eosinophilic lung diseases

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INTRODUCTION

Eosinophilic lung disease, for the purposes of this grand rounds, will be defined as the presence of large numbers of eosinophils in either airway mucosa (asthma) or lung parenchyma. The term eosinophilic lung disease encompasses a diverse group of conditions ranging from a common disease such as asthma to a number of relatively rare diseases (Table 1). PIE syndrome, which stands for pulmonary infiltrates with eosinophilia, has been used to describe many of these diseases but is misleading, since some patients with eosinophilic lung disease do not have eosinophilia. The first part of this protocol reviews clinical aspects of these diseases. Subsequently, the cellular biology of the eosinophil will be reviewed, focussing on regulation of eosinophil formation, eosinophil activation and adherence, and the mechanisms by which eosinophils cause inflammatory injury to the lung. Emphasis will be placed on the role of T cell-derived cytokines as key mediators of eosinophilic disease.

Table 1

EOSINOPHILIC LUNG DISEASES

1. Asthma
2. Churg-Strauss syndrome
3. Allergic bronchopulmonary aspergillosis
4. Parasitic infections
5. Drug induced
6. Eosinophilic pneumonia - acute and chronic

EOSINOPHILIC LUNG DISEASE

ASTHMA

During the past fifteen years concepts of asthma pathophysiology have changed considerably. The traditional view of asthma was that airway obstruction resulted as a consequence of airway smooth muscle contraction, triggered by IgE-induced release of preformed mast cell mediators such as histamine. This concept failed to explain many clinical features of asthma, such as hyper-reactive airways and chronic perennial symptoms. The new paradigm is that obstruction results largely from bronchial inflammation. Although the concept of asthma as an inflammatory disease seems new, in fact pathologists have long appreciated the presence of airway inflammation in fatal asthma and William Osler, in the 1892 edition of his textbook of medicine, remarked

that many cases were caused by "a special form of inflammation of the smaller bronchioles" (1).

Figures 1 and 2 show two clinical observations which contributed towards understanding the inter-relationship of eosinophils, inflammation, and asthma.

Airway hyper-reactivity is a defining feature of asthma and is illustrated on Figure 1. Compared to normal subjects, asthmatics develop bronchospasm at relatively low doses of inhaled bronchoconstrictor agonists such as histamine or methacholine as well as after inhaling other substances such as smoke, cold air, or exercise (2). Hyper-reactivity correlates with frequency and severity of attacks, most asthmatics have hyper-reactive airways, and hyper-reactivity accounts for chronic asthma in which attacks often occur in the absence of exposure to an identifiable aeroallergen (3).

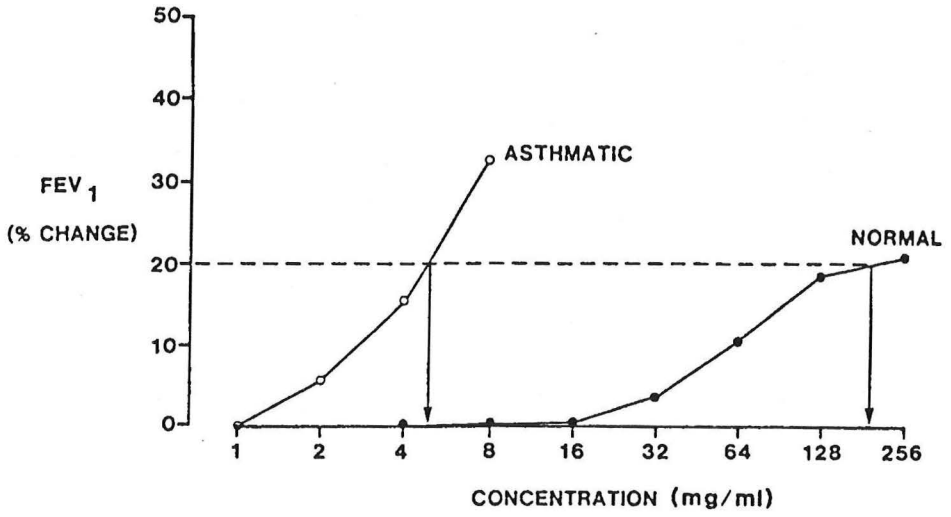


FIGURE 1. A dose-response curve showing the decrease in the FEV1 in response to an increasing concentration of an inhaled bronchoconstrictor agonist such as methacholine.

Figure 2 illustrates a late asthmatic response. Most atopic asthmatics develop immediate bronchospasm after inhaling an antigen to which they are sensitized, and this early response generally resolves within one hour. In about 50% of cases the early response is followed by a late response which starts about four hours after antigen challenge and lasts up to 24 hours. Pretreatment with an inhaled B2 bronchodilator inhibits the early response only, whereas pretreatment with anti-inflammatory drugs inhibits the late response. Cartier in 1982 first recognized the association between late responses and hyper-reactivity while studying a group of atopic asthmatics; hyper-reactivity developed

only in those with late responses, and the late response always preceded the onset of hyper-reactivity (4). Thus, the late response is important for hyper-reactivity, and investigation of the cellular and biochemical characteristics of the late response has established that asthma is an inflammatory disease in which the eosinophil plays a major role.

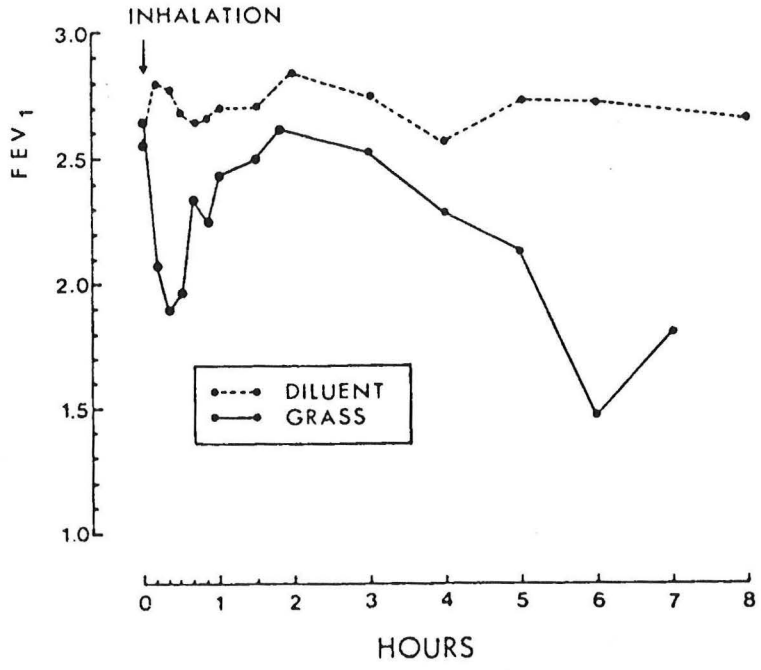


FIGURE 2. An early and late response to an inhaled allergen (grass pollen).

Lancet 2:255, 1983

EOSINOPHILS, INFLAMMATION, AND ASTHMA

Bronchoscopy has been an important tool for asthma research, permitting biopsy of the bronchial epithelium and sampling of the respiratory epithelial surface by bronchoalveolar lavage (BAL). Investigation of the late response has been performed by giving stable, atopic asthmatics an inhaled antigen followed by bronchoscopy 24 to 48 hours later. Results can be summarized as follows:

1. There is a large influx of eosinophils into the airways. In one study, eosinophils comprised 66 $\pm$ 12% of the cells obtained by BAL, compared to less than 1% prior to antigen challenge (5,6).

2. Eosinophils entering the lung appear to have been activated, as judged by the presence of large amounts of eosinophil granule proteins in BAL fluid. Considerable amounts of the two cytokines which activate and recruit eosinophils into tissue, IL-4 and IL-5, are also present (5,7).

3. Bronchial epithelium is disrupted. Detached epithelial cells are present, the albumin content of BAL fluid is increased reflecting increased permeability and epithelial injury, and the albumin content correlates with the number of BAL eosinophils (8).

4. The changes noted in the lungs are mirrored by changes in circulating eosinophils. During and after a late response there is an eosinophilia, and during that time eosinophils become less dense, presumably because they have been activated by cytokines to release granule contents (Figure 3) (9,10). Hypodense eosinophils have an increased number of IgE receptors, are more cytotoxic, and release more LTC4 and superoxide when stimulated. Thus, circulating eosinophils of asthmatics are activated and are more potent inflammatory cells.

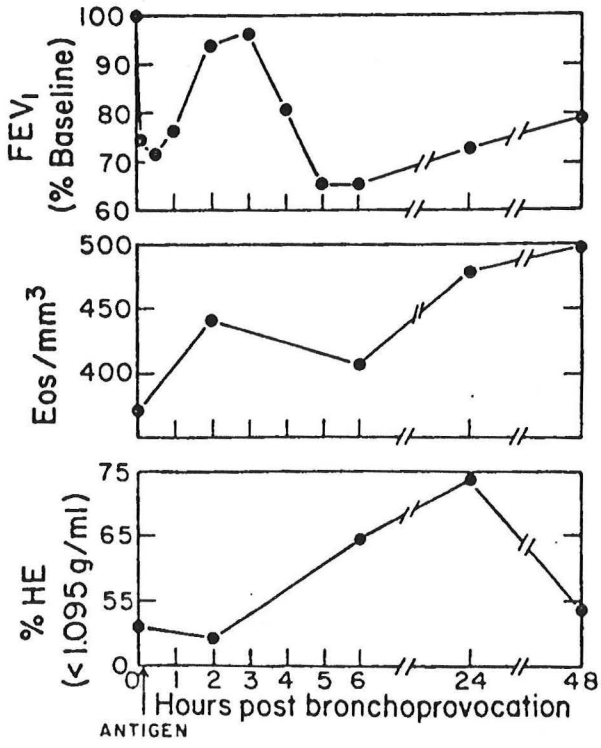


FIGURE 3. Alterations in peripheral blood eosinophils during a late response. % HE refers to % Hypodense Eosinophils

Other investigators have used bronchoscopy to investigate stable asthmatics without a prior antigen challenge to induce a late response. The following results have been obtained:

1. Bronchial biopsies obtained from even mildly symptomatic, newly diagnosed patients show a surprising amount of epithelial injury, with decreased numbers of ciliated epithelial cells, goblet cell hyperplasia, and a marked increase in the number of bronchial wall eosinophils and lymphocytes (Figure 4) (11,12).

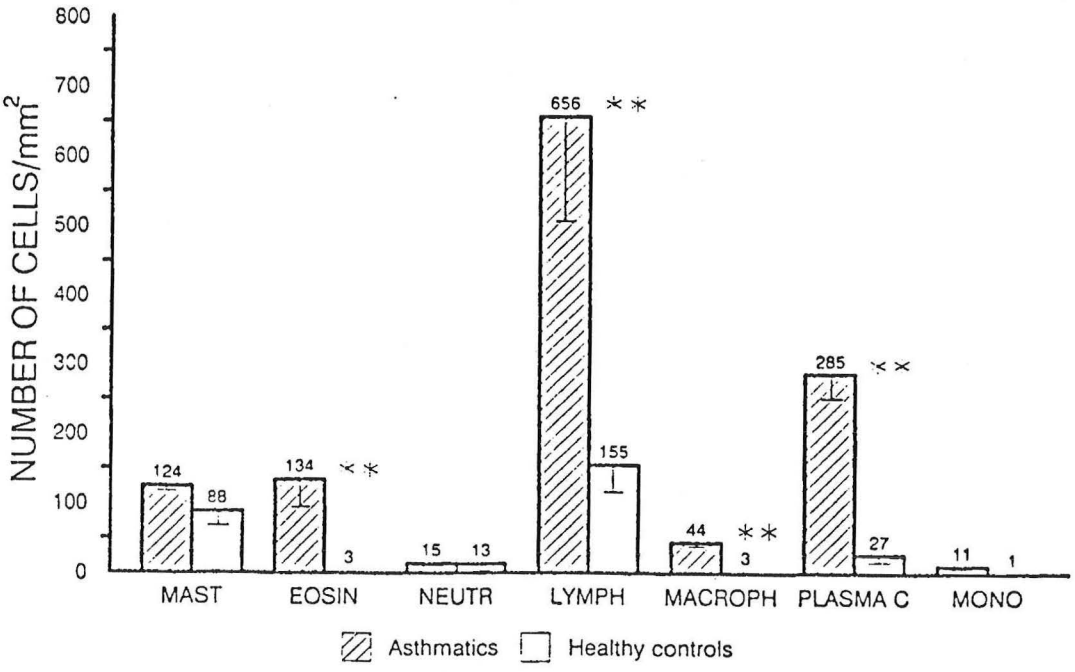


FIGURE 4. Bronchial biopsy results in patients with newly diagnosed asthma.

Am. Rev. Respir. Dis. 147:702, 1993

2. The level of eosinophilia and the number of eosinophils seen in bronchial biopsies or in BAL fluid correlate with asthma severity (Figure 5) (13,14). Additionally, serial biopsies of individual patients have shown that few eosinophils are present when patients are well, symptomatic asthma is accompanied by a marked increase in bronchial eosinophils, and clinical improvement is associated with a decrease in eosinophils to pre-exacerbation levels (15).

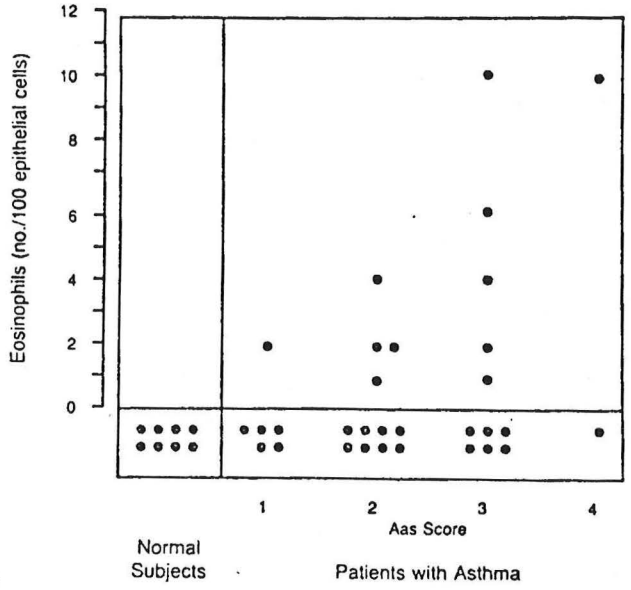


FIGURE 5. Eosinophils in bronchial biopsies of asthmatics. Aas score is a severity index, with 4 being most severe.

N. Eng. J. Med. 323, 1037, 1990

3. Severity of bronchial hyper-reactivity correlates well with the number of eosinophils in biopsies or BAL, the amount of bronchial epithelial injury, and the level of eosinophilic granule proteins in BAL (Figure 6) (16-18).

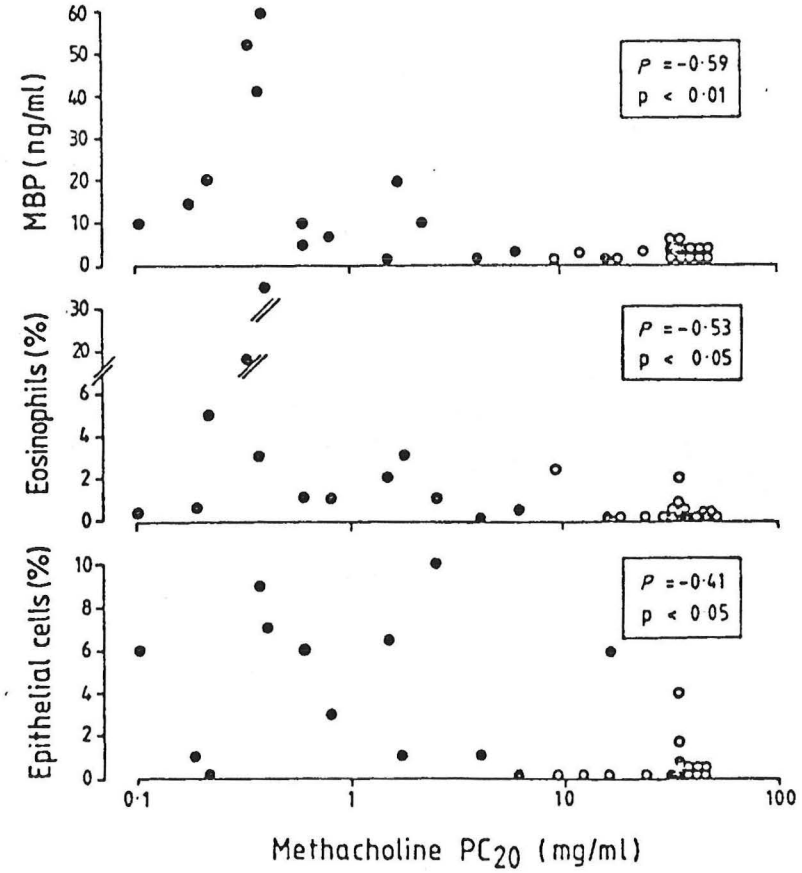


FIGURE 6. Correlation major basic protein (MBP), eosinophil count, and detached epithelial cells in BAL with airway hyper-reactivity.

Am. Rev. Respir. Dis. 137:67, 1988

Carefully controlled experiments in primates, in which sensitized animals develop hyper-reactivity after antigen inhalation, have shown that eosinophils enter bronchial epithelium before hyper-reactivity develops. Depletion of circulating leukocytes by hydroxyurea pretreatment prevents hyper-reactivity (19). Pretreatment with a monoclonal antibody (Mab) against an endothelial cell adhesion molecule, which prevented eosinophil entry into bronchial epithelium, also prevented hyper-reactivity (Figure 7) (20). Thus, a large body of clinical and experimental evidence suggests that eosinophils

are recruited into bronchi during a late response, are activated, and play a key role in producing hyper-reactive airways and asthma symptoms. In recognition of this, some investigators have proposed renaming asthma as eosinophilic bronchitis, a term which more accurately conveys the cellular and inflammatory nature of the disease (21). Many experts believe that epithelial injury results in hyper-reactivity; loss of the epithelial barrier uncovers irritant receptors and mast cells, allowing direct contact with inhaled stimuli. Details of how eosinophils effect inflammation and tissue injury will be reviewed later.

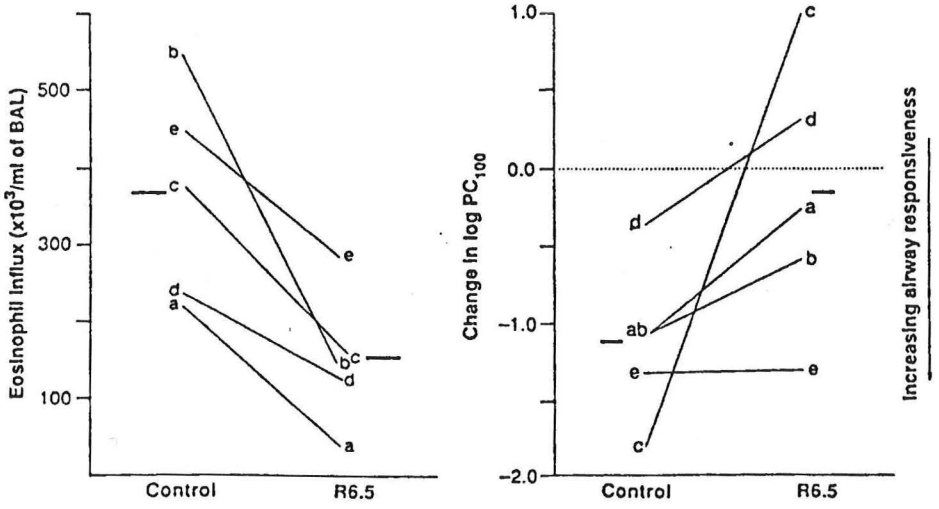


FIGURE 7. Effect of preventing eosinophil entry on airway hyper-reactivity. R6.5 is a monoclonal antibody against the endothelial adhesion molecule ICAM-1.

Science 247:458, 1990

CHURG-STRAUSS SYNDROME

This syndrome was originally described in 1951 in a group of 14 patients, all of whom had asthma. Since then many cases have been described. The three criteria necessary for a diagnosis are asthma, eosinophilia (>1,500 cells/mm^3), and systemic vasculitis involving $\geq$2 extra-pulmonary organs (22). The typical patient presents with a prodromal phase of allergic rhinitis, worsening asthma, and eosinophilia. The vasculitis phase follows and is marked by systemic symptoms (fever, weight loss, malaise), pulmonary infiltrates (which may be transient and, in contrast to Wegener's granulomatosis, rarely cavitate), peripheral neuropathy (40% develop a mononeuritis multiplex), skin rash (papable purpura), gastrointestinal disease (diarrhea, bleeding), and cardiac involvement (pericarditis, heart failure,

electrocardiographic abnormalities) which is the commonest cause of death. Clinically significant renal involvement is rare.

Pertinent laboratory abnormalities include very high serum IgE. 70% have a positive anti-neutrophil cytoplasmic antibody (ANCA) test, which is due to anti-myeloperoxidase antibodies and appears as a perinuclear staining pattern (P-ANCA). Tissue biopsy typically shows a necrotizing small vessel vasculitis with the characteristic prominent collection of mature eosinophils (23). Patients usually improve when treated with high doses of corticosteroids, and cyclophosphamide can be given to the rare steroid-unresponsive patient (24).

ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS (ABPA)

ABPA is another eosinophilic lung disease limited to patients with pre-extant asthma; in common with Churg-Strauss Syndrome, patients with ABPA have long-standing asthma which usually worsens prior to ABPA onset. The seven criteria required for the diagnosis are listed on Table 2.

Table 2

DIAGNOSTIC CRITERIA FOR ABPA

1. Asthma
2. Abnormal chest radiograph
3. Eosinophilia
4. Elevated serum IgE
5. Immediate skin test response to Aspergillus antigens
6. Precipitating antibodies (IgM, IgG, or IgA) to Aspergillus
7. Central bronchiectasis

Aspergillus fumigatis is the usual species causing ABPA, and it is present as a saphropyte, colonizing bronchi but not invading the bronchial wall. Patients often have a history of coughing brown colored mucous plugs, which are brown due to the presence of abundant fungal hyphae (25,26). About half present with a productive cough, fever, and an infiltrate on the chest radiograph and thus are often mis-diagnosed as having a bacterial pneumonia. The chest radiograph may have a distinctive appearance with dense shadows radiating out from the hilum in a gloved finger pattern, which is caused by mucous plugs in large central bronchi. Other radiographic patterns include nodular infiltrates and linear/ring shadows which are formed by thickened bronchial walls (27). ABPA responds rapidly to modest

corticosteroid doses. Patients with established ABPA can be followed by measuring their total serum IgE level, which usually rises prior to an exacerbation (28-30).

PARASITIC INFECTION

Since a major eosinophil function is killing parasites it is not surprising that many parasite-infected patients have eosinophilia and some develop eosinophilic lung disease. These diseases can be separated into two groups - Lofflers Syndrome and Tropical Pulmonary Eosinophilia - based on both clinical features and the type of helminth (worm) infecting the patient.

Lofflers Syndrome, or simple pulmonary eosinophilia, is caused by a number of worms (Table 3). A common feature of these infections is that the worm larvae have a migratory phase during which they exit the circulation through the pulmonary capillaries, enter the airspaces of the lung, and ascend the bronchial tree to eventually be swallowed and enter the gastrointestinal tract. Pulmonary disease caused by these worms represents a larvae-induced pneumonitis and is typically transient, since the migratory larvae either exit the lungs or die (31,32). Typical cases are mild, last for <4 weeks, and present as a febrile illness with wheezing and cough. The chest radiograph shows migratory infiltrates, mild eosinophilia is present, and serum IgE levels are usually normal. Severe cases, in which patients develop marked airway obstruction and respiratory failure, have been reported (33).

Table 3

PARASITES CAUSING LOFFLERS SYNDROME

- Ascaris species
- Ancylostoma duodenale
- Necator americanus
- Toxocara canis and catis (visceral larva migrans)
- Strongyloides stercoralis
- Schistosoma species

Tropical pulmonary eosinophilia is caused by two worms, Wuchereria bancrofti and Brugia malayi. The adult worms dwell in lymphatic vessels and release micro-filariae into the circulation, which lodge in pulmonary capillaries, are opsonized by IgE, and induce an influx of eosinophils into the lung parenchyma. Patients present with lymphedema, fever, cough, wheezing, and nodules or infiltrates on the chest radiograph. Laboratory tests reveal marked eosinophilia (>3,000/mm^3), high IgE levels, and large numbers of eosinophils in BAL fluid (34-36). Unlike Lofflers Syndrome tropical pulmonary eosinophilia is a chronic disease and symptoms may last for months to years. Progression to irreversible interstitial lung disease, with restrictive changes on pulmonary function testing and fibrosis on lung biopsy, has been described (37,38).

DRUG-INDUCED EOSINOPHILIC LUNG DISEASE

A large number of drugs can cause eosinophilic pneumonia (Table 4). Affected patients present similarly to Lofflers Syndrome with fever, wheezes, and radiographic infiltrates. Eosinophilia is usually but not always present (39). Symptoms resolve rapidly and the chest radiograph clears once the offending drug is stopped. Recent additions to the list are tryptophan and inhaled crack cocaine; the former, when ingested in gram amounts as a treatment for insomnia, can also cause an eosinophilia-myalgia syndrome without pulmonary involvement (40,41).

Table 4

DRUGS CAUSING EOSINOPHILIC LUNG DISEASE

- Antibiotics:
  Penicillins, nitrofurantoin,
  streptomycin, sulfonamide, tetracycline
- Bleomycin, methotrexate
- Naproxen, gold, phenylbutazone
- Dilantin, carbamazepine
- Imipramine
- Propylthiouracil
- Phenothiazine, chlorpromazine
- Crack cocaine
- Tryptophan

ACUTE AND CHRONIC EOSINOPHILIC PNEUMONIA

The eosinophilic pneumonias are idiopathic conditions in which eosinophils infiltrate the interstitium and airspaces. Secondary causes of pulmonary eosinophilia such as infection, drug reactions, or a vasculitis must be excluded before a diagnosis of eosinophilic pneumonia can be made. Both chronic and acute presentations have been described.

Criteria for chronic eosinophilic pneumonia include > two weeks of symptoms, radiographic infiltrates, eosinophilia, and exclusion of any other cause of eosinophilic pneumonia. Middle aged females are most often affected, and 40% have asthma or a history of asthma. Cough, fever, dyspnea and weight loss are the usual symptoms, with the mean duration of symptoms in one series being 7.7 months (42,43). 60% have a very characteristic chest radiograph which has been described as resembling a photographic negative of pulmonary edema; dense air-space infiltrates are present in the periphery of the lung, abutting the pleura, but central peri-hilar areas are clear. Lung biopsies show intra-alveolar and interstitial eosinophils and fibrosis, and BAL typically contains >30% eosinophils (44). Many of the eosinophils in lung bioipsies appear degranulated, and large amounts of major basic protein, a component of eosinophil granules, have been detected in lung and pleural fluid samples (45,46). Patients typical improve dramatically and rapidly (<5 days) with corticosteroids, and steroids can be given as a diagnostic and therapeutic trial (). However, relapses occur and a minimum of six months corticosteroid therapy is recommended (43).

Acute eosinophilic pneumonia has only recently been described and is a fulminant disease, with respiratory failure occurring within seven days of initial symptoms. Patients present with fever, dyspnea, severe hypoxemia, and diffuse infiltrates on the chest radiograph. Unlike chronic eosinophilic pneumonia, the chest radiograph does not show a characteristic peripheral infiltrate, eosinophilia is rare, and the disease does not recur. The diagnosis is usually made by bronchoscopy when BAL contains >25% eosinophils (47,48). Patients improve within 48 hours of corticosteroid administration.

EOSINOPHIL CELL BIOLOGY

EOSINOPHIL REGULATION - THE Th2 RESPONSE

Recent advances in immunology indicate that eosinophil production, entry of eosinophils into tissue, and activation is largely controlled by T cells and T cell-derived cytokines. The evolving picture is that CD4+ helper T cells can be divided into

two subgroups, based on both the pattern of cytokines released and the type of immune response which results (49,50). The subgroups are called Th1 and Th2 (for T helper) and both require an antigen presenting cell such as a macrophage to initiate the response. The Th1 response is important for cell mediated immunity against intracellular pathogens (such as Mycobacteria) and results in granuloma formation and delayed-type hypersensitivity (Figure 8). The Th2 response occurs during parasitic and allergic diseases and is associated with eosinophilia and high IgE levels. Each response inhibits the other; gamma interferon suppresses Th2 cells and IL-10, released by Th2 cells, inhibits early stages of Th1 activation (51,52).

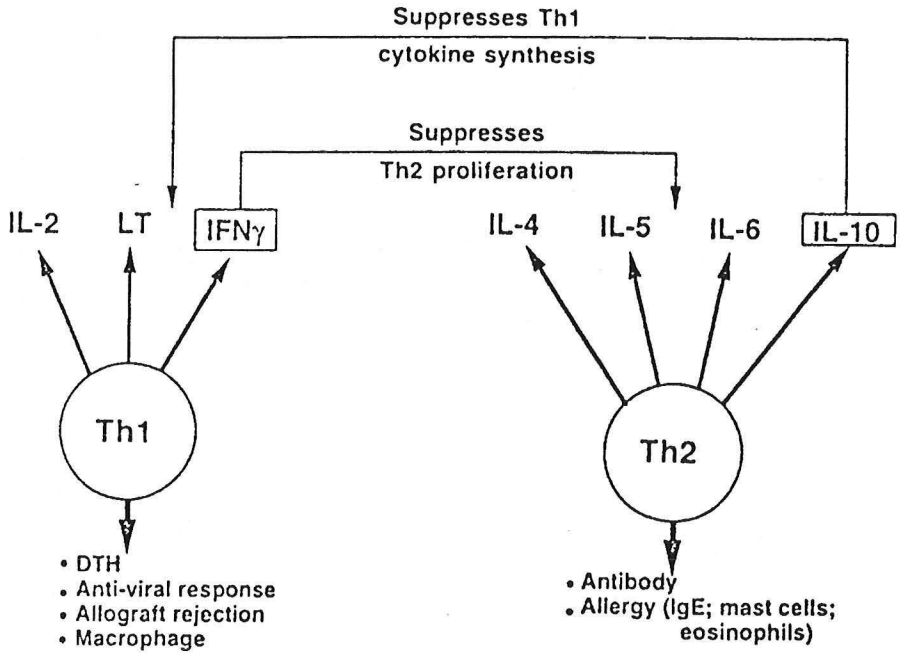


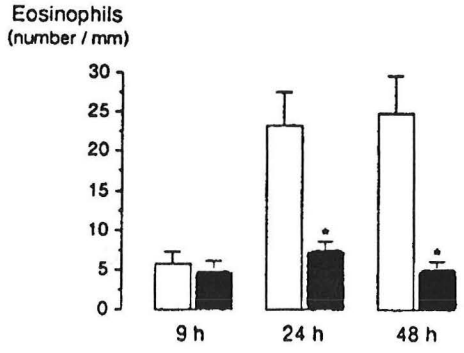
FIGURE 8. Th1, Th2 responses.

Immunology Today, 14:335, 1993

Some of the in vivo evidence suggesting that C4+ cells control eosinophils by producing Th2-type cytokines (particularly IL-4 and 5) is outlined below. Although most of the data has been obtained from studies of allergic reactions and asthma, it is not unreasonable that identical mechanisms occur during other types of eosinophilic lung disease.

1. Mice infected with the parasite Nippostrongylus brasiliensis develop eosinophilia, high IgE levels, and their lymph node/spleen T cells produce large amounts of IL-4 and 5 and little gamma interferon. Treatment with an anti-IL-4 Mab reduces IgE levels, and an anti-IL-5 Mab decreases eosinophilia (53).

2. In a murine model of asthma, in which ovalbumin-sensitized mice inhale aerosolized ovalbumin, a rapid influx of CD4+ T cells and eosinophils into the tracheal epithelium occurs after the inhalational challenge. Pretreatment with either an anti-CD4 Mab (Figure 9) or an anti-IL-5 Mab prevents the increase of epithelial eosinophils, suggesting that CD-4+ cells recruit eosinophils to the respiratory tract by releasing IL-5 (54). Pretreatment with an anti-CD8 Mab had no effect. In the same model, gamma interferon pretreatment prevents the antigen-induced entry of CD-4+ cells and eosinophils, supporting the concept that gamma interferon, an important Th1 cytokine, suppresses Th2 responses (55).



Am. Rev. Respir. Dis. 146:375, 1992

FIGURE 9. The open bars represent control animals and the black bars animals pretreated with an anti-CD4 Mab.

3. Human studies have characterized cytokine production by measuring lymphocyte mRNA at sites of antigen-induced dermal late-phase reactions and in BAL fluid obtained from asthma patients. The results have been consistent; large numbers of T cells are present which are producing the Th2 cytokines IL-3,4,5 and GM-CSF, while little message for gamma interferon or IL-2 is present (Figure 10) (56-59).

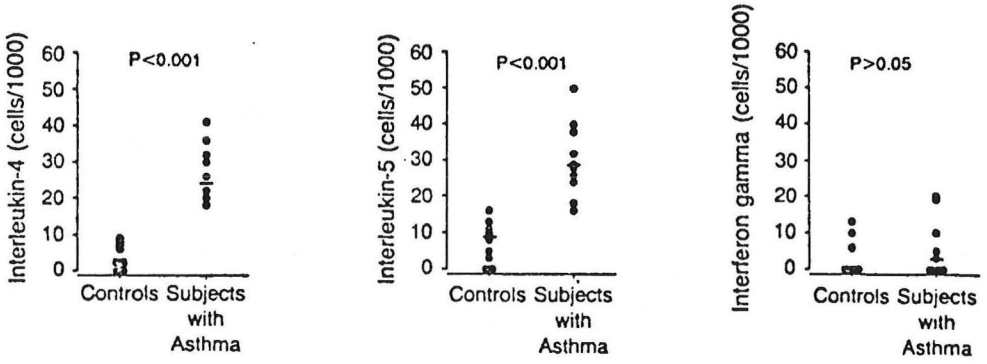


FIGURE 10. IL-4, IL-5 and gamma interferon mRNA expression in BAL T cells obtained from asthma patients.

N. Eng. J. Med. 326:302, 1992

Th2 CYTOKINES - IL-4 AND IL-5

IL-4 has several important effects. It supports mast cell proliferation by acting as a mast cell growth factor, and Il-4 is the principal switch factor promoting IgE and IgG1 production by B cells (60,61). Although IL-4 does not affect eosinophil production, it does promote eosinophil entry into tissue by increasing eosinophil adherence to endothelial cells. In experiments performed with human eosinophils and endothelial cell monolayers, IL-4, at concentrations of 10-1,000 units/ml, markedly (250% increase) and selectively (granulocyte adherence is not affected) increases eosinophil adherence to endothelial cells (62). The mechanisms of eosinophil adherence will be reviewed in a subsequent section. Transgenic mice have been produced which overexpress IL-4. These animals have high IgE levels and develop a characteristic external eye swelling which histologically shows large numbers of eosinophils, edema, and mast cells (63). Mice infected with the parasite Nippostronglus brasiliensis have high IgE levels and eosinophilia, and an IL-4 Mab reduces IgE levels by 90% with no effect on blood eosinophil counts (53). Thus, IgE production and eosinophil adherence are two IL-4 effects relevant to eosinophilic lung disease.

IL-5, the other major player in a Th2 response, has a number of direct effects on eosinophils (Table 5). Human bone marrow, exposed in vitro to IL-5, produces large numbers of colonies containing only eosinophil precursors. Other Th2 cytokines, such as GM-CSF and IL-3, are less selective than IL-5 because they also stimulate granulocyte production, but the effect of GM-CSF and IL-3 on eosinophilopoiesis is additive to IL-5 (64). Transgenic mice expressing large amounts of IL-5 develop a marked (68%) eosinophilia, and in parasite infected mice, which have eosinophilia and high IL-5 levels, eosinophil counts decline when the animals are treated with anti-IL-5 Mabs (53,65). Thus, IL-5 is the major factor responsible for eosinophilopoiesis. Additionally, IL-5 significantly prolongs human eosinophil survival and IL-5 selectively increases eosinophil adherence to endothelial cells (66-69).

Table 5

IL-5 EFFECTS

1. Eosinophilopoiesis
2. Increased eosinophil survival
3. Increased adherence to endothelial cells
4. Eosinophil activation

IL-5 activates eosinophils for enhanced toxicity to parasites and host cells. Human eosinophils, exposed to IL-5 for 3 - 5 days, release granule proteins (MBP, EDN) when exposed to a second stimulus such as immunoglobulin-coated beads (70). IL-5 causes eosinophils to become less dense, and hypodense cells produce more LTC4, consume more oxygen, and are more toxic to parasites and eukaryotic cells (71,72). However, IL-5 activation by itself is insufficient to cause disease, because IL-5 transgenic mice have high IL-5 levels, large numbers of circulating eosinophils, and heavy eosinophilic infiltration of lymph nodes and lung but are apparently healthy (65). It may be that a second stimulus, such as PAF, or an sIgA or IgG opsonized parasite, is necessary to cause tissue injury by IL-5 activated eosinophils.

Evidence supporting a role for IL-5 in human disease comes from asthma patients. BAL fluid, obtained 48 hours after an inhaled antigen challenge given to atopic asthmatics, contains large amounts of IL-5, and the amount of IL-5 present correlates with the number of BAL eosinophils (Figure 11). No IL-3 or GM-CSF was present (5,73). Bronchial biopsies from symptomatic asthmatics show many IL-5 mRNA positive cells, and the number of IL-5 positive cells correlates with the number of recruited eosinophils and with the presence of activated eosinophils, as judged by positive EG2 staining (74). Bronchial biopsies obtained from normal subjects or asymptomatic asthmatics have no IL-5 producing cells. Although Th2 cells are thought to be the major source of IL-5, in one study 69% of BAL eosinophils obtained from asthma patients expressed IL-5 mRNA (58). Thus, it is possible that airway eosinophils may produce IL-5 in an autocrine fashion, resulting in a positive feedback loop.

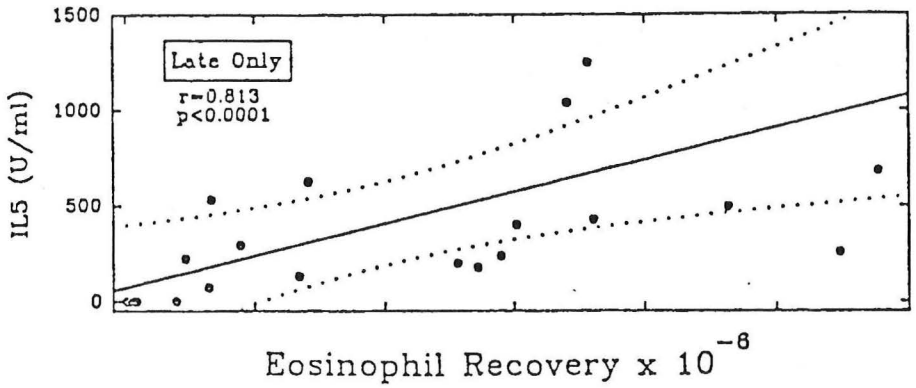


FIGURE 11. Relationship of IL-5 in BAL fluid with eosinophils in BAL.

Am. Rev. Respir. Dis. 144:1279, 1991

EOSINOPHIL-ENDOTHELIAL ADHERENCE

To cause an inflammatory response leukocytes must exit the circulation and enter a target organ. The initial step in this process occurs when leukocytes adhere to venule endothelial cells, followed by diapedesis through the endothelium and movement towards a chemotaxin gradient. Eosinophils, granulocytes, and lymphocytes have evolved sophisticated systems of adhesion molecules which recognize endothelial cell surface ligands; by varying the pattern of adhesion molecule expression, cytokines and other substances can selectively recruit inflammatory cells.

An overview of eosinophil adhesion molecules and their corresponding endothelial ligands is shown on Figure 12. The two most important eosinophil adhesion molecules are the B1 and B2 integrins, CD11b/CD18 and VLA-4, which are regulated by the two Th2 cytokines discussed earlier, IL-4 and IL-5. IL-5 increases the amount of CD11b/CD18 on eosinophils and increases eosinophil adherence to human endothelial cells in a CD11b/CD18 dependent manner (66,69,75,76). Interestingly, although neutrophils also adhere via CD11/CD18, IL-5 has no effect on either neutrophil CD11/CD18 levels or on neutrophil adherence, and IL-5 thus selectively increases eosinophil adherence. Sputum eosinophils from asthmatics, compared to blood eosinophils from the same patients, express increased amounts of CD11b, suggesting that CD11b was upregulated during transit from the pulmonary circulation (77). Bronchial biopsies obtained from atopic asthmatics six hours after inhaled antigen challenge show intense staining for ICAM-1, the ligand for CD11b/CD18, along sub-epithelial blood vessels (78). Convincing evidence comes from a primate model in which Ascaris sensitive monkeys develop hyper-reactive airways after three inhalational challenges with Ascaris antigens. After inhaling Ascaris antigens their airways show intense ICAM-1 expression along the basolateral epithelial cell surface and on sub-mucosal endothelial cells and there is an influx of CD11/CD18+ leukocytes; pretreatment with MabR6.5, an anti-ICAM-1 Mab, prevents both eosinophil entry and the development of hyper-reactivity (20).

Human Eosinophil Adherence to Vascular Endothelial Cells

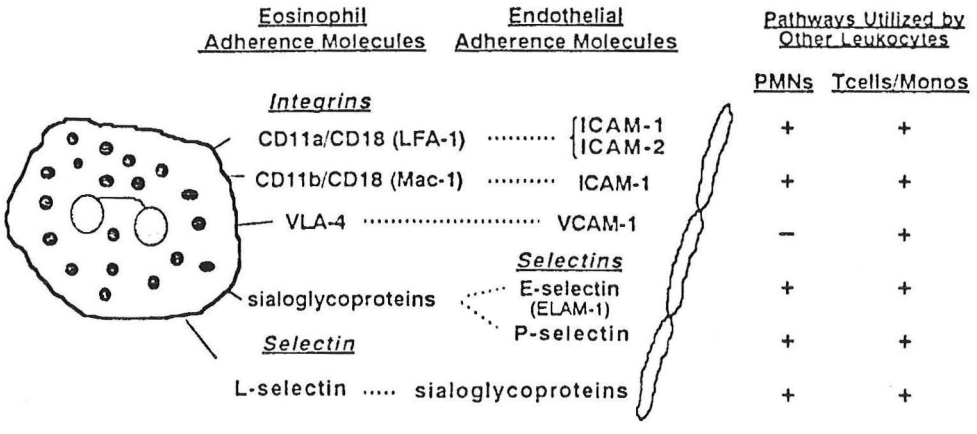


FIGURE 12

Am. J. Respir. Cell Mol. Biol. 8:352, 1993

IL-4 promotes eosinophil adherence through a separate mechanism. VCAM-1, the endothelial cell ligand for VLA-4, is not normally expressed. IL-4 increases endothelial cell VCAM-1 but has no effect on other endothelial cell adhesion molecules; eosinophils (but not granulocytes) express VLA-4. Thus, IL-4 also selectively promotes eosinophil adhesion.

The results of in vivo experiments comparing the relative roles of these two pairs of adhesion molecules are shown on Figure 13. Ovalbumin sensitized mice were given inhaled ovalbumin and the number of tracheal eosinophils measured (79). Pretreatment with anti-ICAM-1 Mab or anti-CD11/CD18 (anti-LFA in the figure label) had little effect. Either an anti-VCAM-1 or an anti-VLA-4 Mab decreased tracheal epithelial eosinophils by >70%, and both also caused a significant rise in blood eosinophil counts. The greatest effect occurred when an anti-VLA-4 and an anti-CD11/CD18 Mab were administered together; blocking both eosinophil adhesion molecules totally prevented eosinophil entry. Similar results have been seen with a model of dermal allergic inflammation (80). Thus, eosinophils rely on two adhesion molecules, which are regulated by two different Th2 cytokines, for entry into the respiratory tract. VLA-4-VCAM-1 are responsible for most eosinophil recruitment and blocking VLA-4 mediated eosinophil adherence with either Mabs or soluble VCAM-1 analogues may eventually become a useful therapy for eosinophilic diseases.

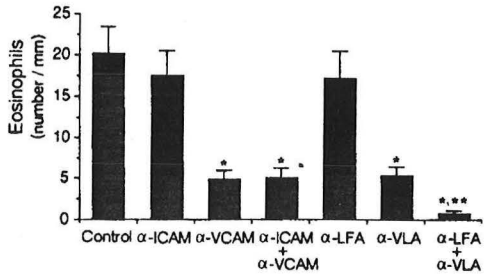


FIGURE 13. Effect of pretreatment with anti-adhesion Mabs.

J. Exp. Med. 179:1149, 1994

EOSINOPHIL CHEMOTAXIS

The adherence mechanisms reviewed in the previous section localize eosinophils along the pulmonary capillary walls; movement out of the vascular space and into the lung requires additional factors such as chemotaxins. Eosinophils respond with directed movement to a number of substances, including C5a, FMLP, PAF, LTB4, IL-5, and GM-CSF. Of these substances PAF, which is produced by many different cells, is the most potent; 10 nM concentrations suffice for eosinophil chemotaxis (81). Inhaled PAF also causes acute bronchoconstriction in humans and recruits eosinophils to respiratory epithelium, and in vitro PAF primes eosinophils for oxygen radical and LTC4 release (82-84). Lymphocyte chemoattractant factor (LCF) is a recently described substance which is even more active than PAF.LCF is released by lymphocytes in response to antigens or histamine, binds only to CD4 + cells (eosinophils, but not neutrophils, have CD4) and causes eosinophil migration at 0.01-0.1 nM concentrations (85). It is likely that LCF, released by the same cells responsible for Th2 cytokines, is a major factor moving eosinophils into lung parenchyma and/or bronchial epithelium.

EFFECTOR MECHANISMS

Eosinophils are capable of releasing a number of pro-inflammatory cytotoxic substances once they are recruited to a tissue site. Many of these products are toxic to helminths as well as to host cells. The numerous large secondary granules, which stain intensely with the acidic dye eosin thus accounting for the name eosinophil, contain four different pre-formed proteins. These granules proteins probably account for most eosinophil-induced inflammation and will be reviewed in a subsequent section.

Non-granule inflammatory mediators include the sulfidopeptide leukotrienes LTC4, LTD4, and LTE4. Eosinophils preferentially produce leukotrienes from arachidonic acid, and phospholipase A2, the enzyme which forms arachidonic acid from cell membrane phospholipids, is activated when eosinophils are stimulated (86). The Th2 cytoines IL-3, IL-5, and GM-CSF all prime eosinophils for enhanced LTC-4 production, as does PAF (87,88). Leukotrienes increase vascular permeability and cause bronchospasm, two key features of asthma. All three leukotrienes are 1,000 fold more potent than histamine in causing bronchoconstriction (89). A LTD4 receptor antagonist called MK-571 inhibits exercise-induce asthma, and administration of the 5-lipoxygenase inhibitor Zileuton to asthma patients causes an immediate and sustained decrease in airway obstruction (90,91). Intact bronchi, removed from birch pollen sensitive asthma patients during lung surgery, constrict and produce large amounts of leukotrienes when challenged ex-vivo with birch pollen.

Pretreatment with a leukotriene synthesis inhibitor prevents the pollen-induced constriction (92). Thus, it is likely that leukotrienes are released by eosinophils within bronchial walls and contribute significantly to both acute brochospasmn and the late response.

Eosinophil granules contain four proteins - major basic protein (MBP), Eosinophil peroxidase (EPO), eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin (EDN). Although all four are released by degranulating eosinophils and are potentially injurious, most of the available evidence suggests that MBP is especially important. MBP comprises 50% of secondary granule content, has no known enzymatic activity, and is present as a discrete electron-dense core within the secondary granule; the other three granule proteins form a less-dense matrix around MBP (93). As its name implies it is an extremely basic protein, having an isoelectric point pH of 10.9 due to the presence of 17 arginines. The effects of MBP are shown on Table 6.

Table 6

MBP EFFECTS

- Helminth parasite killing
- Cytotoxic to respiratory epithelial cells
- Ciliostasis
- Acute bronchoconstriction
- Airway hyper-reactivity

Relatively low (10 ug/ml) MBP concentrations cause ciliostasis, due to inhibition of an ATPase in ciliary axonemes (94). Slightly higher concentrations are toxic to tracheal epithelial cells and cause epithelial desquamation (95,96). MBP's positive charge is important for its cytotoxicity probably because it promotes close apposition of MBP to negatively charged cells. Once attached, MBP is thought to disrupt cell membranes by inserting hydrophobic segments into the membrane, causing an increase in permeability and cell death (97). The importance of MBP's positive charge for toxicity is shown by experiments in which mixing MBP with equimolar amounts of negatively charged substances, such as heparin or poly-L-glutamic acid, ablates its toxicity to helminths and tracheal epithelial cells (98,99). Eosinophils avoid MBP toxicity by initially making pro-MBP, a 207 amino acid protein with an isoelectric pH of 6.2 . The pro portion balances MBP's positive charge by containing 26 acidic amino acids. Pro-MBP is transported to the granule and, once it is sequestered within the dense core area, the pro protein is cleaved to form MBP (97).

Asthmatic patient's sputum contains MBP at concentrations up to 93 ug/ml, and similar concentrations are present in BAL fluid obtained during a late response (5). MBP concentration in BAL fluid correlates with hyper-reactivity in asthma (Figure 14). Primates (cynomolgus monkeys), given 1 mg of pure human MBP by inhalation, develop acute bronchoconstriction and also develop hyper-reactivity to subsequent methacholine challenges (Figure 15). None of the other three granule proteins cause hyper-reactivity (100). Co-administration of acidic polyanions with MBP prevent hyper-reactivity as well as toxicity to tracheal epithelial cells, again suggesting that MBPs' positive charge is essential for its effects. Thus, asthma patients have toxic levels of MBP, and MBP probably causes the epithelial desquamation and hyper-reactivity which characterizes asthma. Since MBP is toxic to fibroblasts and other lung cells, and is present in high concentrations in chronic eosinophilic pneumonia, it is likely to contribute to lung injury in all the eosinophilic lung diseases.

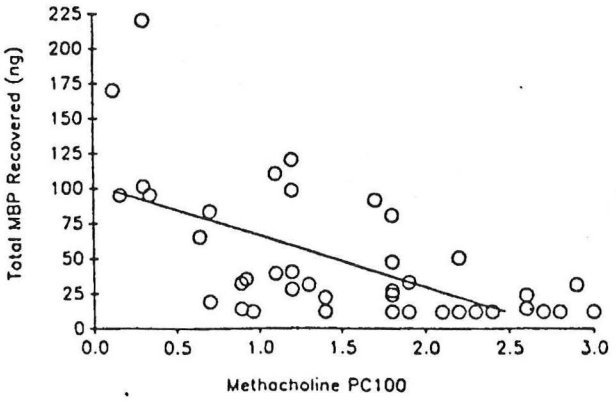


FIGURE 14. BAL MBP content and hyper-reactivity to methacholine.

J. App. Phy. 68:783, 1990

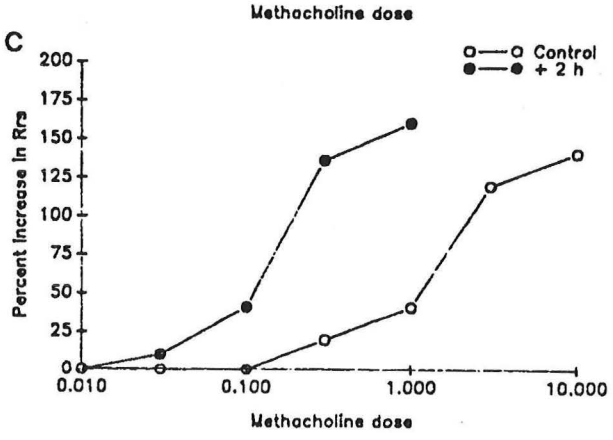


FIGURE 15. Effect of inhaled MBP on airway hyper-reactivity.

J. Clin. Inv. 87, 1472, 1991

The other three granule proteins may also produce eosinophilic injury. EPO is also a very basic protein (isoelectric pH 10.8) which is toxic to epithelial cells by itself; if supplied with hydrogen peroxide and a halide, EPO catalyzes formation of toxic oxidants which considerably enhance EPO cytotoxicity (95). ECP is toxic to tracheal cells but only at a relatively high concentration of >100 ug/ml (95,101). EDN does not injure respiratory epithelium but is toxic to neurons, causing axonal degeneration and vacuole formation in myelin sheaths. The neural toxicity of EDN may explain the common occurrence of neuropathies in the Churg-Strauss syndrome (102).

TREATMENT

Therapy of eosinophilic lung disease depends on the specific cause. For example, antibiotics should be administered for parasitic infections, and obviously drugs causing eosinophilia should be stopped. For most of these diseases, however, there is no specific therapy and the recommended treatment is a corticosteroid. Steroids have numerous beneficial effects, including decreased cytokine production by T cells, down-regulation of endothelial cell adhesion molecules, and decreased LTC4 and tissue entry by eosinophils (103). Corticosteroids also cause a marked drop in the number of circulating eosinophils by decreasing eosinophil survival, which occurs even if IL-5 is also present (104). Most eosinophilic lung disease responds rapidly to relatively small doses given systemically or, in the case of asthma, delivered directly to bronchial epithelium via an inhaler. The advent of inhaled steroids has revolutionized asthma therapy, as most asthmatics can be treated with 1-2 mg of an inhaled steroid per day, and inhaled steroids are now considered first line therapy for asthma patients (105). Some patients fail to respond to usual corticosteroid doses. Recently, T cells obtained from these steroid-resistant patients have been shown to have two defects; the majority have a decreased binding affinity of their steroid receptors, while a small number have a reduced number of steroid receptors (106).

REFERENCES

1. Osler, W. The Principals and Practice of Medicine. Appleton and Co., New York, 1892.

2. Deal, Jr., E.C., E.R. McFadden, Jr., R.H. Ingram, Jr., F.J. Breslin, J.J. Jaeger. Airway Responsiveness to Cold Air and Hypernea in Normal Subjects and in Those With Hay Fever and Asthma. Am. Rev. Respir. Dis. 121:621-628, 1980.

3. Boushey, H.A., M.J. Holtzman, J.R. Sheller, J.A. Nadel. State of the Art Bronchial Hyper-reactivity. Am. Rev. Respir. Dis. 121:389-413, 1980.

4. Cartier, A., N.C. Thompson, P.A. Frith, R. Roberts, M. Tech, F.E. Hargreave. Allergen-induced Increase in Bronchial Responsiveness to Histamine: Relationship to the Late Asthmatic Response and Change in Airway Caliber. J. Allergy Clin. Immunol. 70:170-177, 1982.

5. Sedgwick, J.B., W.J. Calhoun, G.J. Gleich, H. Kita, J.S. Abrams, L.B. Schwartz, B. Volovitz, M. Ben-Yaakov, W.W. Busse. Immediate and Late Airway Response of Allergic Rhinitis Patients to Segmental Antigen Challenge - Characterization of Eosinophil and Mast Cell Mediators. Am. Rev. Respir. Dis. 144:1274-1281, 1991.

6. De Monchy, J.G.R., H.F. Kauffman, P. Venge, G.H. Koeter, H.M. Jansen, H.J. Sluiter, K. De Vries. Bronchoalveolar Eosinophilia During Allergen-induced Late Asthmatic Reactions.

7. Metzger, W.J., D. Zavala, H.B. Richerson, P. Moseley, P. Iwamota, M. Monick, K. Sjoerdsma, G.W. Hunninghake. Local Allergen Challenge and Bronchoalveolar Lavage of Allergic Asthmatic Lungs - Description of the Model and Local Airway Inflammation. Am. Rev. Respir. Dis. 135:433-440, 1987.

8. Collins, D.S., R. Dupuis, G.J. Gleich, K.R. Bateemes, Y.Y. Koh, M. Pollice, K.H. Albertine, J.E. Fish, S.P. Peters. Immunoglobulin E-mediated Increase in Vasvular Permeability Correlates with Eosinophilic Inflammation. Am. Rev. Respir. Dis. 147:677-683, 1993.

9. Frick, W.E., J.B. Sedgwick, W.W. Busse. The Appearance of Hypodense Eosinophils in Antigen-dependent Late Phase Asthma. Am. Rev. Respir. Dis. 139:1401-1406, 1989.

10. Fukuda, T., S.L. Dunnette, C.E. Reed, S.J. Ackerman, M.S. Peters, G.J. Gleich. Increase Numbers of Hypodense Eosinophils in the Blood of Patients with Bronchial Asthma. Am. Rev. Respir. Dis. 132:981-985, 1985.

11. Laitinen, L.A., M. Heino, A. Laitinen, T. Kava, T. Haahtela. Image of the Airway Epithelium and Bronchial Reactivity in Patients With Asthma. Am. Rev. Respir. Dis. 131:599-606, 1985.

12. Laitinen, L.A., A. Laitinen, T. Haahtela. Airway Mucosal Inflammation Even in Patients With Newly Diagnosed Asthma. Am. Rev. Respir. Dis. 147:697-704, 1993.

13. Bousquet, J., P. Chanez, J.Y. LaCoste, G.Barneon, N. Ghavanian, I. Enander, P. Venge, S. Ahlstedt, J. Simony-Lafontaine, P. Godard, M. Francois-Bernard. Eosinophilic Inflammation in Asthma. N. Eng. J. Med. 323:1033-1039, 1990.

14. Broide, D.H., G.J. Gleich, A.J. Cuomo, D.A. Coburn, E.C. Federman, L.B. Schwartz, S.I. Wasserman. Evidence of Ongoing Mast Cell and Eosinophil Degranulation in Symptomatic Asthma Airway. J. Allergy Clin. Immunol. 88:637-648, 1991.

15. Laitinen, L.A., A. Laitinen, M. Heino, T. Haahtela. Eosinophilic Airway Inflammation during Exacerbation of Asthma and its Treatment With Inhaled Corticosteroid. Am. Rev. Respir. Dis. 143:423-427, 1991.

16. Ohashi, Y., S. Motojima, T. Fukuda, S. Makino. Airway Hyper-responsiveness, Increased Intracellular Spaces of Bronchial Epithelium, and Increased Infiltration of Eosinophils and Lymphocytes in Bronchial Mucosa in Asthma. Am. Rev. Respir. Dis. 145:1469-1476, 1992.

17. Wardlaw, A.J., S. Dunnette, G.J. Gleich, J.V. Collins, A.B. Kay. Eosinophils and Mast Cells in Bronchoalveolar Lavage in Subjects With Mild Asthma. Am. Rev. Respir. Dis. 137:62-69, 1988.

18. Kirby, J.G., F.E. Hargreave, G.J. Gleich, P.M. O'Byrne. Bronchoalveolar Cell Profiles of Asthmatic and Nonasthmatic Subjects. Am. Rev. Respir. Dis. 136:379-383, 1987.

19. Gundel, R.H., M.E. Gerritsen, G.J. Gleich, C.D. Wegner. Repeated antigen Inhalation Results in a Prolonged Airway Eosinophilia and Airway Hyper-responsiveness in Primates. J. Appl. Physiol., 68(2):779-786, 1990.

20. Wegner, C.D., R.H. Gundel, P. Reilly, N. Haynes, L. G. Letts, R. Rothlein. Intercellular Adhesion Molecule-1 (ICAM-1) in the Pathogenesis of Asthma. Science, 247:456-458, 1990.

21. O'Byrne, P.M. Asthma as an Inflammatory Disease. Marcel Dekker, Inc., New York, NY, 1990.

22. Lanham, J.G., K.B. Elkon, C.D. Pusey, G.R. Hughes. Systemic Vasculitis With Asthma and Eosinophilia: A Clinical Approach to the Churg-Strauss Syndrome. Medicine 63(2), 65-81, 1984.

23. Chumbley, L.C., E.G. Harrison, Jr., R.A. DeRemee. Allergic Granulomatosis and Angiitis (Churg-Strauss Syndrome) - Report and Analysis of 30 Cases. Mayo Clinic Proceedings 52:477-484, 1975.

24. Lanham, J.G. Churg-Strauss Syndrome. Bri. J. Hos. Med. 47(9), 667-673, 1992.

25. Rosengerg, M., R. Patterson, R. Mintzer, B.J. Cooper,. M. Roberts, K.E. Harris. Clinical and Immunologic Criteria for the Diagnosis of Allergic Bronchopulmonary Aspergillosis. Ann. Int. Med. 86:405-414, 1977.

26. Pierce, A.K. Pulmonary Aspergillosis. Medical Grand Rounds, January 14, 1988.

27. Mintzer, R.A., L.F. Rogers, G.D. Kruglik, M. Rosenberg, H.L. Neiman, R. Patterson. The Spectrum of Radiologic Findings in Allergic Bronchopulmonary Aspergillosis. Radiology 127:301-307, 1978.

28. Wang, J.L.F., R. Patterson, M. Rosenberg, M. Roberts, B.J. Cooper. Serum IgE and IgG Antibody Activity against Aspergillus Fumigatus as a Diagnostic Aid in Allergic Bronchopulmonary Aspergillosis. Am. Rev. Respir. Dis. 117:917-927, 1978.

29. Patterson, R., P.A. Greenberger, R.C. Radin, M. Roberts. Allergic Bronchopulmonary Aspergillosis: Staging as an Aid to Management. Ann. Int. Med. 96:286-291, 1982.

30. Wang, J.L.F., R. Patterson, M. Roberts, A.C. Ghory. The Management of Allergic Bronchopulmonary Aspergillosis. Am. Rev. Respir. Dis. 120:87-92, 1979.

31. Leitch, G.A. Pulmonary Eosinophilia. Basics of RD 7(5) 114-119, 1979.

32. Phills, J.A., A. J. Harrold, G.V. Whiteman, L Perelmutter. Pulmonary Infiltrates, Asthma and Eosinophilia Due to Ascaris Suum Infestation in Man. N. Eng. J. Med. 286(18):965-970, 1972.

33. Feldman, G.J., H.W. Parker. Visceral Larva Migrans Associated With the Hypereosinophilic Syndrome and the Onset of Severe Asthma. Ann. Int. Med. 116(10):838-840, 1992.

34. Ottesen, E.A., T.B. Nutman. Tropical Pulmonary Eosinophilia. Ann. Rev. Med. 43:417-424, 1992.

35. Neva, F.A., E.A. Ottesen. Current Concepts in Parasitology Tropical (Filarial) Eosinophilia. Medical Intelligence 298(20(1129-1131, 1978.

36. Chhabra, S.K., S.N. Gaur. Airway Hyper-reactivity in Tropical Pulmonary Eosinophilia. Chest 93(5):1105-1106, 1988.

37. Rom, W.N., V.K. Vijayan, M.J. Cornelius, V. Kumaraswami, R. Prabhakar, E.A. Ottesen, R.G. Crystal. Persistent Lower Respiratory Tract Inflammation Associated With Interstitial Lung Disease in Patients With Tropical Pulmonary eosinophilia Following Conventional Treatment With Diethylcarbamazine. Am. Rev. Respir. Dis. 142:1088-1092, 1990.

38. Udwadia, F.E. Tropical Eosinophilia - A Correlation of Clinical Histopathologic and Lung Function Studies. Diseases of the Chest 52(4):533-538, 1967.

39. Lynch, J.P., R.A. DeRemee. Immunologically Mediated Pulmonary Diseases. J. P. Lippincott Co., Phila. PA 18:432-449, 1991.

40. Oh, P.I., M.S. Balter. Cocaine Induced Eosinophilic Lung Disease. Short Reports, Mount Sinai Hospital, Canada October, 1991.

41. Strumpf, I.J., R.D. Drucker, K.H. Anders, S. Cohen, O. Fajolu. Acute Eosinophilic Pulmonary Disease Associated With the Ingestion of L-Tryptophan-Containing Products. Chest 99:8-13, 1991.

42. Carrington, C.B., W.W. Addington, A.M. Goff, I.M. Madoff, A. Marks, J.R. Schwaber, E.A. Gaensler. Chronic Eosinophilic Pneumonia. N. Eng. J. Med. 280(15):787-798, 1969.

43. Jederlinic, P.J., L. Sicilian, E.A. Gaensler. Chronic Eosinophilic Pneumonia. Medicine 67(3):154-162, 1988.

44. DeJaegher, P. M. Demedts. Bronchoalveolar Lavage in Eosinophilic Pneumonia Before and During Corticosteroid Therapy. Am. Rev. Respir. Dis. 129:631-632, 1984.

45. Grantham, J.G., J.A. Meadows, G.J. Gleich. Chronic Eosinophilic Pneumonia - Evidence for Eosinophil Degranulation and Release of Major Basic Protein. Am. J. Med. 80:89-94, 1986.

46. Gonzalez, E.B., J.L. Swedo, S. Rajaraman, J.C. Daniels, J.A. Grant. Ultrastructural and Immunohistochemical Evidence for Release of Eosinophilic Granules In Vivo: Cytoxic Potential in Chronic Eosinophilic Pneumonia. J. Allergy Clin. Immunol. 79:755-762, 1986.

47. Allen, J.N., E.R. Pacht, J.E. Gadek, W. B. Davis. Acute Eosinophilic Pneumonia as a Reversible Cause of Noninfectious Respiratory Failure. N.Eng. J. Med. 321:569-574, 1989.

48. Buchheit, J., N. Eid, G. Rodgers, Jr., T. Feger, O. Yakoub. Acute Eosinophilic Pneumonia With Respiratory Failure: A New Syndrome? Am. Rev. Respir. Dis. 145:716-718, 1992.

49. Mosmann, T.R., R.L. Coffman. Th1 and Th2 Cells: Different Patterns of Lymphokine Secretion Lead to Different Functional Properties. Ann. Rev. Immunol. 7:145-173, 1989.

50. Else, K.J., L. Hultner, R.K. Grencis. Cellular Immune Responses to the Murine Nematode Parasite Trichuris Muris II. Differential Induction of Th-Cell Subsets in Resistant Versus Susceptible Mice. Immunology 75:232-237, 1992.

51. Hsieh, C., S.E. Macatonia, C.S. Tripp, S.F. Wolf, A. O'Garra, K.M. Murphy. Development of Th1 $CD4^+$ T Cells Through IL-12 Produced by Listeria-Induced Macrophages. Science 260:547-549, 1993.

52. Mosmann, T.R., K.W. Moore. The Role of IL-10 in Crossregulation of Th1 and Th2 Responses. Immunology Today A49-A53, 1991.

53. Coffman, R.L., B.W.P. Seymour, S. Hudak, J. Jackson, D. Rennick. Antibody to Interleukin-5 Inhibits Helminth-Induced Eosinophilia in Mice. Science 245:308-310, 1989.

54. Nakajima H., I. Iwamoto, S. Tomoe, R. Matsumura, H. Tomioka, K. Takatsu, S. Yoshida. $CD4^+$ T-Lymphocytes and Interleukin-5 Mediate Antigen-Induced Eosinophil Infiltration Into the Mouse Trachea. Am. Rev. Respir. Dis. 146:374-377, 1992.

55. Iwamoto, I., H. Nakajima, H. Endo, S. Yoshida. Interferon Gamma Regulates Antigen-Induced Eosinophil Recruitment Into the Mouse Airways by Inhibiting the Infiltration of $CD4^+$ T Cells.

56. Robinson, D.S., Q. Hamid, S. Ying, A. Tsicopoulos, J. Barkans, A.M. Bentley, C. Corrigan, S.R. Durham, A.B. Kay. Predominant Th2-Like Bronchoalveolar T-Lymphocyte Population in Atopic Asthma. N. Eng. J. Med. 326:298-304, 1992.

57. Kay, A.B., S. Ying, V. Varney, M. Gaga, S.R. Durham, R. Moqbel, A.J. Wardlaw, Q. Hamid. Messenger RNA Expression of the Cytokine Gene Cluster, Interleukin 3 (IL-3), IL-4, and Granulocyte/Macrophage Colony-stimulating Factor in Allergen-induced Late-phase Cutaneous Reactions in Atopic Subjects. J. Exp. Med. 173:775-778, 1991.

58. Broide, D.H., M.M. Paine, G.S. Firestein. Eosinophils Express Interleukin 5 and Granulocyte Macrophage-Colony-stimulating Factor mRNA at Sites of Allergic Inflammation in Asthmatics. J. Clin. Inv. 90:1414-1424, 1992.

59. Bentley, A.M., Q. Meng, D.S. Robinson, Q. Hamid, A.B.Kay, S.R. Durham. Increases in Activated T Lymphocytes, Eosinophils, and Cytokine mRNA Expression for Interleukin-5 and Granulocyte/Macrophage Colony-stimulating Factor in Bronchial Biopsies After Allergen Inhalation Challenge in Atopic Asthmatics. Am. J. Respir. Cell Mol. Biol. 8:35-42, 1993.

60. Pene, J., F. Rousset, F. Briere, I. Chretien, J. Bonnefoy, H. Spits, T. Yokota, N. Arai, K. Arai, J. Banchereau, J.E. DeVries. IgE Production by Normal Human Lymphocytes is Induced by Interleukin 4 and Suppressed by Interferons Gamma and α and Prostaglandin E_2. Proc. Nat. Acad. Sci. 85:6880-6884, 1988.

61. Del Prete, G., E. Maggi, P. Parronchi, I. Chretien, A. Tiri, D. Macchia, M. Ricci, J. Banchereau, J. DeVries, S. Romagnani. IL-4 is an Essential Factor for the IgE Synthesis Induced In Vitro by Human T Cell Clones and Their Supernatants. J. Immunol. 140:4193-4198, 1988.

62. Schleimr, R.P., S.A. Sterbinsky, J. Kaiser, C.A. Bickel, D.A. Klunk, K. Tomioka, W. Newman, F.W. Luscinskas, M.A. Gimbrone, Jr., B.W. McIntyre, B.S. Bochner. IL-4 Induces Adherence of Human Eosinophils and Basophils But Not Neutrophils to Endothelium - Association With Expression of VCAM-1. J. Immunol. 148:1086-1092, 1992.

63. Tepper, R.I., D.A. Levinson, B.Z. Stanger, J. Campos-Torres, A.K. Abbas, P. Leder. IL-4 Induces Allergic-like Inflammatory Disease and Alters T Cell Development in Transgenic Mice. Cell 62:457-467, 1990.

64. Clutterbuck, E.J., E.M.A. Hirst, C.J. Sanderson. Human Interleukin-5 (IL-5) Regulates the Production of Eosinophils in Human Bone Marrow Cultures: Comparison and Interaction With IL-1, IL-3, IL-6, and GMCSF. Blood 73(6):1504-1512, 1989.

65. Dent, L.A., M. Strath, A.L. Mellor, C.J. Sanderson. Eosinophilia in Transgenic Mice Expressing Interleukin 5. J. Exp. Med. 172:1425-1431, 1990.

66. Neeley, S.P., K.J. Hamann, S.R. White, S.L. Baranowski, R.A. Burch, A.R. Leff. Selective Regulation of expression of Surface Adhesion Molecules Mac-1, L-selectin, and VLA-4 on Human Eosinophils and Neurtophils. Am. J. Respir. Cell mOl. Biol. 8:633-639, 1993.

67. Bevilacqua, M.P., R.M. Nelson, G. Mannori, O. Cecconi. Endothelial-Leukocyte Adhesion Molecules in Human Disease. Annu. Rev. Med. 45:361-378, 1994.

68. Resnick, M.B., P.F. Weller. Mechanisms of Eosinophil Recruitment. Am. J. Respir. Cell Mol. Biol. 8:349-355, 1993.

69. Walsh, G.M., A. Hartnell, A.J. Wardlaw, K. Kurihara, C.J. Sanderson, A.B. Kay. IL-5 Enhances the In Vitro Adhesion of Human Eosinophils, But Not Neutrophils, in a Leucocyte Integrin (CD11/18)-Dependent Manner. Immunology 71:258-265, 1990.

70. Kita, H., D.A. Weiler, R.Abu-Ghazaleh, C.J. Sanderson, G.J. Gleich. Release of Granule Proteins From Eosinophils Cultured With IL-5[1]. J. Immunol. 149:629-635, 1992.

71. Weller, P.F. The Immunobiology of Eosinophils. N. Eng. J. Med. 324(16):1110-1117, 1991.

72. Spry, C.J.F., A.B. Kay, G.J. Gleich. Eosinophils 1992. Immunology Today 13(10):384-387, 1992.

73. Ohnishi, T., H. Kita, D. Weiler, S. Sur, J.B. Sedgwick, W.J. Calhoun, W.W. Busse, J.S. Abrams, G.J. Gleich. IL-5 is the Predominant Eosinophil-active Cytokine in the Antigen-induced Pulmonary Late-phase Reaction. Am. Rev. Respir. Dis. 147:901-907, 1993.

74. Hamid, Q., M. Azzawi, S. Ying, R. Roqbel, A.J. Wardlaw, C.J. Corrigan, B. Bradley, S.R. Durham, J.V. Collins, P.K. Jeffrey, D.J. Quint, A.B. Kay. Expression of mRNA for Interleukin-5 in Mucosal Bronchial Biopsies from Asthma. J. Clin. Inv. 87:1541-1546, 1991.

75. Weller, P.F., T.H. Rand, S.E. Goelz, G. Chi-Rosso, R.R. Lobb. Human Eosinophil Adherence to Vascular Endothelium Mediated by Binding to Vascular Cell Adhesion Molecule 1 and Endothelia. Leukocyte Adhesion Molecule 1. Proc. Nat. Acad. Sci. 88:7430-7433, 1991.

76. Dobrina, A., R. Menegazzi, T.M. Carlos, E. Nardon, R. Cramer, T. Zacchi, J.M. Harian, P. Patriarca. Mechanisms of Eosinophil Adherence to Cultured Vascular Endothelial Cells - Eosinophils Bind to the Cytokine-induced Endothelial Ligand Vascular Cell Adhesion Molecule-1 via the Very Late Activation Antigen-4 Integrin Receptor. J. Clin. Inv. 88:20-26, 1991.

77. Walker, C., S. Rihs, R.K. Braun, S. Betz, P.L.B. Bruijnzeel. Increased Expression of CD11b and Functional Changes in Eosinophils After Migration Across Endothelial Cell Monolayers. J. Immunol. 150:4061-4071, 1993.

78. Montefort, S., C. Gratziou, D. Goulding, R. Polosa, D.O. Haskard, P.H. Howarth, S.T. Holgate, and M.P. Carroll. Bronchial Biopsy Evidence for Leukocyte Infiltration and Upregulation of Leukocyte-Endothelial Cell Adhesion Molecules 6 Hours After Local Allergen Challenge of Sensitized Asthmatic Airways. J. Clin. Inv. 93:1411-1421, 1994.

79. Nakajima, H., H. Sano, T. Nishimura, S. Yoshida, I. Iwamoto. Role of Vascular Cell Adhesion Molecule 1/Very Late Activation Antigen 4 and Intercellular Adhesion Molecule 1/Lymphocyte Function-associated Antigen 1 Interactions in Antigen-induced Eosinophil and T Cell Recruitment Into the Tissue. J. Exp. Med. 179:1145-1154, 1994.

80. Weg, V.B., T.J. Williams, R.R. Lobb, S. Nourshargh. A Monoclonal Antibody Recognizing Very Late Activation Antigen-4 Inhibits Eosinophil accumulation In Vivo. J. Exp. Med. 177:561-566, 1993.

81. Casale, T.B., R.A. Erger, M.M. Little. Platelet-activating Factor-induced Human Eosinophil Transendothelial Migration: Evidence for a Dynamic Role of the Endothelium. Am. J. Respir. Cell Mol. Biol. 8:77-82, 1993.

82. Zoratti, E.M., J.B. Sedgwick, M.E. Bates, R.F. Vrtis, K. Geiger, W.W. Busse. Platelet-activating Factor Primes Human Eosinophil Generation of Superoxide. Am. J. Respir. Cell Mol. Biol. 6:100-106, 1992.

83. Cuss, F.M., C.M.S. Dixon, P.J. Barnes. Effects of Inhaled Platelet Activating Factor on Pulmonary Function and Bronchial Responsiveness in Man. Lancet 189-192, 1986.

84. Chung, K.F., P.J. Barnes. Effects of Platelet Activating Factor on Airway Calibre, Airway Responsiveness, and Circulating Cells in Asthmatic Subjects. Thorax 44:108-115, 1989.

85. Rand, T.H., W.W. Cruikshank, D.M. Center, P.F. Weller. CD4-mediated Stimulation of Human Eosinophils: Lymphocyte Chemoattractant Factor and Other CD4-binding Ligands Elicit Eosinophil Migration. J. Exp. Med. 173:1521-1528, 1991.

86. White, S.R., M.E. Strek, G.V.P. Kulp, S.M.Spaethe, R.A. Burch, S.P. Neeley, A.R. Leff. Regulation of Human Eosinophil Degranulation and Activation by Endogenous Phospholipase A_2. J. Clin. Inv. 91:2118-2125, 1993.

87. Owens, Jr., W.F., J.Petersen, K.F. Austen. Eosinophils Altered Phenotypically and Primed by Culture With Granulocyte/Macrophage Colony-stimulating Factor and 3T3 Fibroblasts Generate Leukotriene C_4 in Response to FMLP. J. Clin. Inv. 87:1958-1963, 1991.

88. Takafuji, S., S.C. Bischoff, A.L. DeWeck, C.A. Dahinden. IL-3 and IL-5 Prime Normal Human Eosinophils to Produce Leukotriene C4 in Response to Soluble Agonists. J. Immunol. 147:3855-3861, 1991.

89. Griffin, M., J. W. Weiss, A. G. Leitch, E.R. McFadden, Jr., E.J. Corey, K. F. Austen, J.M. Drazen. Effects of Leukotriene D on the Airways in Asthma. N. Eng. J. Med. 308:436-439, 1983.

90. Israel, E., P. Rubin, J.P. Kemp, J. Grossman, W. Pierson, S.C. Siegel, D.Tinkelman, J.J. Murray, W. Busse, A.T. Segal, J. Fish, H.B. Kaiser, D Ledford, S. Wenzel, R. Rosenthal, J. Kohn, C. Lanni, H. Pearlman, P. Karahalios, J.M. Drazen. The Effect of Inhibition of 5-Lipoxygenase by Zileuton in Mild-to-Moderate Asthma. Ann. Int. Med. 119(11):1059-1065, 1993.

91. Manning, P.J., R.M. Watson, D.J. Margolskee, V.C. Williams, J.I. Schwartz, P.M. O'Byrne. Inhibition of Exercise-induced Bronchoconstriction by MK-571, A Potent Leukotriene D_4-Receptor Antagonist. N. Eng. J. Med. 323:1736-1739, 1990.

92. Dahlen, S., G. Hansson, P. Hedqvist, T. Bjorck, E. Granstrom, B. Dahlen. Allergen Challenge of Lung Tissue From Asthmatics Elicits Bronchial Contraction That Correlates With the Release of Leukotrienes C_4, D_4, and E_4. Proc. Nat. Acad. Sci. 80:1712-1716, 1983.

93. Hamann, K.J., R.L. Barker, R.M. Ten, G.J. Gleich. The Molecular Biology of Eosinophil Granule Proteins. Int. Arch Allergy App. Immunol. 94:202-209, 1991.

94. Hastie, A.T., D.A. Loegering, G.J. Gleich, F. Kueppers. The Effect of Purified Human Eosinophil Major Basic Protein on Mammalian Ciliary Activity. Am. Rev. Respir. Dis. 135:848-853, 1987.

95. Motojima, S., E. Frigas, D.A. Loegering, G.J. Gleich. Toxicity of Eosinophil Cationic Proteins for Guinea Pig Tracheal Epithelium In Vitro. Am. Rev. Respir.. Dis. 139:801-805, 1989.

96. Frigas, E., D.A. Loegering, G.J. Gleich. Cytotoxic Effects of the Guinea Pig Eosinophil Major Basic Protein on Tracheal Epithelium. Lab. Inv. 42(1):35-43, 1980.

97. Barker, R.L., G.J. Gleich, L.R. Pease. Acidic Precursor Revealed in Human Eosinophil Granule Major Basic Protein cDNA. J. Exp. Med. 168:1493-1498, 1988.

98. Barker, R.L., R.H. Gundel, G.J. Gleich, J.L. Checkel, D.A. Loegering, L.R. Pease, K.J. Hamann. Acidic Polyamino Acids Inhibit Human Eosinophil Granule Major Basic Protein Toxicity - Evidence of a Functional Role for ProMBP. J. Clin. Inv. 88:798-805, 1991.

99. Butterworth, A.E., D.L. Wassom, G.J. Gleich, D.A. Loegering, J.R. David. Damage to Schistosomula of Schistosoma Mansoni Induced Directly by Eosinophil Major Basic Protein. J. Immunol. 122(1):221-227, 1979.

100. Gundel, R.H., L.G. Letts, G.J. Gleich. Human Eosinophil Major Basic Protein Induces Airway Constriction and Airway Hyper-responsiveness in Primates. J. Clin. Inv. 87:1470-1473, 1991.

101. Young, J.D., C.G.B. Peterson, P. Venge, Z.A. Cohn. Mechanism of Membrane Damage Mediated by Human Eosinophil Cationic Protein. Nature 321:613-616, 1986.

102. Durack, D.T., S. M. Sumi, S.J. Klebanoff. Neurotoxicity of Human Eosinophils. 76(3):1443-1447, 1979.

103. Boumpas, D.T., G.P. Chrousos, R.L. Wilder, T.R. Cupps, J.E. Balow. Glucocorticoid Therapy for Immune-mediated Diseases: Basic and Clinical Correlates. Ann. Int. Med. 119:1198-1208, 1993.

104. Wallen, N., H. Kita, D. Weiler, G.J. Gleich. Glucocorticoids Inhibit Cytokine-mediated Eosinophil Survival. J. Immunol. 147(10):3490-3495, 1991.

105. Barnes, P.J. A New Approach to the Treatment of Asthma. N. Eng. J. Med. 321(22):1517-1526, 1989.

106. Sher, E.R., D.Y.M. Leung, W. Surs, J.C. Kam, G.Zieg, A.K. Kamada, S.J. Szefler. J. Clin. Inv. 93:33-39, 1994.