterial infection develop OME?” “Why, despite positive cultures, are antibiotics no more effective than placebo[175] in patients with chronic otitis media?” “Why do patients with OME have 4-5 times the expected incidence of allergies?”[93],[148],[175] “Why is OME more typical of older children who have
reached an age at which they had been expected to have outgrown an immature ET morphology?” “Why do 20% of children require a second set of tympanostomy tubes or develop otorrhea?”[55] We asked: “To what degree is allergy a risk factor?” “If allergy itself is not the ‘actual cause’ of an effusion, is it more than a mere ‘contributing factor’?” or “Is allergy a catalyst essential to the formation of persistent effusion?” This thesis was designed to ascertain the role of allergy, if any, in the pathogenesis of OME and help answer some of those questions.

Aims of the Investigation

I. To determine the degree of involvement of various inflammatory cells (eosinophils, neutrophils and mast cells) in middle ears with chronic effusion and to determine if there was a difference in the inflammatory response in an OME ear depending on whether or not the patient was atopic.

II. To determine whether there is evidence of tryptase as a marker of mast cells in either middle ear effusion or in biopsies of middle ear mucosa in patients whose atopic status is determined by in vitro testing.

III. To investigate the feasibility of a quantitative determination of eosinophils and neutrophils in the middle ear mucosa by using specific immunocytochemical markers, in order to study the extent of the involvement of these cells in patients with OME and to examine the use of plastic embedding techniques to enhance cell identification.

IV. To document in biopsy material the expression of eosinophils as well as cells containing IL-5 messenger RNA and CD3+ T cells in the middle ear mucosa of children with persistent OME, determine the patient’s relative atopic status, and compare these results with biopsies from normal controls.

Materials and Methods

Classification of Patients: (I-IV)
The term otitis media (OM) encompasses a variety of situations, varying from an isolated solitary episode to persistent “infection” that appears to resist all forms
of therapy. The nature of each individual infection may be characterized by either its duration (acute or chronic) or by the nature of the fluid (serous, purulent, or mucoid).

Middle ear disease runs across a spectrum that extends from acute to refractory. One must consider the frequency of episodes as well as the general history of the disease process. In distinction to an acute infection, an acute history of disease is short, self-limited, or episodic. Typical is a solitary episode in an adult or child (e.g., an infant under three years of age whose acute infection always clears rapidly and completely on antibiotics). Another episode may occur in one to twelve weeks. This is the child whose disease is ideally treated by tubes if frequency warrants intervention, none of whom were included in Studies I-IV.

A history of chronic disease is defined as a patient with multiple episodes of chronic OM. (Excluded is the isolated but persistent infection.) Often it involves an infant or child with recurrent infections every two to three months who upon re-examination fails to show resolution. Typically the patient with a chronic disease history presents as a preschooler or elementary school child, age four to ten. This might also be the child who had recurrent acute otitis media (RAOM) as an infant yet now presents anew with otherwise asymptomatic “infections” first identified on school screening tests because of persistent hearing loss. To be classified as having a chronic disease history, their “ear infection” must not have resolved despite two or three months of medical therapy. This refractory patient has a history of long-standing, often multiple episodes of OM, each of which may have been acute or chronic with fluid which may be mucoid, serous, or of mixed viscosity. The overall history of this patient’s progress is one of resistance to conventional medical and surgical therapy and constitutes 30% of all cases of otitis media. It was these patients who were the objects of our investigations.
Experimental Design (Study I - IV)

Study I: A randomized, prospective comparison study of levels of ECP, MPO, tryptase and total serum IgE. Levels of inflammatory mediators from eosinophils, neutrophils and mast cells were measured in effusion from 97 patients whose atopic status was determined by in vitro testing to 12 inhalants and 5 foods. Mediator levels were measured 1) to determine the involvement of these cells in the middle ear of patients with OME and 2) to see if that inflammatory response differed depending on whether or not the patient was atopic.

Study II: Immunohistologic study of mast cells in middle ear mucosa and a qualitative random study of tryptase in middle ear effusion. Tryptase was measured in middle ear effusions from 38 individuals (i.e. 44 ears, including 8 pairs) with refractory OME. Subjects included 18 children (aged 32 months to 6 years), 15 children of school age (6-18 years), and 5 adults (aged 55 to 69) selected in a random, prospective manner. Biopsies were taken from the promontory of the middle ear of a second cohort of five children (ages 5.2 to 16 years) with eight diseased ears selected randomly from children undergoing M&T. All five patients had serum ELISA testing. Biopsies from four other patients undergoing routine tympanoplasty for dry perforations served as controls.

Study III: Immunohistologic study of eosinophils and neutrophils in middle ear mucosa. Biopsies from 5 OME patients were evaluated using monoclonal antibodies against specific granule proteins of specific antibodies against specific granule proteins to quantitatively determine the frequency with which eosinophils and neutrophils occur in tissue and mucus in the middle ear. Dual markers were used to determine which marker was better: for eosinophils; eosinophil cationic protein (EG2), and eosinophil peroxidase (EPO), for neutrophils: myeloperoxidase (MPO) and human neutrophil lipocalin (HNL). Atopy was determined by in vitro testing. Five subjects undergoing tympanoplasty for dry perforations served as controls.

Study IV: Immunohistologic study of CD3+ T cells and IL-5 producing cells and eosinophils in middle ear mucosa. Middle ear biopsies from 7 consecutively acquired patients with persistent, uncomplicated OME were examined using the techniques of immunocytochemistry (CD3+, MBP) and in-situ hybridization (IL-5 mRNA). Non-atopic stapedectomy patients with no history of OM served as controls (n=7). Atopic status of OME patients was determined by in vitro testing.
**Patient Selection: (I-IV)**

All patient groups (I-IV) were selected in a random prospective manner. The issue of patient selection so as to avoid bias is crucial. Patients were referred by local pediatricians and family physicians to the author who is a solo otolaryngologist, practicing in an area which draws from a population of 48,000 living in 9 communities who constitute the primary catchment area of the Franklin Memorial Hospital. Demographic data collated by the Maine Health Data Organization (mandated and funded by the State) on referrals by town of origin, diagnosis and choice of treating hospital was reviewed. The 1994-5 data indicates that 75.5% of all patients requiring M&T living in the 9 surrounding communities had their procedure locally by the author at FMH. An additional 6.5% had been referred 75 miles away to the state’s academic center. Our patients represent 81% of those patients receiving otolaryngologic care at a community hospital. This represented a penetration of 66-73% from 4 towns, 80% from Farmington, and 100% from 4 other communities. There seemed to be no indication of pre-selection on the part of referring physicians.

The clinical course of each patient’s ear disease was categorized by evaluating the duration of effusion and frequency of infection. Ears that typify recurrent acute otitis media that quickly resolve between infections were excluded from these studies. On the opposite end of the disease spectrum are patients who exhibit refractory OME. This group was the specific focus of study. None were immunodeficient nor exhibited congenital malformations. Middle ear effusion was collected at the time patients underwent routine M&T. All had documented hearing loss, flat tympanograms and effusion of a minimum of two months duration unresponsive to antibiotic and/or decongestants. Among OME patients was a sub-type distinguished as purulent OME (PUR-OME)(I,II,IV). In addition to having persistent effusion, this group included patients with a superimposed acute ear infection within two weeks prior to their M&T. Patients with a hyperemic tympanic membrane or pus at the time of myringotomy were also categorized as PUR-OME. With the understanding that no ear with an effusion is truly normal, a Control group referred to patients with essentially normal ears who experienced eustachian tube dysfunction following an upper respiratory infection or airplane ride. Allergy testing occurred after the patients were entered into each of the 4 studies to avoid preselection bias. Mediators were measured at an outside lab with no information regarding patient history or testing results so as to be a “single blind” study.
Collection of Fluid (I - IV)

The effusion from each ear was collected quantitatively in a Juhn Tym-Tap® and diluted with precisely 2 ml of normal saline. Note was made as to whether the fluid was thin, mucoid or purulent. Supernatants of diluted, centrifuged specimens were pipetted, stored at -70°C and later sent to Sweden where they were measured for ECP, MPO, and/or tryptase. The 2 ml. of diluent solution in 14 samples contained lithium chloride at a concentration of 5mmol/L. This served as an exogenous marker that was measured by means of atomic absorption to accurately determine sample volume.[97]

Diagnosis of Atopy (I-IV)

We chose in vitro testing for our study. Its sensitivity of 90% makes it likely that most atopic patients were identified, with a minimal number of false negatives.[177] All patients were evaluated for atopy by in vitro testing following M&T. Atopy was determined without knowledge of the mediator results so as to have a singly blind study.

In vitro testing: Specific IgE antibodies to allergens were measured initially by Nordex RAST and the last 5 years by Pharmacia Immuno-CAP™ (Pharmacia&UpJohn Diagnostic AB, Uppsala, Sweden) or Micro-ELISA (Thabest-IgE™, Molecular Medicine, Inc., Denville, New Jersey). Testing of serum was performed in all patients to the same battery of 10 inhalant, 2 mold, and 5 food allergens (Der p I. and Der f I., cat, dog, Timothy and meadow fescue grass, long and short ragweed, birch and oak trees, Alternaria and Hormodendrum plus milk, egg, soy, corn, and wheat.) Thabest-IgE™ is an immunoperoxidase immunoassay which allows semiquantitative measurement using micro-ELISA technology for in vitro diagnosis of atopic disorders.[5] Thabest-IgE™ employs indirect standardization with reference to the World Health Organization Second International Reference Preparation 75/502 for Human Serum Immunoglobulin E. The numerical data is expressed in Standard IgE Units(SIU). Thabest-IgE™ values under 40 SIU may not be reliable in any quantitative sense and are considered non-reactive. The range below 40 SIU corresponds to approximately 0.4 IU of IgE and was regarded as negative for atopy. There is excellent correlation between ELISA and RAST tests(r=0.88).[5] The possibility of including false positives was addressed by requiring each patient to be positive to two allergens in order to be considered atopic. Initially, specific RAST testing was performed only on those patients with a total serum IgE level greater than 20 U/ml, because RAST has a low level of sensitivity in patients with low levels of allergic sensitization.[109],[176] In vivo results were reported using modified RAST Class scores. Class I-V were considered atopic.
Total serum IgE was also measured in all patients (I-IV) but due to its limited value in screening for atopic disease it was not used to prove any criteria in these studies,[87],[24]

**Clinical Allergy: (II)**
To be able to correlate mediator levels with a clinical history, patients were classified as either having atopy with effusion (+AE) or not having atopy with effusion (-AE). For the atopy to be related, the season of those allergens must have corresponded with the chronology of the patient’s ear disease. A patient whose positive ELISA test was obviously unrelated to the season of his otologic disease (birch and grass allergy in a 11 year old with otitis only in the winter) was classified as atopy/not related (+AE/NR).

**Titration of Mediators (I-IV)**
ECP in effusion was measured by a double antibody radioimmunoassay (Kabi Pharmacia Diagnostic AB) with a polyclonal rabbit antibody as previously described. The ECP standards were calibrated against a pure ECP according to the method of Venge, et al.[169] The interassay coefficient of variation was 3% to 8% and levels under 1 µg/L are undetectable. Myeloperoxidase was measured with a double antibody radioimmunoassay (Pharmacia&UpJohn Diagnostic AB). The assay was run according to the instructions provided by the manufacturer. The interassay coefficient of variation varied between 6% to 10% and levels under 8 µg/L were undetectable. Tryptase in the effusion was measured by a double antibody radioimmunoassay (Tryptase RIACT, Pharmacia&UpJohn Diagnostic AB, Uppala, Sweden).

Stability of ECP and MPO in middle ear fluid was verified by incubating a known amount of ECP (2 to 200 µg/L) and MPO (8 to 1000 µg/L) into one of the ear samples for one hour. This was then assayed for the two proteins. The recovery for ECP was 101.5%±5.3% (SD) (n=6) and for MPO 102.9%±5.3 (SD) (n=7). [172] Effusion mediator levels were considered to be abnormally elevated if ECP > 10 µg/L (= control mean + 2 SD), MPO > 300 µg/L (= control mean + 2 SD), or tryptase > 2 µg/L (= non atopic mean + 2 SD).

**Biopsies of Mucosa (II,III,IV)**
Biopsies of the mucosa from the promontory of the middle ear were taken following approval of the Franklin Memorial Hospital (Farmington, Maine) Committee on Ethics and Human Experimentation and with parental consent. Biopsies were obtained using a micro cup forceps through the myringotomy incision. Studies I(ECP) and IV(MBP, IL-5) reports on biopsies that were fixed in conventional manner in formalin. Material for Studies II(tryptase) and
III(EG2, EPO, MPO, HNL) were embedded in plastic and prepared according to
the unique protocols described briefly below so as to enhance cell architecture.

Tryptase Immunohistochemistry (II): The sections were incubated with
monoclonal antibody AA1 at a concentration of 0.04 mg/ml in a humid
chamber for 30 min. The incubation was terminated by washing in PBS with 0.2
% BSA. The antigen-antibody complex was visualized by using a commercial
APAAP (alkaline phosphatase-anti alkaline phosphatase) kit (K670, Dako) with
fast red substrate, according to the instructions given in the manual. Bound
alkaline phosphatase resulted in a bright red color identifying cells positive to
antitryptase. In the negative controls the primary antibody was omitted.

Immunohistochemistry on plastic sections (III): The biopsies were fixed in
glycol methacrylate (GMA) embedding kit. The sections were rehumidified with
phosphate-buffered saline (PBS) containing 0.2% bovine serum albumin (BSA)
prior to addition of the monoclonal antibodies against MPO (final concentration,
0.11 mg/ml), HNL (final concentration, 0.02 mg/ml), EG2 (final concentration,
0.004 mg/ml) and EPO (final concentration, 0.02 mg/ml). Sections were
incubated with monoclonal antibodies at room temperature in a humid chamber
for 30 min. and the incubation was terminated by washing in PBS containing
0.2% BSA. The antigen antibody complex was visualized with a commercial
APAAP (alkaline phosphatase anti alkaline phosphatase) kit with fast red
substrate (Dako). The instructions given in the manual were carefully followed.
Bound alkaline phosphatase resulted in a bright red color identifying positively
stained cells. In the negative controls the primary antibody was omitted. The
samples were then counterstained with Mayer’s hematoxylin (Darmstadt,
Germany) for 6 min. The cover glasses were mounted with Dako Fluorescence
Mounting Medium. The samples were observed with a Nikon Eclipse E800
microscope. Fuji 200 film was used for the color prints.

Staining for ECP(I,IV) and MBP(IV): The tissue was immersion-fixed in 4%
formaldehyde in 0.1 M phosphate buffer, pH 7.3, embedded in paraffin,
sectioned at 20µm in a cryostat and subjected to immunoperoxidase staining for
the demonstration of ECP. The ECP antiserum was a gift from P. Venge,
Uppsala, Sweden. The site of antigen-antibody reaction was demonstrated by
the peroxidase-antiperoxidase (PAP) procedure of Sternberger. Antibody
dilution for ECP was 1:1200. Conventional staining controls as detailed by
Sternberger as well as absorptional (specificity) controls were employed.
Consecutive sections were stained with hematoxylin and eosin to describe the
tissue.
Controlling for bias

Concerns have been expressed regarding the lack of apparent “controls” in these studies. As stated earlier, there is no true normal patient with an ear effusion to use for comparison. All four studies were conducted in a single blind fashion. Because of ethical considerations, biopsies of the middle ear of normal children were not done. Patients were chosen prospectively with no knowledge of their atopic status and before results of mediator levels were known. The variable studied in each paper was attempted to be controlled for as follows:

In Study I and Study II the levels of ECP, MPO, and/or tryptase were measured among patients of all ages. Results were compared between both atopic and nonatopics (Fig. 2,3,4). As stated in Study II: “This study was not designed to necessarily prove a clinical relation of allergy to OME and therefore no large comparative nonatopic group was recruited.” Study II did include a group of normal controls for the biopsy portion.

Study III compared histologic sections of atopic OME patients with effusion to biopsies taken from normal dry ears undergoing tympanoplasty.

Study IV compared cell counts in the mucosa of patients with OME to biopsies taken from normal ears undergoing stapedectomy. Non-atopics were not chosen as controls in looking for either eosinophils or mast cells as this seemed a non-sequitur.

A further criticism occurs over the unusually high percentage of patients with OME and atopy reported in these studies which may represent sampling error. The demographic data from the Maine State Coalition discussed under “Patient Selection” and the power of statistics suggests otherwise. More likely our results reflect the added sensitivity afforded by modern technology. Another explanation is the bias created by the great delay in referral for care. In the United States, despite the presence of standards dictating appropriate referral interval for patients with chronic ear disease, there is a significant delay in referral of patients with OME to an otolaryngologist. At one university referral center, the rate of adherence to Agency for Health Care Policy and Research Guidelines was 0%. The average duration of effusion in patients with chronic OME was 5.2 months. Forty-seven percent of patients with OME had a history of recurrent effusion averaging 22.7 months, with 92% having hearing loss and 16% speech delay.[65] Some of my patients had effusion for years before referral.

Statistical Analysis
Statistical analyses were carried out by means of nonparametric tests. The Mann-Whitney U test with Bonferroni’s correction was used to compare the different groups (serum vs ear ECP in Study III; atopic vs non-atopic in Study I, II). Results are given as means ± SEM. Fisher’s Exact test was used to examine the difference between variables (II). Comparison between cell counts (III) in the biopsies from patients with OME and in control biopsies was performed with the non-parametric Mann Whitney test. The Spearman rank correlation coefficient was used to quantify the relationship between the cell counts in middle ear tissue and mucus and between cell counts obtained by using two different markers for identification of a cell type. (III) Medians for paired values were compared using Wilcoxon’s non-parametric test. (I,II) All statistical calculations were performed by means of the statistical package, InStat® (GraphPad Software, San Diego, CA.) using a Macintosh Quadra 650 personal computer.

**Results**

**Study I** This study is the first to our knowledge to correlate the expression of markers of eosinophil, neutrophil, and mast cell activity found in middle ear effusion from patients with OME to their atopic status as characterized by in vitro testing.
Mediator levels in effusions: The inflammatory response by eosinophils, neutrophils and mast cells in the middle ear(Figs. 2,4,3) was distinctly different depending on the patient’s atopic status (p<0.001). ECP was elevated (>10 µg/l) in 68 of 79 (86.1%) of atopic patients (mean 165.8µg/L). (Fig. 2) Tryptase was elevated (mean 4.8 µg/L) in the effusion from 23 of 36 atopic patients (64%). Tryptase was below 2µg/L in all 7 non-atopic patients as well as in 1 PUR-OME and 12 atopic patients. (Fig. 3) There was no correlation of tryptase to either MPO or ECP(Spearman p>0.05). Most atopics had a serum IgE<100µg/L. (Fig. 5)
The highest levels of MPO were found in ears which had a superimposed infection at the time of myringotomy (PUR-OME). Neutrophils were significantly active in all atopic ears, producing mean MPO levels 53 times higher than that measured in non-atopics. (Fig 4) Pus in the middle ear produced the highest eosinophil and neutrophil mediator levels, but had no apparent effect on mast cell involvement, although the sample size was small.
Figure 4: Myeloperoxidase levels in middle ear effusions.
Line at mean = 115 µg/L non-atopic, 6231 µg/L atopic

Elevated ECP was related to a patient being atopic (Fisher p=0.001) and was found to serve as a marker of atopy among patients with non-purulent effusion with a positive predictive value of 97.1% [68 (true positives)/70 (true positives plus false positives) + AE] and a diagnostic sensitivity of 86.1% [68 (true positives)/79 (true positives plus false negatives)] in correlating with atopy in a patient with effusion. (Table II) The response of all three inflammatory cells in the middle ear was distinctly different between atopic and non-atopics (p<0.001).

<table>
<thead>
<tr>
<th>Disease Status</th>
<th>Atopic</th>
<th>Non-atopic</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECP &gt; 10</td>
<td>68</td>
<td>2</td>
</tr>
<tr>
<td>ECP &lt; 10</td>
<td>11</td>
<td>16</td>
</tr>
</tbody>
</table>

Table II– The proportions of normal and raised ECP in middle ear effusions of atopic and non-atopic patients with OME

**Disease Status**
Most atopics had a serum IgE<100µg/L. (Fig. 5) Total IgE did not differ between atopics (mean 93.8µg/L) and non-atopics (mean 42.2µg/L) (p=0.28).

Among the 11 atopics with a seemingly false negative low ECP, (Fig. 2) 8 had their effusion in a season unrelated to their allergy (i.e. grass allergy with OME only in the winter) or +AE/NR. This was also seen in atopics with low tryptase values (Fig 6). Therefore, neither ECP nor tryptase were elevated just because the patient was an atopic.

Evaluation of the type and number of antigens to which atopic patients had a positive RAST class 2 or greater revealed that for inhalants: 42% reacted to 2-5 antigens; and 39% had antibodies to 6 or more. Additionally, 70% reacted at class 2 or greater RAST to both molds, 6% to just one, and 51% had significant
reactions to foods. Thirty-seven percent demonstrated antibodies to 1 or 2 foods and 14% to 3-5 foods.

Study II

**Effusion Results**

This study is the first to our knowledge to demonstrate the active degranulation of mast cells and secretion of tryptase in the middle ear effusion of patients with OME in whom their atopic status was characterized. Eighty-seven percent of the 33 patients with chronic OME were deemed to be atopic by in vitro testing. Of the 61% with elevated tryptase in their effusion, 95.6% had positive ELISA tests and 94.4% had elevated ECP in their ears as well. The fluid in these ears was purulent in three, mucoid in 19 and thin-serous in 17 including all five controls. All 4 biopsy patients were also atopic.

**Granulocyte Mediator Levels in Effusions**: Tryptase was below the level of detection in all control patients as well as in 3 of 7 PUR-OME and 8 of 26 OME patients. (Fig. 6) There was no significant difference between the means for PUR-OME and OME so the results were combined as “Diseased Ears”. Mean tryptase for diseased ears (4.63 µg/l) was significantly greater than for controls (p<0.01).

Tryptase was elevated in the effusion from 23 of 39 diseased ears (60.6% patients). Ninety-five percent were found to be atopic (+AE) based on +ELISA. Mean ECP was elevated (>10 µg/l) in 34 of 39 (87.2%) of diseased ears (mean 122.5 µg/l). There was a strong correlation of ECP to levels of tryptase (p=0.01, r=0.52). Elevated effusion ECP levels corroborated the ear disease as being allergic.\textsuperscript{[72]} The ratio of the two mediators did not differ between PUR-OME and OME patients, but did differ between diseased and control groups. Nine patients (2 controls and 7 diseased) had +ELISA but no otologic symptoms referable to their allergy season (+AE/NR).

**Correlation of Atopy and Tryptase:** Among 44 OME ears in Study II, 4 were from non-atopics, 32 from atopics, and 8 from +AE/NR. Sixty-three percent of ears (20 of 32) from atopics had elevated levels of tryptase. (Fig. 6)
Figure 6  Tryptase levels in 39 OME and 5 control ears

**Biopsy Results. Tryptase (II), ECP (III):** Gross examination of the nine diseased ears as seen through the myringotomy incision found the mucosa to be edematous and inflamed. The mucosa in the four normal controls was thin, pale, smooth and shiny. Microscopically, the middle ear cavity of control subjects was lined with a single cuboidal epithelium with a thin subepithelial layer containing fibroblasts and a few mononuclear cells. This contrasted with the lining of the inflamed middle ears of the diseased patients which showed a more varied structure. In some biopsies the epithelium was completely or partly pseudostratified. In most cases the epithelium was thickened and edematous and consisted of one or two layers of cuboidal cells. Inflammatory cells were seen infiltrating the subepithelial connective tissue and often a thick mucus layer covered the epithelium. This mucus layer contained scattered mono and polymorphonuclear cells. It was difficult to differentiate between the different
cell types based on morphology alone. Immunocytochemical labeling with monoclonal antibodies against the granule proteins and/or mediators allowed easy identification of the different cells. Even in cases when the cells were badly damaged, an unequivocal identification of the cell type by immunocytochemistry was possible.

Mast cells as well as their chief mediator, tryptase, were present in the mucosal biopsies of 6 of 9 ears from 8 patients with chronic effusion, all of whom were atopic to an average of 10 allergens. A few neutrophils and occasionally some eosinophils, but no mast cells were present in the middle ear tissue of the control subjects. The numbers of both neutrophils and eosinophils increased in the diseased middle ears. Anti-tryptase antibody (AA1) staining confirmed the presence of mast cells in the mucosa and submucosa in atotics compared to controls(Fig. 7).

Monoclonal stain for AA1 also indicated a release of tryptase from degranulating mast cells into the mucous blanket covering the epithelium in 80% of diseased atopic patents, a finding totally absent in the normals. The mean number of mast cells per high powered field was 2.7 and the mean number of eosinophils was 5.1 among those ears staining positive for granulocytes. The ratio of Mast/Eos in ear biopsies was 0.53.

![Figure 7](image_url)  
Figure 7  Anti-tryptase antibody(AA1) staining of mast cells(circled).

**Study III**
In addition to the use of dual monoclonal antibodies to ensure correct identification of the eosinophils,(III) and neutrophils(III) the plastic embedding used in this study provides superior structural preservation compared to the
cryosections used in previous studies. Because thinner sections can be cut from plastic embedded material, plastic embedding is preferable to paraffin embedding. The different cell types could be identified without problems and cell counts could be easily made. In the middle ear tissue of the control subjects, occasionally some eosinophils and also a few neutrophils were present. On the other hand, the numbers of both eosinophils and neutrophils were increased in the diseased middle ear. The number of both cell types was normally higher in the mucus than in the tissue, and there was a significant positive correlation between the number of inflammatory cells in the mucus and the number of cells in the mucosa. It appeared that, compared to the controls, the number of neutrophils was elevated more than the number of eosinophils. This was most obvious in the mucus.

The differences between OME patients and controls were statistically significant for all markers both in tissue and in mucus, with the exception of EPO in tissue which did not give significantly different results. The most significant differences were obtained with HNL as neutrophil marker. There was a statistically significant positive correlation between the number of neutrophils and the number of eosinophils both in mucus and in tissue. This correlation was especially evident if HNL was used as a marker for neutrophils and EPO as a marker for eosinophils (mucus: $r = 0.76$, $p = 0.006$, tissue: $r = 0.75$, $p = 0.007$).

We found significantly more eosinophils and neutrophils in 9 OME ears than in the 5 control subjects. The eosinophils seemed to have degranulated or were degranulating; i.e., releasing their markers, while the neutrophils were generally structurally well preserved. In particular, neutrophils found in the mucus did not show degranulation. The number of cells stained with MPO was generally higher than the number of cells labeled with HNL. However, the cell counts obtained with the different antibodies against MPO and HNL showed a significant positive correlation (in mucus: $r = 0.71$, $p = 0.014$; in tissue: $r = 0.83$, $p = 0.0028$). Also for the cell count with the different antibodies against EPO and EG2 a significant positive correlation was found (in mucus: $r = 0.85$, $p = 0.006$; in tissue: $r = 0.74$, $p = 0.025$).

**Study IV**

Seven subjects were enrolled on a consecutive basis without bias. They all tested positive for allergen specific IgE to multiple allergens. When middle ear biopsies were examined using immunocytochemistry and in-situ hybridization, it was noted that there was a significantly elevated expression (as number of positively staining cells per mm$^2$) of T cells, eosinophils and IL-5 mRNA (Fig. 8) as compared with controls.
Expression of TH-2 markers in Middle Ear Biopsies: CD3 expression in the epithelium of middle ear biopsies from patients with OME was 31.7 (SEM 4.3). Control biopsies had a mean count of 10.0 (SEM 1.3)(p <0.01). There was essentially no expression of MBP in biopsies from 7 control patients vs 3.9 cells per mm² (SEM 0.8) in 8 OME ears.(p<0.01).The mean expression of IL-5 mRNA in biopsies OME patients was 7.3 (SEM 1.0) compared to 1.0 (SEM 0.5) in controls (p<0.01).
Discussion

The concept that otitis media with effusion is in some way related to allergy is not new. As outlined in the Introduction Section, this has been a consideration since Proetz noted a relationship between patients with allergic rhinitis and chronic OM 65 years ago[126] and Koch observed eosinophilia in the otorrhea from 222 children, “supporting the contention that the middle ear takes part in allergic reactions similar to those seen in the nose and sinuses”.[89] In 1965 Fernandez and McGovern[126] suggested that an allergic mechanism, while not the major cause of chronic otitis media, is a predisposing factor in as many as 85% of children with acute otitis. Shambaugh suspected allergy as an etiology of chronic draining mastoid cavities or middle ears of patients with OME, citing empirical data. He cautioned that “surgical mastoidectomy, simple or radical is not indicated. With competent allergic diagnosis and management, preferably by the otologist trained in allergic methods, the otorrhea is finally brought under control.”[143] Sprinkle’s review of the literature on the pathophysiology of OME found “solid evidence....to suggest that Type I immune injury can be considered a major contributing factor to persistent middle ear effusion.” He also stated that Type III hypersensitivities that require the presence of microorganisms were “very important,” and Type IV may also “play a role in causing and potentiating serous otitis media in man.”[150]

Most negative opinion regarding the “allergy connection” is based on the results published by Suehs.[154] Using the technology of 1952, he was unable to observe any eosinophils in 50 patients, nor did Senturia[141] who in 1960 also did not find eosinophils in MEE of patients suspected of having allergic otitis media. Senturia[141] and Sade[137] attributed the basic cause of OME to infection in the nasopharynx with retrograde contamination of the middle ear and edema of the eustachian tube. Their thinking has dominated otology to this day.

Epidemologic studies in Japan[164] and Sweden[74] have shown a significant relation of allergy to OME. Although only 6 to 20% of the general population are atopic and among atopics only 21% have OME, over 87% of OME patients were found to be atopic and/or have allergy symptoms.[164] Irander[74] found that among 54 Swedish infants 38% with OME had respiratory tract allergy. Infants with allergy symptoms were 5 times more likely to develop OME than non-atopics! Karma found similar results in Finland where allergy posed a risk factor of 4.4 for children failing to clear an acute otitis.[77] Among our own patients in Study I, 62% had documentation of additional atopic signs and symptoms including asthma 22%, allergic rhinitis 48%, eczema 4%, and chronic nasal congestion 8%. It is notable though that several studies have refuted a relationship between allergy and OME. In contrast to the above studies these were epidemiological studies in which the diagnosis of allergy was determined
from questioners of allergy of among siblings and parents. \[100],[48]\ Demonstration of any relationship of otitis to allergy depends on determining if the patient is atopic. Thus, there seems to be a discrepancy between those studies and other studies that attempted to objectively establish the relationship between OME and allergy either by investigating the prevalence of other allergic symptoms in the actual patients or by the demonstration of specific IgE in the patients.

Eustachian tube dysfunction, either extrinsic or intrinsic, is regarded as the underlying pathophysiologic event which leads to chronic middle ear disease. Obstruction of the ET in humans has been clearly demonstrated to result from antigen challenge.\[50],[14],[2]\ The most frequently cited objection to the hypothesis that allergy might in any way attribute to middle ear disease is that an allergen is unlikely to get into the middle ear itself due to the structural gatekeeper function of the E.T.\[43],[73]\ Several possible mechanisms of immune response in the ear have been proposed. These do not rely on the premise of direct allergen transport to the middle ear, but rather are dependent on an understanding of both humoral and cell mediated immunology. The middle ear is not a privileged site, devoid of immune response mechanisms as was taught in the 1960’s. Middle ear mucosa, which evolves from the same ectoderm as the rest of the upper respiratory epithelium, has in recent years been found in animal studies to have the same active intrinsic immunologic responsiveness to antigenic stimuli as do the nasal tract, sinuses and bronchi.\[18],[159]\ Chronic inflammation in the middle ear generates mucosal hypertrophy, fibrosis, osteolysis,\[116],[111]\ cartilage damage\[102]\ and hearing loss.\[56]\ If an allergen does not have direct access to the middle ear then how is it that such a hypersensitivity reaction may occur? Physicians who understand immune-mediated diseases acknowledge that in asthmatics an immune mediated response by respiratory mucosa to an allergen may produce general edema, bronchospasm, increased mucous production and effusion within pulmonary alveoli\[75]\ even though some allergens may not actually penetrate the airway beyond the nose or bronchi. The current hypothesis of a common mucosal immune system suggests that activated T lymphocytes are able to migrate from one mucosal site to another. Exposure of one epithelial surface by an infectious or antigenic agent leads to secretion of locally produced antibodies.\[171]\ This is demonstrated classically in eczema or asthma secondary to peanut allergy. Current studies support the hypothesis that an abnormally increased absorption of allergen is pathogenic in airway diseases of rhinitis and asthma.\[57]\ It is now recognized that human nasal airway mucosa is the focus of absorption of allergens as well as microorganisms which activate the mucosal defense systems. Secretory immunity is the best defined effector mechanism of
antigen presentation and stimulation of the immune system in humans and is elegantly described by the work of Jahnsen.[75]

The great difficulty in attempting to demonstrate that the middle ear might be involved in allergic disease is the ethical impossibility of performing direct challenge studies. Therefore we are forced to rely on circumstantial evidence in the form of animal experiments, histologic studies and clinical outcomes. The mechanisms that regulate the activity and accumulation of eosinophils and mast cells and their mediators which lead to tissue injury in the atopic patient are governed by the Th2 type of lymphocyte with activation of cytokines such as IL-4 and IL-5.[33],[75] The middle ear has clearly been demonstrated to be an immunoreactive site in many animal models.[18],[106],[92],[105],[84],[42] Takeuchi has concluded that the T-cell antigen receptor is involved in the regulation of all antigen specific T-cell responses in human middle ears. [159] He also raised the possibility that antigens retained in MEE following an acute infection are responsible for activating T-cells and subsequent cytokine production. It has been proposed that the late-phase reaction in the ear is under the control of cytokines and is produced by cells in the tubo-tympanic cavity[18] instead of selective recruitment of lymphocytes from the circulation, as previously thought.

Evidence based medicine proves that various well established past explanations for chronic OME are myths. It is still taught that “Otitis will stop once the eustachian tube grows to a normal size” even though Sade showed that the ET of OM patients does not have an “immature morphology”. The pharyngeal and isthmus portions of the ET from OM patients is no different in size than that of normal children.[138] Parents are told that their otitis prone child cannot equalize the pressure in their middle ears, yet the fact is that there is no organic obstruction or stenosis of the ET in OME patients. Actually only 11% of active OME patients have abnormally high opening pressures.[157] Finally, there is the myth of antibiotics. Rosenfeld and Bluestone[134] have shown that meta analysis of all treatment studies demonstrate antibiotics to be no better than placebo. In fact 70% of effusions resolve whether treated or not. They noted that their review included no studies of allergy management because there are no Double Blind Placebo Controlled studies involving allergy management of otitis to review. Our concern is those 30% of children who’s effusions do not resolve despite conventional therapies and time. However, several studies have suggested that conventional allergy management may reduce the incidence of relapses or complications of OME.[66],[101],[110],[127]

There are few studies in which humans or animals have been challenged with an allergen which have resulted in the development of OME, in part because of the lack of an natural model, and in part by design. Experiments with Rhesus
monkeys produced ET obstruction after a four day challenge with ragweed. These animals failed to develop OME, perhaps because MEE does not form in monkeys without transection of the tensor veli palatini muscle.[28] Experiments with chinchillas and mice are more successful in producing MEE.[106],[73],[156] Surgical obstruction of the ET will only produce OME in 25% of chinchillas.[106] Fireman and Bluestone demonstrated that exposure of the human nose to antigen leads to ET obstruction and is dose dependent. The authors were careful to point out that: “The fact that OME did not occur in our preselected adult patient cohort was anticipated and an expectation of our experimental design.” They chose allergic adults who had no history of OME “so as to avoid creating complications”.[2]

Some animal experiments do support the concept that an immune-mediated response is localized within the middle ear itself, involving eosinophils, mast cells and neutrophils. Ryan has shown that in middle ears of mice local immunoregulation mediated by cytokines may be responsible for the amplification and differentiation of IgA-producing cells located in the middle ear mucosa. Ears with a chronic immune response showed extensive mucosal hyperplasia consisting of lymphocytes and neutrophils in the submucosa with neutrophils, macrophages and diffuse eosinophilic material in the effusions. This is accompanied by numerous IL-5+ cells in the subepithelial tissue and effusion in the chronic stages.[18] In asthma IL-5 has been shown to be a critical cytokine associated with eosinophil and lymphocyte recruitment following exposure to allergen.[155] Nakata also demonstrated high levels of histamine and prostaglandins in MEE that he suggested are local products rather than the result of a transudate from plasma.[106] Study IV demonstrated IL-5 in human MEE. In an elegant study using a rat model, Labadie[92] was able to demonstrate that allergen presentation to the middle ear of allergic rats caused ET dysfunction which resulted in middle ear effusion. Pollock[124] showed that when allergen at a dose 1/50 of that needed to cause local inflammation is presented to the nose it results in failure of those allergic rats to be able to demonstrate normal ET function.

An immune response involving IgG, complement activation, neutrophils, eosinophils and mast cells has been implicated in the generation of immune-mediated OME by Suzuki.[156] The perpetuation of chronic inflammation by cell mediators is further supported by the experiments of Nakata who was 100% successful in creating effusion when he either exposed the middle ear of pre-immunized animals to antigen or when he injected effusion fluid itself into a sterile ear. He concluded that middle ear “effusion itself induces inflammatory reactions in the middle ear cavity.”[106] Prostaglandins[106] and leukotrienes (LTC4)[81] have also been demonstrated in human ears. Study I similarly
demonstrated neutrophils, eosinophils and mast cells and their mediators in human MEE in atotics.

Miglets[103] and Yamashita[178] performed histologic studies which showed that allergen presentation to the middle ear did result in inflammation, but were reluctant to attribute this to allergy because of the lack of eosinophils. Mogi reviewed animal studies and concluded that although the Eustachian tube “is involved, both functionally and morphologically, in Type I allergic reactions of the nose and that the tubal dysfunction evoked by nasal allergic reactions is transient.” He postulates that since this inflammation does not culminate in the development of MEE, then “it is unlikely that Type I allergy is a casual factor” in the development of OME “even if the middle ear is an allergic shock organ.”

One might argue that what in fact Mogi describes as subjects being in a “chronic state of disease” is exactly what constitutes an atopic patient: namely an individual whose eosinophils, neutrophils and mast cells are primed so that when exposed to an antigen they react and release their various mediators, in contrast to the same cells in a non-atopic individual which do not react on similar exposure.[45],[153] Fireman concluded that the interaction between viral infection and nasal allergy might enhance certain responses and that such an enhancement might explain why allergic rhinitis is a risk factor for otitis media.[51]

The etiological relationship of allergy to OME is very controversial especially since from 1958 to 1984 Suehs,[154] Senturia,[142] Boor,[22] Siirala,[145] Lim,[96] and Reisman[131] could not find any clinical basis of allergy in the formation of OME. Typical was the guarded negative conclusion by Boedts whose study “failed to support the concept of allergy as a major causative factor in OME....although it may occur as a complication of an allergic (nasal) disease.”[21] Several reviews include critical analyses of decades of studies, both clinical and histological, that were designed to evaluate the relation between allergy and OM.[8],[44],[112],[178],[21],[164],[136] Unfortunately many reports including those published since the discovery of IgE are often less than critical, quote old data,[19],[93] are not based on present day standardized methods of allergy testing, apply allergy treatments to all patients regardless of diagnosis, or narrowly define their clinical population of allergic to include only those with a history of rhinitis.[13],[178],[149] Tomonaga criticized many of the aforementioned works. He found 21% of 605 allergy patients had OME, but among 259 OME patients, 87% were atopic by skin testing, although only 50% of these had nasal allergy.[164] He determined the incidence of allergies among patients with OME to be four to five times that expected in a similar age-adjusted population of normal individuals. This suggests that the relationship may be more than coincidental and is supported by the high prevalence among our patients.
Since OME often appears to be a disease of atotics, it seemed logical to ask: “Is OME an allergic disease?” As stated, an allergic response involves activation of Th-2 cells by environmental antigens with resultant up regulation and production of IL-4 and 5 and an expression of IgE on mast cells and eosinophils with a resultant release of tryptase, ECP and other mediators of inflammation.[151] This combination of events has heretofore not been described in OME. Clinically, the diagnosis of Type I hypersensitivity is based on the detection of allergen specific IgE by means of skin testing and/or in vitro testing. This thesis includes studies I,II,IV which are the first to our knowledge to correlate the expression of markers of eosinophil, neutrophil, mast cell, CD+3, and IL-5 mRNA activity found in middle ear effusion and/or mucosal biopsies from patients with OME to their atopic status.

Prior studies which led otolaryngologists to believe that less than 30% of OME was related to allergy were based on unusually narrow definitions of atopy requiring both rhinitis and a total IgE > 100µg/L or results of prick testing.[16] Our data shows that the mean total IgE among atotics was 93.8µg/L with two-thirds of atotics with OME having a serum IgE < 100 µg/l.(Fig. 5) Otitis is thus similar to rhinitis in having no relation to total IgE, unlike asthma which does show correlation.[30] Our reported prevalence of atopy of 81% among the group of OME patients in our studies(I,II) may reflect the increased sensitivity of modern in vitro testing as compared to results obtained from prick tests or questionnaires. This percentage of allergy among OME patients is consistent with prior reports based on in vitro testing by McMahan (88%),[101] Tomonaga (72%),[164], Nsouli (86%),[110] Corey (60%),[32] and Psifidis (78%).[127] and as discussed in the Methods Section, does not reflect a selection bias.

**Histologic Studies:** The fact that conventional histology does not readily detect degranulated or activated neutrophils, mast cells or eosinophils leads to various conclusions[102],[116],[112],[156] and is the major reason this controversy has been perpetuated. There is also disagreement as to whether mediators in middle ear effusion come from the plasma or local tissue. Using animal studies Nakata found few eosinophils in the effusion of immunized chinchillas in the acute phase of inflammation but also concluded that “middle ear effusion is a local product of the middle ear mucosa rather than a transudate from plasma”. Histopathologic examination of effusion demonstrates that eosinophils and neutrophils are integral components in these secretions,[82],[116] but not necessarily present in the mucosal lining. Despite Koch’s initial description of cytologic changes in chronic OM, in which he found eosinophils in the mucosa from 52 of 62 cases of allergic otitis,[89] eosinophil involvement is not usually
observed and mast cells are not described in the middle ears of children with OME.

Previous biopsies of middle ear mucosa from atopic patients have shown that the active degranulation of eosinophils correlated with elevated levels of effusion mediators. The flat epithelial cells found in middle ear mucosa are known to be able to change into either secretory or ciliated cells when challenged by pathological stimuli; this was also seen in our earlier biopsy study and was confirmed in Study III. These studies suggest that the inflammation observed in the promontory mucosa of 25 patients with OME, proven by in vitro testing to be atopic, appear to be accompanied by the continual recruitment and activation of eosinophils and mast cells. The histologic findings in the ears from normal subjects correlated with the descriptions by previous investigators.

The high numbers of eosinophils and the high density of EG2/MBP staining deposits within the tissue and mucous as well as the appearance of mast cells in middle ear epithelium is an indication of disease and not a normal feature. Anti-tryptase antibody staining showed that mast cells are present in the mucosa and submucosa in atopics as compared to the controls. Study II found 61% of 33 chronic OME patients had extensive activation of mast cells in their middle ears. The presence of mast cells and eosinophils or their mediators in the target organ is essential, although not pathognomonic, for a disease process to be considered allergic.

Inflammation is exclusively an in vivo phenomenon that only occurs in living tissues with an active microcirculation. Perpetuation of that inflammation, regardless of origin, is the crucial difference between recurrent acute otitis media and OME. A basic question is whether the middle ear inflammation was the result of infection or allergy or both. In Study I atopy seemed to have a significant relationship to whatever was producing a response from eosinophils and mast cells in the middle ear. This is a significant observation not only in regards to the association to allergy but also because human eosinophils are much more toxic than neutrophils, making them particularly harmful to host tissues. Demoly notes that “chronic sinusitis, which occurs in some patients with allergic rhinitis, can be distinguished on the basis of T cells and intraepithelial mast cells.” He found 8 times more T cells in patients with allergic rhinitis than in patients with nonallergic rhinitis. In Study IV we have demonstrated that 8 of 8 atopic patients with chronic OME had increased CD3+ T cells, vs none of the controls. This, coupled with the presence of degranulating mast cells and EG2 positive eosinophils in the mucosa, is
further evidence that the inflammatory response described in the middle ears of our patients(I-IV) may represent a Th-2 mediated disease.

In addition to the cytotoxic effects of ECP itself, these mediators are always accompanied by the other proteases, lysozomal enzymes and oxidizers that are released simultaneously from their respective cells. This may be why the atopic patient continues to produce additional fluid, as compared to the nonatopic patient. This was seen in allergic mice which produced twice the amount of MEE as non-allergic mice on antigen challenge.[92] ECP, known to decrease ciliary function, also impairs ET clearance. The very high concentrations of mediators released by eosinophils and mast cells may also account for the great destruction, osteitis and granulation tissue described on histologic examination of temporal bones from patients with chronic OM.[102] The destructive potential of these mediators in effusion is often overlooked, as is hearing loss, yet serves as a further justification for the removal of this fluid at the time of myringotomy and for prevention of its reoccurrence by appropriate medical management e.g. allergy management.

Purulence in the middle ear has previously been shown to elevate both eosinophil and neutrophil mediators.[72],[68] In Studies I and II gross pus had a similar elevating effect on ECP and MPO(Fig. 2,4) but did not appear to have an effect on mast cell activity(Fig. 3,6). Our findings are in concordance with studies of the histopathology of both serous OM and OME which have demonstrated that eosinophils and neutrophils are integral components in these infiltrates.[82],[116] One of the unique features of the middle ear response demonstrated in Study I was the involvement of neutrophils. Most atopic patients had increased levels of MPO in addition to ECP in their ear effusion, even though there was no evidence of acute inflammation. Paired samples(I) confirmed that in an acutely infected ear purulent OM was associated with a significant elevation of both mediators, but with a disproportionally greater elevation of MPO(Fig. 4), in the infected side as compared to the nonpurulent ear.[72] Yet even in the non-purulent ear the levels of MPO were much higher in atopics than non-atopics. The late asthmatic response in animal models is dependent on neutrophil availability and neutrophil chemotactic factor. This factor has been demonstrated in both atopic and nonatopic subjects.[31] Neutrophils from atopics produce more superoxide in the absence of stimuli than neutrophils from nonatopic patients. The total capacity of cells from atopics for oxidative metabolic activity apparently is not abnormal, but perhaps reflects a greater responsiveness to low levels of stimulation. Styrt found that “the neutrophils from atopics may be both easier to stimulate and more difficult to suppress than cells from normals.”[153] It was therefore expected that neutrophils would be present in the purulent OME,[85] but we were surprised to
find that neutrophils were so active in non-acute OME. Neutrophils however, are reported in cutaneous IgE-late-phase reactions in the nose and skin.\[39\],[114]. In Study I, purulence in the middle ear was shown to elevate both eosinophil and neutrophil mediators, although MPO rose twice as high in purulent ears as in OME ears. One explanation for the increased MPO we report in non-purulent MEE is that an increase in neutrophils may occur as a result of weak stimulation of these cells as bacteria are being cleared from the site of inflammation.\[61\] Recent studies have indicated that bacterial mRNA present in otherwise “sterile” MEE may serve as a stimulus to T cell activation.\[159\] Endotoxins have been demonstrated in 52 to 87% of effusions.\[107\],[37\] Atopy may thus contribute to the elevated levels of MPO (Study I) by causing the atopic child to respond differently to the products of acute inflammation due to its primed inflammatory cells.

It is our contention that the middle ear thus behaves like the rest of the respiratory tract and that what has been learned about the atopic response in the sinuses and lungs may be applied to the study of the middle ear to help in understanding OME. For the middle ear to emulate the respiratory tract and as such be capable of an allergic response it is essential that the cells and mediators necessary for the recognition of antigen, production of specific IgE and eventual release of inflammatory mediators and cytokines associated with a Th-2 response are indeed present in the middle ear effusion and mucosa of patients with OME.

Determining whether or not inflammatory mediators in the middle ear are produced locally is important in supporting or rejecting the hypothesis that the middle ear might be involved in an allergic Th-2 response. Just as our earlier studies showed no relation between levels of serum and effusion ECP\[72\] or specific IgE,\[70\] the concept that active immunologic processes may be a localized phenomena in the middle ear is supported by these studies which demonstrate local release of ECP(I,III) and tryptase(II) in middle ear mucosa, as well as CD3+ cells and IL-5 mRNA expression.(IV) A specific immune response in the middle ear has been established in animal models\[94\],[103],[92\] as well as in humans. Lymphocytes necessary for antibody production have been shown to be recruited nonspecifically into the mucosa in otitis media. IL-5 is increased during the late-phase reaction of chronic middle ear disease.\[18\] T lymphocytes are common in serous or mucoid effusions.\[115\] The finding of CD3+ T cells has been thought to be a marker which can be used to distinguish between allergic and non-allergic sinusitis.\[62\] IL-5 is produced predominately by stimulated Th-2 and not Th-1 cells.\[46\] Having CD3+ cells and more importantly, cells actually manufacturing IL-5 mRNA in our biopsies(IV), provide strong evidence that the middle ear is actively participating in a TH-2
response. Similarly, in Studies I,II tryptase was not present in the effusion of any non-atopic patient, nor was it present in all atotics, suggesting that elevated effusion tryptase reflects a local activation of mast cells, not a general systematic response to being atopic.(II) Eight patients with paired samples had different values of tryptase from their opposite ears (i.e. patient AL: 16µg/l left, 1.5 µg/l right) which also suggested a local response. Among the 13 patients (16 ears) with apparent undetectable levels of tryptase, 56% had evidence of atopy as signified by increased effusion ECP and positive serum ELISA. Thus not all atotics always show mast cell mediator release, again evidence for local, Th-2 allergic-like response in the middle ear.

**Future Studies:**

Patients may be atopic and by definition capable of making IgE antibodies to common allergens in their environment, although not necessarily symptomatic. Only if those antibodies are associated with disease of a specific target organ is the patient allergic.[119] Just as it is difficult to know with certainty which positive antigens are responsible for the actual disease in allergic rhinitis or asthma without direct challenge tests, the same is true of otitis. Clinical proof of this mechanism will require either provocative challenge studies or a large randomized therapeutic trial. Determination of whether local elevated IgE titers and the presence of mast cells and their products are directly due to an atopic reaction will also require advanced research involving direct challenge studies or immunohistostaining of middle ear mucosal biopsies. The development and use of newer technologies to improve on the sensitivity of in vitro testing so as to more accurately identify which patients are atopic will improve our ability to know in which OME patients allergy may be contributing to the disease.

The hypothesis that viral and/or bacterial infections may induce OME is not contradictory to an allergy hypothesis. Perhaps only in an atopic individual does an initial viral or bacterial infection lead to the chronic recruitment of inflammatory cells with the resultant release of their potent inflammatory mediators, attractants, cytokines, and other factors that results in the production and maintenance of effusion in a sterile ear. Given the economy of nature, it is difficult to explain the presence of plasma cells,[121] IL-5 +mRNA cells,(IV) CD3+ T cells,(IV) eosinophils,(I,II,III) mast cells,(II) specific IgE,[70] tryptase(I,II) and ECP(I,II) in any middle ear, let alone 80% of those tested, if a Th-2 immune-mediated “allergy-like” response were not involved.

The fact that 93.5% of our 77 consecutive children with persistent effusion were atopic(I) suggests that whatever the initiating event responsible for otitis might be, it results in more chronic disease in atotics and constitutes an important justification for the need to develop a new treatment approach for this disease.
As an atopic child is known to be more likely to develop asthmatic bronchitis following a viral infection, so too an atopic with otitis may be more prone to develop chronic effusion. Certainly many children in day care get upper respiratory infections and acute otitis media. Irander suggests that it is predominantly those who are atopic who progress to chronic OME.\(^{[74]}\) The observation that the inflammatory pattern in the middle ears of atopics differs markedly from that seen in non-atopics(Figs. 3,4,6,) deserves further investigation. The paucity of traditional randomized clinical trials dealing with OME, especially as it might relate to allergy, clearly indicates the need to devise alternate methods to study this disease. It may be that current methods used to control chronic OME, otorrhea, and draining mastoid cavities will continue to fail as long as the effects of allergy on middle ear mucosa are ignored. There is a need to develop outcome research instruments and methods to validate the efficacy of various treatments, including allergy management.

**Conclusions**

Eighty-five percent (85%) of 131 OME patients randomly selected for these four studies were atopic by in vitro testing. The 20 clinical studies cited in Table I demonstrate that there is a relationship of allergy to OME. Twelve of these studies present evidence that allergy management of chronic OME, when applied to patients proven by RAST, ELISA and/or IDT to be atopic, has a superior outcome to M&T or antibiotic/decongestant therapy alone; with effectiveness ranging from 82% to 100%. These empirical studies challenge scientists to investigate whether there is more direct evidence of allergic Th-2 like activity in the middle ear which might explain such results. Elevated effusion ECP(Fig. 2) and tryptase (Fig. 3,6) values in patients with non-purulent OME, coupled with the presence of ECP(I,II), degranulated eosinophils(I,III), tryptase(I,II), mast cells(II), CD3+ T cells(IV), and mRNA + cells for IL-5(IV) in the middle ears of atopic individuals but not nonatopic subjects supports our stated hypothesis that 1) there is a relation of atopy to OME, and that 2) the middle ear epithelium of atopics has all the components required to behave in a manner similar to that of the rest of the upper respiratory system and is itself capable of an allergic immune response.

These studies suggest on a cellular level that the mediators measured in effusion arise from cells identified in the local tissue lining the middle ear cleft. They refute the null hypothesis as they confirm that many of the participants essential to a Th-2 driven immune response are indeed present in the majority of ears with chronic effusion. They suggest that the inflammation within the middle ear associated with the maintenance of many cases of OME is “allergy like”. When viewed in the context of the Th-2 paradigm for allergic inflammation, the results are compelling. The Th-2 model purports that an increased expression of Th-2
cytokines (e.g. IL-4 and IL-5) is associated with T cell and eosinophil infiltration and IgE production (i.e. Type I hypersensitivity). The demonstrated expression of IL-5 mRNA in middle ear mucosa is support of an allergic etiology in refractory OME.

Two findings in this thesis may be keys to the understanding of the development of OME. First is the fact that atopics have elevated levels of inflammatory mediators in contrast to nonatopics and the other is the fact that neutrophil mediators in addition to the “Th-2-mediators” are raised in atopics only. (Fig. 4) The first finding tells us that there is some unique quality associated with being atopic. This quality is most likely related to a patient’s allergen sensitivity and its being exposed to that allergen. Indeed, Labadie[92] showed in animal studies that allergen-sensitized and challenged animals responded by producing more middle ear fluids to a secondary stimulus than non-sensitized and challenged animals. Labadie also showed that allergen challenge per se did not induce any fluid production, which is in agreement with others. The unique reactivity of atopic ears has also been documented in man with the demonstration of higher fluid levels of the adhesion molecule VCAM-1 than found in nonatopics.[111]

Our second finding regarding elevated MPO in effusion tells us that the response in the atopic ears is not restricted to those cells and mediators that are involved in the classical allergic reaction in which neutrophils are usually non-participants. Thus, the findings of MPO as a sign of neutrophil involvement suggest that the reaction accountable for middle ear effusion may be a more general inflammatory response to some inciting agent, but that this response is so much more prominent in the primed atopic ear. The relationship between allergy and OME, therefore, may be indirect and the allergic reaction one of the mechanisms that may prime the middle ear to a vigorous and prolonged response to microbial products.

Identification of factors responsible for the chronicity of otitis media is an essential step in understanding the disease. We present effusion and mucosal biopsy studies demonstrating levels of ECP, tryptase, and/or IL-5 mRNA cells, CD3+ T cells, eosinophils, and mast cells which indicate that many of the mediators and cells essential to the production of a Th-2 immune mediated response are present in ears with chronic effusion. The increased levels of MPO in atopic patients further suggest that the general inflammatory response to putative inciting agents such as bacterial and viral products may be altered in atopy. These studies support the hypothesis that the exaggerated inflammation within the middle ear associated with most cases of OME is possibly the result of an atopic response within the middle ear itself.
Remembering that children with hearing loss due to OME constitute potentially the largest group in the world with a reversible learning disorder, the significance of these studies as regards the treatment and ultimate prevention of this chronic disease cannot be understated. These findings should be relevant to a physician’s diagnostic and therapeutic approach to patients with chronic MEE. Physicians must become disengaged from the discussions of “What to do?” regarding antibiotics, adenoids, tubes, etc. long enough to pursue the “Why?”. Having evidence that otitis media with effusion may be a sign of allergy (although not pathognomonic) and having evidence that the middle ear mucosa, like a “5th sinus,” appears to be capable of responding in a manner similar to the rest of the respiratory tract and participate in a Th-2 like allergic response, the standard evaluation for a patient demonstrating persistent effusion with or without purulence warrants including a full evaluation for food and inhalant allergies. To be efficacious, antibiotics should be used for infection; antihistamines, steroids, and immunotherapy should be reserved for allergy.

**Summary**

I. Most striking in this random prospective study of 97 individuals is the very different response seen among groups of atopic vs non atopic patients (p<0.001) by all 3 inflammatory cells in what clinically appears to be identical disease. Histologically, OME in the 2 groups involves 2 very distinct disease processes. These findings provide evidence that eosinophils and mast cells, both essential to a Th-2 driven immune response, are active in the majority of ears from atopics with chronic OME.

II. This study was the first to our knowledge to provide confirmation on a cellular level that the mast cell mediator tryptase measured in effusion of atopic patients arises from actively degranulating mast cells identified in the local tissue lining the middle ear cleft. Mast cells and their mediator tryptase, both essential to the production of a Th-2 immune response, are present in ears with chronic effusion. This study provides further evidence in support of the hypothesis that middle ear mucosa itself is similar to that of the rest of the upper respiratory tract and is capable of an allergic response.

III. This study demonstrated that plastic embedding techniques provide superior structural preservation compared to the cryosections used in previous studies. Because thinner sections can be cut from plastic embedded material, this would make it preferable to paraffin embedding. The different cell types could be identified without problems and cell counts could be easily made. This study also shows that by the use of specific antibodies it is possible to quantitatively determine the frequency with which eosinophils and neutrophils occur in tissue and mucus in the middle ear. Such a quantitative determination is
necessary to allow conclusions about the possible role of these inflammatory cells in pathologic conditions such as OME. The results suggest that eosinophils and neutrophils occur with a higher frequency in the middle ear of atopic children with OME as compared to non-OME controls. The present study shows a covariation between the occurrence of eosinophils and neutrophils in OME and supports our earlier findings.[72],[69]

IV. This study demonstrated for the first time the expression of IL-5 mRNA in the middle ear of patients with refractory OME. It is of note that increased expression of IL-5 mRNA cells, CD3+ cells and eosinophils (MBP) were present in the majority of OME ears and furthermore, that all children with these cell markers were atopic. This data suggests that IL-5 and other Th-2 type mediators may perpetuate the inflammatory response and contribute to the inability of these patients to clear their effusions. The presence in OME patients’ mucosa of activated eosinophils and IL-5 lends further support to the hypothesis that a TH-2 inflammation takes place in OME.

Our results indicate that atopy may be a major determinant for the development of OME.
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“The truth can wait,
for it lasts a long time.”

Schopenhauer - 1836
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