Cytokine and Chemokine Interactions in Allergic Airway Inflammation

Emma M. Campbell and Nicholas W. Lukacs

Introduction

Asthma is a disease associated with the upper airways and characterized by episodes of airway reactivity and bronchospasm. Numerous studies have suggested that an underlying inflammation of the bronchi plays a central role in the clinical expression and pathogenesis of this disease. The classical model by which inflammatory events result in changes in airway hyperreactivity is initiated by the binding of specific allergens to sensitized mast cells within the lung, via the high-affinity immunoglobulin E (IgE) receptor FcεRI (Metzger and others 1986). This binding induces a degranulation of the mast cells, releasing a variety of preformed mediators including tumor necrosis factor-α and interleukin (IL)-4 as well as those synthesized de novo such as platelet-activating factor (PAF) and leukotrienes, which collectively induce an immediate bronchoconstriction, vasodilatation leading to edema, and recruitment of leukocytes (Galli 1997). Although this phase commonly resolves fairly rapidly, the asthmatic patient frequently experiences a second decline in lung function that can persist for days (O’Byrne and others 1987). This decline is associated with an accumulation of leukocytes around the large airways that may lead to end-stage disease, including smooth muscle cell hypertrophy and airway thickening.

It is interesting that inflammation has been observed even in patients presenting symptoms associated with mild atopic asthma, which may predispose them to more severe disease later in life. A number of studies suggest that the degranulation of eosinophils causes the release of cationic proteins that can denude the airway epithelium, possibly exposing sensory nerve terminal endings. This process may allow stimuli access to the nerves and initiate airway reactivity, thus establishing a causative link between inflammatory events and physiological changes (Lefort and others 1994). The temporal events surrounding the establishment and maintenance of the late-phase response have been the focus of considerable research over the past 15 yr. What has emerged is a complex and often controversial picture of the importance of participating cells (mast cells versus T cells versus eosinophils) as well as particular cytokine mediators.

Models of Allergic Asthma

The suitability of any animal model of allergic airway inflammation is assessed by its ability to mimic human asthma. Thus, the ideal model would exhibit early and late antigen-induced airway responses, reversible bronchial hyperresponsiveness, peribronchial eosinophilia, and chronic lung remodeling (Corrigan and Kay 1992). In this regard, the guinea pig has traditionally been the species of choice for many investigators and has been especially invaluable in elucidating mechanisms governing changes in airway physiology (Frew and others 1990; Watson and others 1993). However, in recent years, murine models of allergic airway disease have been extensively employed because we have learned that the mouse can also be sensitized to a number of different antigens and develop an associated lung inflammatory response. The increased use of the mouse has been due largely to the availability of species-specific cytokines as well as the development of gene knockout mice. In addition, the murine immune system has been relatively well characterized as being similar to the human system, and, in practical terms, the mouse is cost effective for experimentation.

A number of murine models of allergic airway disease utilizing several different types of allergens and pharmacological challenge have been successfully established. Sensitization to a range of allergenic proteins (for example, ovalbumin [Gonzalo and others 1996] and Schistosoma mansoni egg antigen [Lukacs and others 1997] as well as allergens from the cockroach [Campbell and others 1998]) have been employed to induce inflammatory airway responses. These responses are characterized by an early lung neutrophilic infiltration, followed by a delayed eosinophilic infiltrate akin to the late-phase reaction in human asthma (Corrigan and Kay 1992). Associated with these characteristics is the presence of elevated levels of a number of cytokines including IL-4 (Cory and others 1996; Lukacs and others 1994) and IL-5 (Foster and others 1996). In addition, a number of mediators, including histamine and eosinophil peroxidase, can be detected in bronchoalveolar lavage (BAL) of sensitized mice after allergen challenge, indicating that mouse

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1Abbreviations used in this paper: BAL, bronchoalveolar lavage; CC, conserved cysteine residues; CXC, juxtaposed cysteine residues; IgE, immunoglobulin E; IL, interleukin; INFγ, interferon gamma; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; PAF, platelet-activating factor; RANTES, regulated upon activation normal T cell expressed and secreted; Th, T-helper.
models are useful for allergy studies inasmuch as they also develop an accompanying severe airway hyperreactivity.

Various laboratories have identified differences in mediator involvement between varying animal models, which may be due to differences in the sensitization and challenge protocols used. For example, it has been suggested that in some murine models, the mast cell may function to augment responses induced by low levels of antigen; however, this augmentation is comparatively redundant after stronger procedures of immunization and challenge (Falli 1997). The most common model used in many laboratories employs an ovalbumin-induced allergic airway response. Although this model has identified several important mechanisms and provided a better understanding of the allergic airway response, the model has not been standardized and can differ drastically from laboratory to laboratory. Furthermore, few studies have focused on temporal changes in cytokine profiles after antigen challenge. We have developed and characterized murine models of allergic airway inflammation using multiple antigens that by themselves induce strong T-helper (Th1)2-type responses. In an original model, we utilized a soluble antigen from the eggs of the helminth parasite _S. mansoni_. These antigens have been shown to provide a useful model of Th2-driven responses in the mouse. This airway model is somewhat unique in that the initial immunization involves injection of _S. mansoni _eggs from which the soluble antigen is secreted over a prolonged period (7 days) in situ, negating the need for an adjuvant (Lukacs and others 1994).

In a second model, antigens from the cockroach (Campbell and others 1998) have been employed to induce an allergic airway model. This latter model has clinical relevance given that nearly 60% of all inner city asthmatics have highly elevated IgE levels specific for these antigens. Mice sensitized with cockroach allergen (10 µg given intraperitoneally with incomplete Freund’s adjuvant) also develop a strong Th2-type response with an associated peribronchial eosinophilia. Intratracheal administration of allergen after an initial sensitization period (day 17) induces a strong allergic response localized to the peribronchial region of the airway. The intratracheal administration allows a known dose of antigen to be delivered into the airways, thus standardizing the model and circumventing problems of absorption of the allergen in the mucosal membranes of the nasal passages, which are commonly associated with delivery by aerosolization. Utilizing these two different models, we have been able to begin to decipher the temporal changes in cytokines and chemokines, as well as to identify specific cell populations that are involved at various stages of disease progression.

Assessment of the severity of the inflammatory response can be performed by enumerating eosinophils around the airways using histological preparations of the lung or within the airways using differential cell counts of BAL cytospin preparations. However, monitoring changes in airway physiology in allergic animals is arguably the most clinically relevant marker of the disease. Typically, this change is determined by the observation of a hyperreactive response after administration of a bronchoconstrictor agent such as methacholine. In recent years, such measurements in the mouse have been possible through the development of whole body plethysmography (Buxco, New Haven, Connecticut) specifically designed for low tidal volumes (Martin and others 1988). With our models and specific sensitization protocols, we have consistently observed at least five-fold increases in airway hyperreactivity as a result of single allergen challenges compared with vehicle control challenged mice (Figure 1). This level of increase allows an easier assessment of mediators that may influence the airway response because it is possible to evaluate the attenuation of hyperreactivity in the airway.

**Cytokine Cascades during Allergic Airway Responses**

The induction and maintenance of allergic airway inflammation appear to be dependent on the complex interactions between a number of cytokines and chemokines. Numerous studies to date suggest that the onset of the asthmatic response...
is driven by CD4+ T lymphocytes, and their level of activation has been shown to correlate with disease severity (Azzawi and others 1990; Walker and others 1991). Functional diversity of these cells has been demonstrated by the observation that they can differentiate into Th1 or Th2 phenotypes (Mosmann 1986), characterized by the production of differing profiles of cytokines. Those T cells involved in a "pro-allergic" response appear to secrete a cytokine milieu associated with the induction of a classical Th2 response in which upregulation of IL-4, IL-5, and tumor necrosis factor-α are observed (Lukacs 1996). IL-4 appears to be pivotal as the cytokine driving toward this Th2 response (Kopf and others 1993; Swain and others 1990). However, it also induces IgE class-switching in B cells (Morawetz and others 1990) and upregulates the adhesion molecule VCAM-1 (Schleimer and others 1992), thereby promoting eosinophil extravasation from the blood into the interstitium. Accordingly, the neutralization of IL-4 responses using specific receptor antagonists and neutralizing antibodies (Lukacs and others 1994) can attenuate airway eosinophilia and hyperreactivity in animal models of allergic airway inflammation. Interestingly, IL-13 has similar activities to IL-4 binding to a common receptor subunit (IL-4Ra) (Smertz-Bertling and Duschl 1995), which may account for some of the compensatory activity observed in vivo in the absence of IL-4. Recent work using a soluble receptor IL-13 receptor protein (IL-13R-Fc) that did not bind IL-4 suggests an independent role for IL-13 in mucus hypersecretion and eosinophil accumulation associated with a murine model of allergic airway disease (Grunig and others 1998; Wills-Karp and others 1998).

Another Th2-induced cytokine, IL-5, is particularly important in the induction of eosinophilia in allergic individuals. IL-5 is essential for the terminal differentiation and survival of the eosinophils (Yamaguchi and others 1990) and is important in the systemic release of these cells from the bone marrow into the blood (Dent and others 1992). IL-5 not only causes release of eosinophils from the bone marrow but also acts as a "priming" agent, potentiating the actions of eosinophil-activating mediators, including PAF and chemokines (Lundahl and others 1998; Schweizer and others 1994). Inhibition of IL-5 in vivo has demonstrated that this therapy has both immediate and long-term benefits in allergy/asthma models, indicating it as an attractive target for therapy.

It is generally accepted that the balance between Th2 cytokines and those of a Th1 phenotype is critical in determining the evolution of an inflammatory response. Th2 cytokines largely sustain allergic processes, with the one exception of IL-10, which inhibits the inflammation because it is able to downregulate cytokine production (Zuany-Amorim and others 1995). Th1 cytokines (particularly interferon gamma [IFN-γ]) inhibit the induction of a Th2 response, and recombinant IFN-γ accordingly has been demonstrated to inhibit inflammatory responses in a murine model of allergic airway disease. A similar effect has been observed for IL-12, although these effects were largely through IFN-γ-dependent mechanisms (Gavett and others 1995; Kips and others 1996). In addition, an inflammatory lesion is not static; rather, it is modified by changes to the structural cells within the site as well as new cells trafficking into the site. Many of these leukocytes themselves secrete cytokines. For example, eosinophils produce IL-4, IL-10 (Nakajima and others 1996), and IL-12 (Grewe and others 1998).

Studies in the cockroach allergen model yielded similar results as previously reported for Th2-type cytokines IL-4 and IL-13, with differential effects on the temporal expression of airway hyperreactivity. Interestingly, although anti-IL-4 blocked airway hyperreactivity at both early (8-hr) and late (72-hr) time points, anti-IL-13 treatment only attenuated airway hyperreactivity at the late time point and not at the earlier time point (Figure 2). Thus, it appears that these two

**Figure 2** T-helper(Th2)-type cytokines have differential temporal effects on airway hyperreactivity in cockroach allergen-induced airway hyperreactivity. Data are expressed as the mean ± SE of at least five mice/group. Background airway resistance was similar between groups, ranging from 1.4 to 1.7, and was subtracted from each of the readings.
Th2-type cytokines participate in the response in a different fashion, even though they both may bind to a common receptor. In addition, preliminary evidence indicates that anti-IL-13 does not alter the eosinophil influx in a fashion similar to IL-4 (data not shown). Additional studies are under way to examine the temporal expression of the two cytokines as well as the cellular sources.

**Differential Roles of Chemokines during the Induction of Allergic Airways Disease**

In the late 1980s, a family of small molecular weight proteins (8 to 10 kD) was identified. Each member of the family has the ability to induce the migration of defined subsets of leukocytes. These chemotactic cytokines or chemokines have since been recognized as divisible into two main groups according to whether the first of two cysteine residues in their primary structure are adjacent (CC1) or juxtaposed (CXC1), that is, separated by one amino acid (Premack and Schall 1996). A number of the CC chemokines are implicated in the pathogenesis of allergic inflammation by virtue of their ability to attract/activate lymphocytes, monocytes, eosinophils, and/or mast cells/basophils (Alam 1997), as well as the fact that their production is upregulated during human asthma (Holgate and others 1997; Lamkhioued and others 1997; Powell and others 1996). Eotaxin, regulated upon activation normal T cell expressed and secreted (RANTES1), macrophage inflammatory protein (MIP)-1α, monocyte chemoattractant protein (MCP)-1, MCP-3, and MCP-4 are all potent eosinophil chemoattractants (Jose and others 1994; Rot and others 1992; Schall and others 1990; Stellato and others 1997; Weber and others 1995). This set of chemokines binds to two different receptors on eosinophils—CCR1 and CCR3. CCR1, which binds RANTES, MCP-3, and MIP-1α, is expressed on eosinophils at much lower levels than CCR3 and is thought to play a lesser role in the recruitment of these cells (Daugherty and others 1996; Post and others 1995).

Interestingly, the expression of CCR3 is not solely confined to eosinophils, but also to basophils (Uguccioni and others 1997) and Th2-type cells (low levels) (Sallusto and others 1998), and thus has become a primary focus of therapeutic targeting. At the time of this writing, studies to examine the in vivo effects of blocking the CCR3 receptor have not yet been performed; however, neutralization of its ligands in murine and guinea pig models of allergic inflammation demonstrate attenuated cell recruitment and hyperreactivity (Lukacs and others 1997; Rothenberg and others 1995). Although considerable biological redundancy exists within the CC chemokine subfamily, studies have demonstrated that an orchestrated production/use of individual chemokine ligands may be involved in the development of an allergic response (Campbell and others 1998).

Using the cockroach antigen model of allergic airway inflammation, we observed a characteristic peribronchial eosinophilia and associated airway hyperreactivity after a single airway allergen challenge. This response was exacerbated after a secondary rechallenge of the allergen, including synergistic increases in eosinophils and airway hyperreactivity (Campbell and others 1998). To our surprise, eosinophilia was differentially mediated either by MIP-1α after a single allergen challenge or by eotaxin after a secondary rechallenge. Thus, multiple chemokines are necessary at various stages of the allergic response for the recruitment of eosinophils, which may explain why the coordinated production of a number of chemokines and receptors is necessary during an inflammatory response. The associated hyperreactivity of the secondary, but not the primary, challenge response was attenuated by administration of anti-eotaxin antibodies, which also reduced eosinophil peroxidase levels in the BAL. Cationic proteins have previously been demonstrated to be implicated in the development of airway hyperreactivity by denuding epithelium (Laitinen and others 1985; Lefort and others 1994). Thus, a major role of eotaxin during chronic allergic disease may be necessary to degranulate the eosinophils, subsequently leading to tissue damage and airway remodeling.

Additional studies have demonstrated a role for MCP-1 in the induction airway hyperresponsiveness in murine models of allergic airway diseases induced by *S. mansoni* egg antigen as well as cockroach allergens. Antibodies against murine MCP-1 (JE) are able to block changes in airway resistance (assessed by administration of the bronchoconstrictor methacholine) in sensitized mice when administered 1 hr before a single allergen challenge. In a number of associated studies in our laboratory, we have identified MCP-1 both as an important T cell and monocyte recruitment factor during allergy and an inducer of local mast cell activation. Likewise, using a MCP-1 receptor −/− animal (CCR2 −/−), we have demonstrated similar reductions in airway inflammation and hyperreactivity responses. In fact, direct instillation of MCP-1—but not MCP-3, MCP-5, MIP-1α, or eotaxin—induced airway hyperresponsiveness in normal mice, which, at 1 hr, was associated with increased histamine concentration in the BAL of these animals. CC chemokines can induce histamine-releasing factors in vitro, and such activation is a potential mechanism for our in vivo observations (Alam and others 1992; Rot and others 1992; Rothenberg and others 1997).

In summary, MCP-1 mediates the activation and migration of a number of cell populations including monocytes, T cells, mast cells, and basophils, ultimately leading to airway hyperreactivity. MCP-1 and its receptor, CCR2, have also been targeted as a potential therapeutic target for attenuating asthmatic disease. Thus, a number of chemokines and receptors appear to be relevant targets for therapy (Table 1).

**Conclusion**

Multiple mediator pathways appear to be involved in the induction and maintenance of allergic airway disease, ultimately leading to the accumulation of eosinophils around the airways. This complex cascade of events offers multiple
opportunities for therapeutic intervention, and even though common pathways of activation are utilized, inhibition of a single factor may not be effective at halting disease progression. It is possible, however, that inhibition of receptors with multiple ligands, such as many of the CC chemokine receptors, will provide a viable therapeutic approach. It is clearly necessary to further elucidate the mechanisms controlling these responses. The coordinated use of clinical and animal model research is imperative to better understand disease processes.

References


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Table 1 CC chemokines and their receptors in asthma as possible therapeutic targets

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<th>Receptor</th>
<th>Function</th>
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<td>Eotaxin</td>
<td>CCR3</td>
<td>Eosinophil recruitment/activation</td>
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<tr>
<td>MCP(^b)-1</td>
<td>CCR2</td>
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\(^b\)CC, conserved cysteine residues; MCP, monocyte chemoattractant protein; MDC, monocyte-derived chemokine; TARC, thymus and activation-regulated cytokine; TCA, T cell chemoattractant.


