

Review article

The role of the macrophage in asthma

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Accumulating evidence implicates cells of the mononuclear phagocyte lineage in the pathogenesis of bronchial asthma. The evidence for involvement of peripheral blood monocytes (PBM) and alveolar macrophages (AM) in the pathogenesis of asthma stems from functional studies on peripheral blood monocytes and analysis of the fluid and cellular phases of bronchoalveolar lavage (BAL) samples, and, more recently, from histologic material obtained from endobronchial biopsy *in vivo*.

Peripheral blood monocytes

In 1975, Capron et al. produced evidence of IgE receptors on macrophages by showing the role of IgE antibodies in macrophage-dependent cytotoxic damage to parasites (12). This receptor is similar, if not identical, to the Fc_εR found on T and B cells and is referred to as Fc_εR₂, as it differs in both structure and function from the Fc_εR₁ found on mast cells and basophils (77). The most important functional difference is that the Fc_εR₂ on macrophages is of a lower affinity (i.e., an estimated dissociation constant for monomeric IgE binding to macrophages and U937 cells of 10 μM) and is preferentially activated by immune complexes (40). Approximately 5–10% of PBM and AM bear Fc_εR₂ (56, 77). This percentage rises to 20% in mildly atopic asthmatic subjects and

can be downregulated by corticosteroid treatment (58).

PBM from asthmatic subjects have enhanced complement receptor expression (CRE) in the presence or absence of casein, as compared with normal controls (42). Furthermore, the numbers of PBM that form rosettes with complement-coated sheep erythrocytes are increased in asthmatic subjects after allergen bronchoprovocation, but not after histamine-induced bronchoconstriction (13). In asthmatic subjects who respond to corticosteroids (corticosteroid-sensitive (CS) asthmatic subjects), these levels revert to normal after 7 days' treatment with prednisolone, whereas in patients who fail to improve clinically with corticosteroids (corticosteroid-resistant (CR) asthmatic subjects), these levels remain elevated (42). In addition, it has been shown that *in vitro* colony formation in soft agar by PHA-stimulated mononuclear cells is inhibited by methylprednisolone in CS subjects, but not in CR subjects. The origin of this *in vitro* resistance was found to be monocyte-derived, rather than lymphocyte-derived (67). We have shown that the expression of the activation antigens CR-1, CR-3, and class II molecules on cultured PBM is elevated in asthmatic subjects, as compared with normal control subjects (89). Furthermore, this enhanced receptor expression is suppressed by hydrocortisone

in CS, but not in CR, asthmatic subjects, suggesting ongoing PBM activation in the presence of corticosteroids.

Interactions between monocytes and granulocytes

Activated PBM generate a number of proinflammatory molecules which can influence the activity of other cell types. For instance, supernatants from activated PBM modulate arachidonic acid metabolism by the cyclooxygenase and lipoxygenase pathway in macrophages, fibroblasts, and granulocytes (21, 22, 40, 43). Furthermore, they activate granulocytes for certain cytotoxic functions (20, 85, 86). For instance, LPS-stimulated monocyte supernatants can augment the antibody-dependent cytotoxic killing of *Schistosoma mansoni* larvae by human eosinophils (27). At low antibody concentrations, control medium-treated eosinophils adhered only in small numbers to the larvae and killed 2–10% of the schistosomula. In contrast, eosinophils treated with conditioned media adhered in large numbers to schistosomula and showed high helminthotoxicity (40–80%). The supernatants themselves were not toxic to the larvae and were shown to mediate these effects via enhanced eosinophil degranulation.

In view of the evidence implicating the role of PBM in the mechanisms of allergic inflammation and the suggestion that eosinophils are crucial effector cells in the tissue destruction seen in bronchial asthma, we have assessed the ability of monocyte-derived molecules to modulate eosinophil function. Eosinophils cultured in the presence of 50% PBM-derived culture supernatants remained approximately 67% viable for 7 days. In the absence of PBM supernatant, only 15% of cells remained viable for the same period of time (Fig. 1). The supernatants of atopic asthmatic persons contained more of the viability-enhancing activity (active component) than did those of nonatopic persons (10). On separation of the supernatant on C18 Sep-Pak columns, a major eosinophil viability-enhancing activity was eluted in the aqueous fraction, suggesting that it was a hydrophilic molecule. Neutralization by specific antibodies indicated that the activity was completely inhibited by antiserum against granulocyte-macrophage colony-stimulating factor (GM-CSF) and was unaffected by antibodies specific to interleukin (IL)-3 or IL-5. A second, minor viability-enhancing activity was contained in the 100% methanol fraction, indicating the presence of a more hydrophobic molecule.

GM-CSF is an acidic glycoprotein with a pI of 4.5 and a mol. mass of 22 kDa. It is eluted from size exclusion columns with an apparent mol. mass of 15–40 kDa because of variations in its glycosylation and from anion exchange between 0.10 and 0.20 M

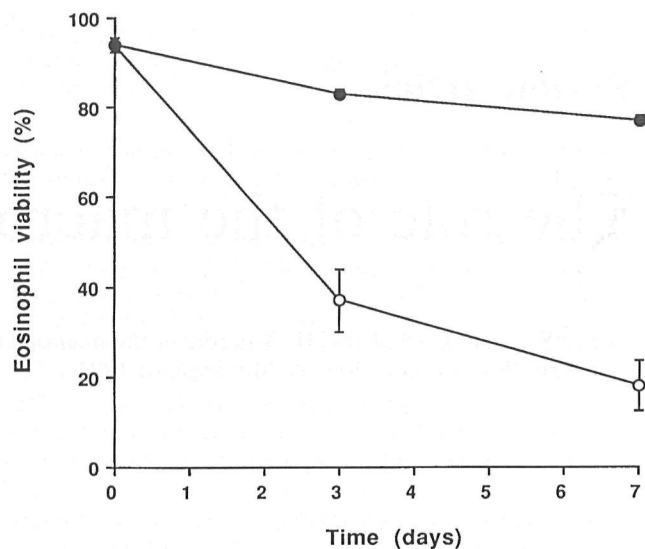


Fig. 1. Survival of eosinophils cultured in presence of peripheral blood cell mononuclear cell-derived culture supernatants (closed circles) and buffer alone (open circles) (With permission, J Allergy Clin Immunol 1991; 88: 226–35).

NaCl (62). The glycoprotein stimulates the proliferation and differentiation of normal granulocytic and monocytic stem cells and modulates the function of mature granulocytes, leading to enhancement of expression of the granulocyte functional antigens 1 and 2, Mo 1, Leu M5, and C3bi (13, 49).

GM-CSF induces histamine release from basophils (33) and enhances eosinophil survival in culture (63). Thus, the presence of GM-CSF in the lung may precondition eosinophils for enhanced proinflammatory functions upon subsequent stimulation, and, either alone or in concert with other cytokines, it may lead to eosinophil colony formation from bone-marrow progenitors. GM-CSF may play an important role in the amplification of the eosinophilic inflammation which is characteristic of asthmatic airways.

Corticosteroid-resistant bronchial asthma

Wilkinson et al. have shown that cultured PBM from asthmatic subjects generate an activity which primes neutrophils for enhanced LTB₄ generation and superoxide release (NPA: neutrophil priming activity) (88). The *in vitro* generation of this activity is inhibited in a dose-dependent and rank-order fashion by corticosteroid treatment of PBM of patients with CS asthma with no significant suppression seen in PBM isolated from patients with CR asthma (45). Moreover, the degree of *in vitro* suppression by corticosteroids of NPA correlated significantly with *in vivo* airways responsiveness to oral prednisolone (47). This activity is a monocyte-derived 3-kDa moiety

which is heat- and pronase-sensitive and stains with Coomassie brilliant blue.

To assess whether the inability of glucocorticosteroids (GCS) to suppress the generation of NPA by PBM derived from CR persons was selective, or whether it involved other well-characterized monocyte-derived cytokines, we cultured PBM from CS and CR asthmatic subjects at different times in the presence or absence of lipopolysaccharide (LPS) and a range of concentrations of dexamethasone and hydrocortisone. Generation of TNF- α , IL-1 β , and GM-CSF in the supernatants was measured by ELISA; NPA by its capacity to enhance LTB $_4$ generation from neutrophils stimulated with the calcium ionophore A23187; and mRNA levels of the cytokines by Northern blot analysis. In contrast to NPA, there was failure to suppress cytokine generation by corticosteroids in the absence of LPS. There was marked enhancement of production of each cytokine in the PBM-primed supernatants in the presence of LPS, a production which was inhibited by hydrocortisone and dexamethasone in a dose-dependent and rank-order fashion. The dose-response curves for inhibition by steroids of cytokine release were not significantly different between CS and CR subjects. IL-1 β synthesis was least sensitive to inhibition by corticosteroids, followed by those of GM-CSF and TNF- α . There was no upregulation of NPA by LPS as measured by bioassay. TNF- α mRNA was found to be minimally expressed in the absence of stimulation, but it was markedly upregulated by LPS. An amount of 1 μ M hydrocortisone had no measurable effect on either base-line or LPS-induced TNF- α mRNA expression in three CS and three CR persons and led to minimal suppression in one CS and one CR person (47).

These results confirm those of Beutler et al. demonstrating that GCS regulation of TNF- α generation is predominantly at the posttranscriptional level (6). mRNAs for IL-1 β and GM-CSF were extracted at 24 h. There was no detectable mRNA for either cytokine in the absence of LPS. An amount of 5 μ g/ml LPS markedly upregulated both mRNAs, which were suppressed by 1 μ M hydrocortisone in both CS and CR asthmatic subjects. These results suggest a selective resistance of NPA to GCS suppression in CR asthma.

The rank order of suppression by GCS of NPA suggested glucocorticoid receptor (GR)-mediated inhibition and prompted an analysis of the ligand-binding function of the GR (45). PBM were separated in suspension, and competitive binding studies were done with tritiated dexamethasone on nuclear extracts. Analysis of specific binding revealed no significant difference in dissociation constants or receptor numbers (R_o) between CS and CR asthmatic subjects (45) (Fig. 2). This implies a normal ligand-

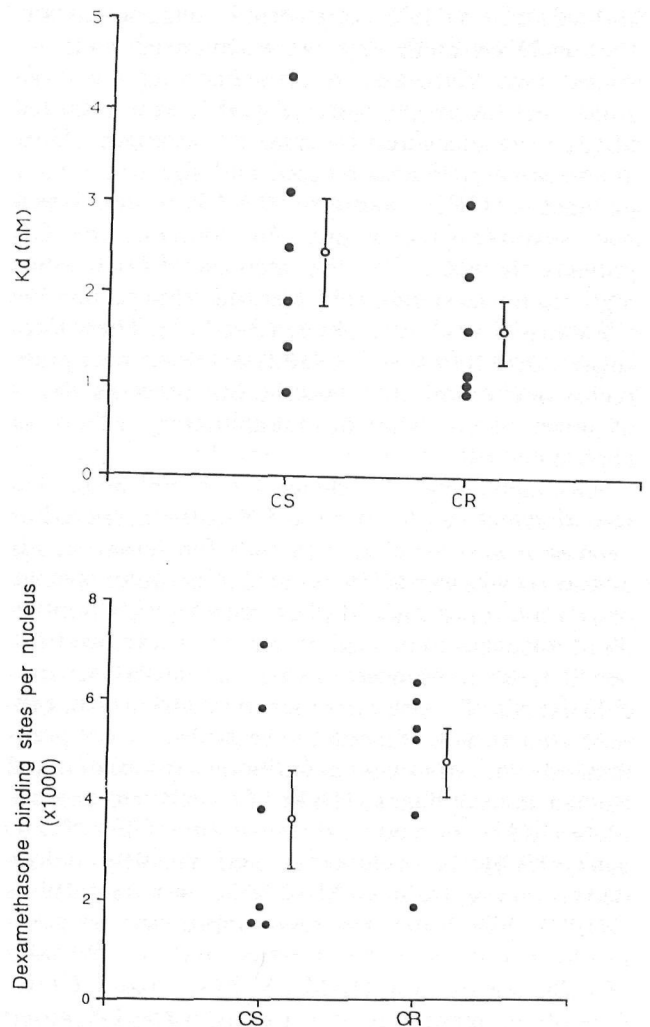


Fig. 2. Dexamethasone binding affinity (K_d) (upper panel) and total numbers of dexamethasone nuclear receptors (R_o) (lower panel) in peripheral blood monocytes from asthmatic subjects classified as corticosteroid sensitive (CS; $n = 6$) or corticosteroid resistant (CR; $n = 6$). Dexamethasone K_d was 2.45 ± 0.58 (mean $\pm$ SEM) in CS group and 1.6 ± 0.35 (mean $\pm$ SEM) in CR group ($P = 0.14$). R_o in CS group was 3605 ± 984 (mean $\pm$ SEM) binding sites per nucleus and 4757 ± 692 (mean $\pm$ SEM) binding sites per nucleus in CR group ($P = 0.23$). Bars represent mean $\pm$ SEM (With permission, Am Rev Respir Dis 1991; 143: 1020-4).

binding function of the GR. As binding studies were done on nuclear extracts, the PBM defect in CR asthma must lie distal to receptor occupancy and its subsequent translocation to the nucleus.

In collaboration with Peter Barnes of the National Heart, Lung, and Blood Institute, we have used gel retardation assay to test the hypothesis that defective glucocorticoid receptor/glucocorticoid response element (GR/GRE) interaction can account for GCS resistance (1). PBM derived from six CS and six CR subjects were cultured for up to 1 h with 1 μ M dexamethasone. Nuclear proteins were extracted by detergent lysis and incubated ^{32} P-

end-labeled dsGRE-constructed oligonucleotide. Protein-DNA complexes were separated from unbound oligonucleotides by nondenaturing polyacrylamide gel electrophoresis (PAGE), and retarded bands were measured by laser densitometry. Dexamethasone produced a rapid and significant 80% increase in GRE binding in the CS group, which was sustained for 60 min. In contrast, the CR patients showed a 20–30% increase in GRE binding, which was significantly less than that seen in the CS subjects at all time points ($P < 0.01$). These data suggest that defective GR-GRE or GR-transcription factor interaction may underlie the molecular basis of resistance to the anti-inflammatory effects of corticosteroids.

Although there is evidence for *in vitro* defects in mononuclear cell function in CR asthma, there has been no *in vivo* correlation to date. Furthermore, it is unknown whether GCS resistance is organ specific or is a more generalized phenomenon. In a double-blind, placebo-controlled, crossover study, we have tested these hypotheses, using immunohistochemical analysis of the cutaneous, tuberculin-driven, cell-mediated immune reaction in response to oral prednisolone with monoclonal antibodies (mab) directed against macrophages (HAM-56), activated eosinophils (EG2), primed and T-memory (CD45RO+) cells (UCHL1), monocytes and recently blood-derived macrophages (MAC387), and neutrophils (NP57) (46). There was less suppression by prednisolone of the site of induration and of infiltration of cells positive for HAM-56, EG2, and UCHL1 phenotype in the CR group than in the CS group ($P = 0.036$). There was no suppression of MAC387 and NP57 positive cells in either group. These data indicate for the first time that there is an *in vivo* defect in mononuclear cell function in CR asthma with reduced suppression by prednisolone of macrophage, T-cell, and activated eosinophil infiltration into the cutaneous tuberculin response. It also indicates that the phenomenon of glucocorticoid resistance is not confined to the lung in these subjects, but is a more generalized phenomenon.

Bronchoalveolar lavage (BAL)

Alveolar macrophages (AM) constitute the majority of cells recovered by BAL both in normal and in asthmatic subjects (28). Studies on BAL specimens have shown that cultured AM have an increased respiratory burst as detected by chemiluminescence, as compared with normal controls (14). Analysis of BAL fluid from asthmatic patients after antigen challenge reveals increased amounts of β -glucuronidase, whereas corresponding macrophage intracellular levels were decreased, as compared with normal controls (61, 83). This suggests that the macrophage

secretory process may be activated by allergen. Metzger et al. have shown that the total number of peroxidase-positive AM obtained from BAL fluid is increased at 48 and 96 h after allergen challenge, suggesting that a population of monocytes has entered the lung from the local vascular compartment (60). Rankin and colleagues showed that normal AM can be activated by monoclonal IgE and specific antigen to generate both LTC₄ and LTB₄ (68, 69).

Interactions between alveolar macrophages and granulocytes

In view of the evidence that AM secrete molecules which can influence the functions of inflammatory granulocytes (64) and the compelling evidence for the participation of eosinophils in airways inflammation, we have studied the interactions of AM and eosinophils in asthmatic subjects. Incubation of eosinophils with supernatants from AM from asthmatic patients, followed by stimulation with A23187, resulted in enhancement of the capacity of eosinophils to secrete LTC₄ (mean enhancement $169 \pm 37\%$, $n = 31$) (36) (Fig. 3). AM supernatants derived from normal persons had no enhancing effects, as compared with culture medium. Enhancement was greatest when eosinophils were preincubated with a 1:6 dilution of AM supernatants for 5 min at 37°C and then stimulated with 5 μ M A23187 for 5 min at 37°C. There was an inverse correlation between percent enhancement and base-line LTC₄ production ($r = 0.48$; $P < 0.01$).

Partial purification of the enhancing activity by HPLC on a TSK G3000 SW column revealed that it had a mol. mass of approximately 30 kDa, and the activity was eluted in two consistent peaks at 0.17 M and 0.2 M NaCl from anion exchange HPLC (TSK DEAE 5 PW column, pH 7.4). The activities were distinct from IL-1 β and TNF- α . The major activity which eluted at 0.2 M NaCl was further resolved by RP-HPLC on an C18 Spherisorb column with a slope gradient of 0° to 100% acetonitrile. A single peak of activity was eluted at 41% acetonitrile. The activity was inhibited by trypsin digestion and heat, and was neutralized by incubation with specific antibodies against human GM-CSF. This suggested that the major active component is identical or closely related to GM-CSF. This finding is supported by the observation that pretreatment of eosinophils with recombinant GM-CSF primed the cells for enhanced LTC₄ generation after stimulation with A23187 or unopsonized zymosan. The priming may be mediated by augmented release of arachidonic acid from membrane phospholipids (27, 86) caused by enhancement of the rate of membrane depolarization which is induced by subsequent stimulation

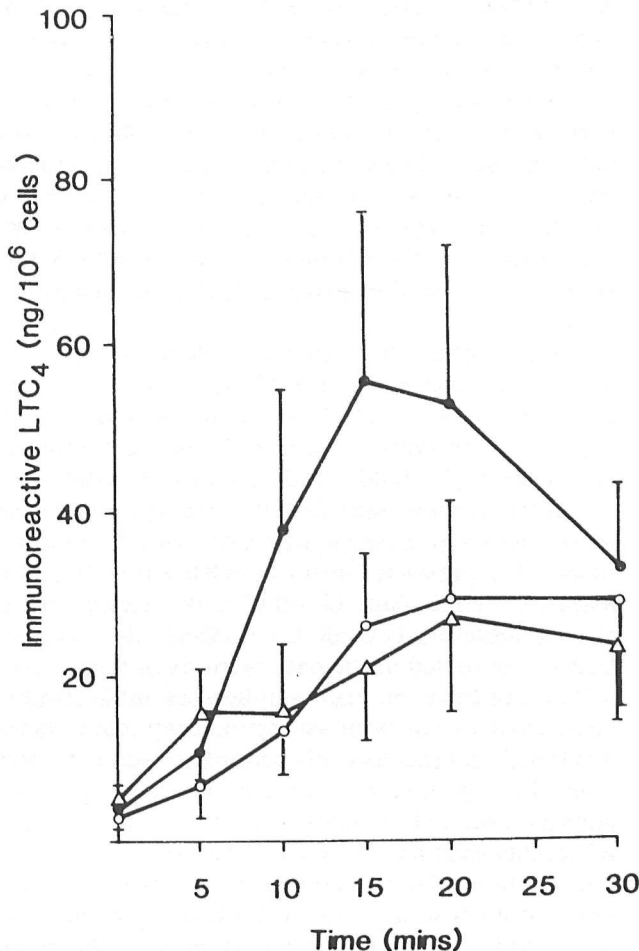


Fig. 3. Effect of pretreatment of eosinophils for 5 min with 1/6 dilution of AM supernatants derived from asthmatic patients (closed circle), normal persons (open circles), or culture medium (open triangle) on time course of LTC₄ generation by eosinophils obtained from same donors after addition of 5 μ M A23187. Each point represents mean $\pm$ SEM ($n=4$) (With permission, Am Rev Respir Dis 1989; 140: 1340-7).

(18). The enhancement was dose-dependent and the greatest enhancement of LTC₄ occurred at 1 ng/ml GM-CSF for A23187 stimulation and at 10 ng/ml GM-CSF for stimulation with unopsonized zymosan. The presence of GM-CSF in the lung may lead to induction and maintenance of the eosinophil inflammatory state characteristic of asthmatic airways, through enhanced eosinophil survival and eosinophil colony formation from bone-marrow progenitors, and may condition eosinophils for enhanced inflammatory functions.

The existence of low-affinity Fc ϵ r₂ (56, 57) suggests that macrophages and their monocyte precursors may be directly involved in allergic responses, in addition to their role in presenting antigens to T cells (72). Monocytes and macrophages are necessary for the generation of cell-mediated immune responses by processing and presenting antigens to

antigen-specific lymphocytes, committing them to antigen-specific division and cytokine generation (84). Human AM from normal subjects function poorly as accessory cells for the presentation of recall antigens to autologous peripheral blood T cells (29, 31, 35, 82), and this may be important for the appropriate control of immune and inflammatory processes within the lung (51, 70). Furthermore, there is evidence suggesting an association between AM accessory function and the presence of BAL lymphocytes in bronchial asthma (30). The cause of this association remains to be determined, but it might be due to infiltration into the asthmatic lung of a functionally distinct subset of site- and allergen-specific CD₄ cells with a specific pattern of cytokine release (41).

Spiteri et al. have isolated phenotypically and functionally distinct macrophage subpopulations from human BAL (79). Using the monoclonal antibodies RFD1 and RFD7, they can distinguish between phagocytes (RFD7+), antigen-presenting cells (RFD1+), and suppressor macrophages (RFD1 + RFD7+) (66). All three subpopulations have been identified within the human alveolar macrophage pool (78). The significance of the presence of the three subsets is highlighted by the observation that their proportions within BAL altered dramatically with lung inflammation, suggesting that T-cell responsiveness might be influenced by relative changes within this heterogeneous AM population (78, 80).

Bronchial histology

Postmortem studies of the airways of patients who died from severe asthma show epithelial damage, plugging of the airway lumen by cellular debris, mucosal edema, thickening of the epithelial basement membrane, smooth-muscle hypertrophy, and concentrated inflammatory cell infiltrate in the bronchial wall (25, 34). More recent data obtained from biopsy specimens of human bronchus confirm the presence of airway inflammation in subjects with mild to moderately severe asthma (4, 5, 8, 24, 37, 38, 44, 50), suggesting that the inflammation of the bronchi is an important factor in the mechanism of asthma (3, 7, 11, 23, 34).

To assess the histochemical features of the cellular infiltration in asthmatic bronchi, we obtained bronchial biopsies from 16 mildly asthmatic patients and six normal subjects (65), and looked for the presence of inflammatory cells by immunohistochemical analysis. In biopsies from asthmatic subjects, there was a significant increase in the number of primed T cells (UCHL1 positive) over that of non-asthmatic normal subjects ($P<0.01$). There was also a significant increase in the number of activated eosi-

nophils (EG2 positive) and of cells expressing the HLA class II antigen (LN3 positive) ($P < 0.02$ and $P < 0.01$, respectively). This suggests that not only is there an increase in cell numbers in asthma, but also the inflammatory cells are more activated. There was, however, no difference in the neutrophil numbers, and very few B cells were detected. Immunohistochemical analysis with HAM 56 (anti-CD68; pan-macrophage marker) showed that the submucosa had a significantly larger macrophage population in asthmatic subjects than in controls ($P < 0.002$). In asthmatic subjects, there was also an increase in cells that stained with MAC387 and antimuramidase antibodies ($P < 0.02$ and $P < 0.05$, respectively), which are markers for macrophages with phenotypic characteristics of monocytes and monocyte-like tissue macrophages (Fig. 4). Furthermore, the HAM56-positive macrophage population was much larger than that recognized by MAC387 and antimuramidase, suggesting that the macrophage population in the bronchial wall is heterogeneous. Double staining

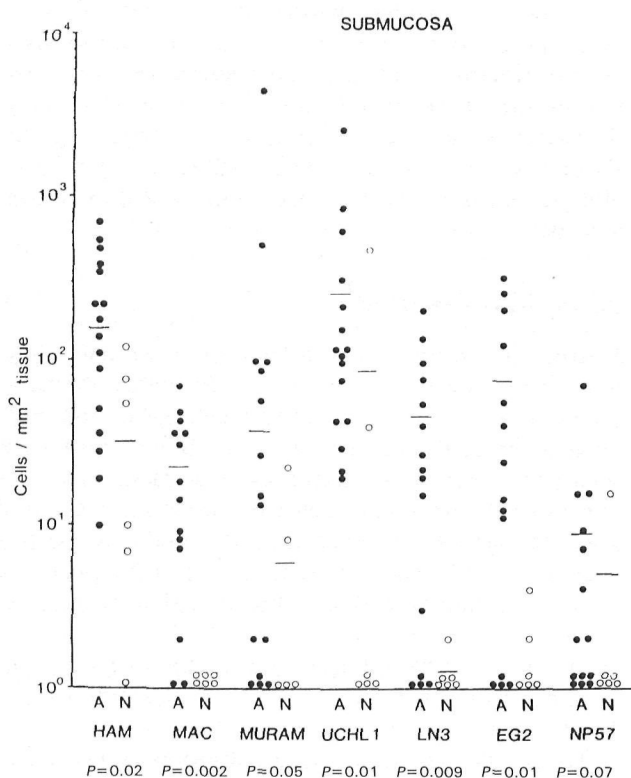


Fig. 4. Individual cell counts in submucosa for each antibody used on biopsies obtained from asthmatic (A) and normal subjects (N). HAM = HAM56: panmacrophage marker; MAC = monoclonal antimonocyte monoclonal antibody; MURAM = polyclonal antimuramidase (lysozyme); UCHL1 = antimemory T-cell monoclonal antibody; LN3 = anti-HLA-DR monoclonal antibody; EG2 = antieosinophil cationic protein-monoclonal antibody; NP57 = antineutrophil elastase monoclonal antibody. *P* values given were obtained with Mann-Whitney U test (With permission, Am Rev Respir Dis 1992; 145: 918–19).

with HAM56 and LN3 showed that class II was expressed on submucosal macrophages as well as on other submucosal cell types. The percentage of macrophages expressing class II antigen was 47%, and 46% of the total cells expressing class II were macrophages. These results suggest that macrophages in asthmatic bronchial biopsies are activated and that lung macrophages may play a role in the mechanism of the chronic immune-mediated response seen in the airway mucosa of asthmatic subjects.

Analysis of the epithelium revealed that there was a significant increase in the MAC387 staining in the asthmatic biopsies ($P = 0.04$). There was also an increase in the number of primed T cells and activated eosinophils ($P = 0.001$ and $P = 0.01$, respectively). No difference was seen in NP57 staining. However, there was a significant upregulation of LN3 staining (class II expression) in basal epithelial cells in the asthmatic epithelium ($P = 0.02$). Bronchial epithelium is therefore activated in asthma; this suggests that it is involved in the pathogenesis of the disease.

The presence of high numbers of inflammatory cells, such as eosinophils and macrophages, in the bronchial submucosa of asthmatic subjects (65) might be explained by increased levels of cytokines such as GM-CSF. Using polyclonal and monoclonal antibodies against GM-CSF for immunohistochemical analysis of airway biopsies obtained by fiberoptic bronchoscopy, we have shown that there are seven times more GM-CSF staining cells in the bronchial submucosa of asthmatic patients than in that of normal subjects. Approximately two-thirds of cells staining for GM-CSF are macrophages, and approximately three-quarters of macrophages stain positively for GM-CSF. In addition to the staining of submucosal inflammatory cells for GM-CSF, the epithelial cell stains very strongly for this cytokine (76). The extent of staining, as quantitated by hue saturation-intensity color-image analysis, indicated that asthmatic airway epithelial cells stained more strongly with a polyclonal or monoclonal antibody against GM-CSF than did those of normal subjects ($P = 0.013$ and $P = 0.003$, respectively). Administration of inhaled beclomethasone dipropionate 1000 μ g daily for 8 weeks resulted in a significant reduction in GM-CSF expression in the airway epithelium in patients given corticosteroids, as compared with those given placebo. There was a correlation between the suppression of GM-CSF staining by inhaled corticosteroids and the increase in FEV₁ and decrease in carbachol responsiveness ($r = 0.61$; $P < 0.05$ and $r = 0.8$; $P < 0.01$, respectively). These data suggest a strong involvement of GM-CSF in the pathogenesis of bronchial asthma. This might be due to GM-CSF involvement in the survival (63), differentiation (59) and activation (32, 36, 49, 73,

81), and chemotaxis (26, 87) of inflammatory cells such as macrophages and eosinophils.

The present findings are consistent with *in vitro* data showing the production of GM-CSF, IL-1, IL-6, and IL-8 by cultured airway epithelial cells in the presence and absence of stimulation by IL-1 β or TNF- α (17, 52, 53, 55, 74).

The substantial macrophage infiltration in the airways of asthmatic subjects (65) led to a study (75) of possible chemoattractant factors produced by the airway epithelium with a specificity for monocytes such as monocyte chemoattractant protein-1 (MCP-1) (48, 71). MCP-1 is an 8–15-kDa basic peptide (74) which is encoded by the human homologue of the mouse JE gene, the first PDGF early-response gene to be identified (15). It belongs to the IL-8 gene superfamily of low-mol.-mass cytokines – the chemokines. However, it belongs to the same subfamily as RANTES and MIP-1- α and - β , the c-c superfamily, which is distinct from the IL-8/NAP-1 C-X-C subfamily. MCP-1 is known to activate different inflammatory cells, such as monocytes (15, 48, 71), and can be produced by different cell types, including monocytes (39, 90), macrophages (16, 54), and pulmonary alveolar macrophages (2, 9, 19).

In our study of this subject (75), we used a monoclonal and a polyclonal antibody to detect MCP-1 expression on bronchial biopsies from asthmatic and normal subjects. Both antibodies showed an upregulation of MCP-1 expression in the asthmatic bronchial epithelium. There was also upregulation of MCP-1 expression in the submucosa of the asthmatic bronchial biopsies, many of the positive cells having the histologic characteristics of macrophages. These increased levels of MCP-1 in asthmatic airways suggest that they may play a role in macrophage recruitment and activation, and thereby contribute to the inflammation of bronchial asthma. We have therefore shown that the bronchial epithelial cell produces two cytokines, namely, GM-CSF and MCP-1, which show specificities for the monocyte. The observation that IL-1 β , a major cytokine produced by monocytes, can lead to upregulation of cytokine production by the epithelial cell suggests that there may be an important amplification network between the infiltrating monocyte/macrophage and the airway epithelial cell in asthma.

In conclusion, there is increasing evidence of the central role of cells of monocyte/macrophage lineage in the pathogenesis of bronchial asthma. This evidence comes from studies on peripheral blood monocytes, BAL fluid and cells, and, more recently, airway immunohistochemistry. Elucidation of the mechanisms of macrophage interactions may eventually lead to new approaches in antiasthmatic therapy.

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