

Determinants of the immune-inflammatory response in allergic airway inflammation: Overview of antigen presentation and cellular activation

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Under normal circumstances the lung is in a state of immunologic homeostasis, a condition in which exposure to innocuous antigens does not lead to immune-inflammatory responses. This is the only reasonable solution to the dilemma faced by the lung: the need to interact with the external environment and the need to avoid responding to most of the environmental antigens to which it is exposed. In allergic diseases, such as asthma, this homeostasis is undermined, and immune-inflammatory responses to harmless aeroallergens are activated. We describe the changes in antigen presentation and cellular activation observed in a model of allergic airway inflammation. Further, we present a summary of our work that investigated the impact of the airway cytokine microenvironment on the development of immune responses in the respiratory tract. (*J Allergy Clin Immunol* 2000;106:S206-12.)

Key words: Microenvironment; allergic airway inflammation; antigen presentation; cytokines; GM-CSF; inhalation tolerance; T cells

Airway eosinophilia is a characteristic feature of asthmatic inflammation in humans and murine models of allergic airway inflammation. However, the accumulation of eosinophils in the airway is the terminal step in a sequence that involves complex cellular interactions, both in the airway itself and in associated lymph nodes. Although the critical role of T lymphocytes in the development of experimental allergic inflammation has been well established,¹ it is clear that the T cells themselves are unable to recognize antigen unless it is adequately presented to them by antigen-presenting cells (APCs). Thus, the tripartite interaction between antigen, APC, and T cell defines the central axis around which the specific immune response is generated. The molecular sig-

Abbreviations used

APC: Antigen-presenting cell
BAL: Bronchoalveolar lavage
DC: Dendritic cell

nals delivered to the T cell at this stage can critically determine the nature of the immune-inflammatory response that will ensue. The objective of this review is to discuss data examining the conditions under which immune responses to aeroallergens might be generated and the changes in APC and T-cell phenotype that are associated with these responses.

TRADITIONAL MURINE MODELS OF ALLERGIC AIRWAY INFLAMMATION

Although there are intermodel variations in timing and dosage, most murine models of allergic airway inflammation use similar pathways for allergic sensitization and the induction of eosinophilic immune-inflammatory responses in the airways. In the sensitization phase, a model protein allergen (most commonly ovalbumin) is applied intraperitoneally on 1 or more occasions, usually with aluminum hydroxide as an adjuvant. After the sensitization phase, airway inflammation is induced by the introduction of antigen into the respiratory tract. In the particular variation on this theme used in our laboratory, 8 µg ovalbumin and 4 mg aluminum hydroxide are injected intraperitoneally on 2 occasions, 5 days apart, followed 1 week later by aerosol ovalbumin.² These interventions reproduce a number of characteristic features of human allergic asthma, including the expression of allergen-specific IgE, the elaboration of T_H2-associated cytokines (eg, IL-4, IL-5, and IL-13), the development of eosinophilic inflammation of the airways, and the induction of bronchial hyperreactivity.

ANTIGEN PRESENTATION

Naive T cells and T_H cells of any experience can normally be activated only by professional APCs (dendritic cells [DCs], macrophages, and B cells). These cells are virtually the only cells in the body to express MHC class II molecules and adequate levels of T-cell costimulatory

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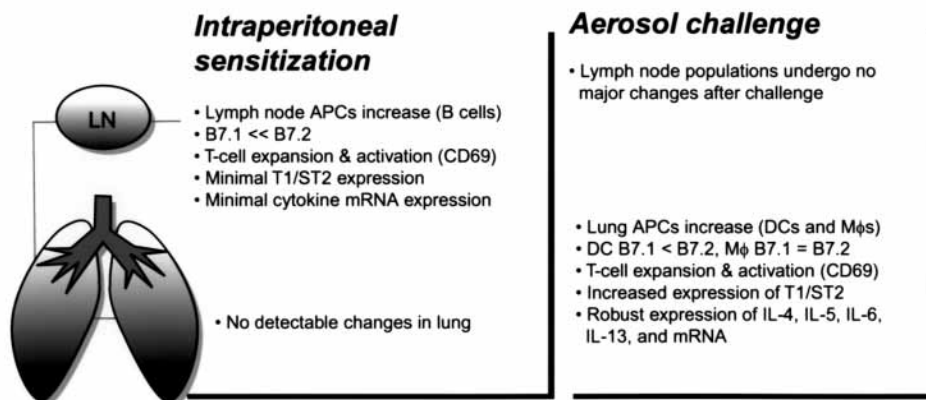


FIG 1. Overview of antigen presentation and cellular activation in a traditional model of allergic airway inflammation. Flow cytometric analysis was used to ascertain the phenotypic changes in the APC and T-cell compartments in the lymph nodes and lungs of mice throughout a model of allergic airway inflammation. The key observations are summarized, in both lymph nodes and lungs, and after intraperitoneal sensitization and after aerosol challenge (unpublished data).

molecules (such as B7.1 and B7.2). Resting APCs in the mucosae normally sample the antigens in their environment by pinocytosis and express relatively low levels of costimulatory molecules. On activation, APCs shut down their antigen uptake and migrate to the draining lymph nodes, during which time they upregulate MHC and costimulatory molecule expression, thereby enhancing their antigen-presenting capabilities. Hence, T-cell-mediated immune-inflammatory responses can only be generated after APCs have been activated. We have performed a detailed spatial and temporal analysis of surface marker expression by APCs in the model of allergic airway inflammation described earlier. Lungs and lymph nodes were analyzed in naive mice at various time points after the second intraperitoneal injection, on the day of challenge but before antigen aerosolization, and at various time points after aerosol challenge (unpublished data). A summary of our observations is shown in Fig 1.

It has been previously observed that the preferential lymphatic drainage of the peritoneal cavity is through the thoracic lymph nodes.³ Indeed, we observed a considerable increase in the cellularity of the thoracic lymph nodes that peaked 24 hours after the second intraperitoneal injection. Flow cytometric analysis of lymph node cells after sensitization revealed a large expansion of the B-cell compartment, with modest increases in the DC and macrophage populations. There was a general increase in antigen-presenting capacity during sensitization, as indicated by the increased expression of B7 molecules. In particular, B7.2 expression was increased (in absolute numbers and proportion of cells expressing B7.2) in both B cells and DCs, compared with naive levels. However, no statistically significant changes were observed in the expression of B7.1 on any APC subset in the thoracic lymph nodes. Thus, the preferential upregulation of B7.2 compared with B7.1 suggests that B7.2 may be more important in the induction of T_H2-mediated immune responses.

Throughout the process of sensitization, flow cytometric analysis of lung cells revealed no differences in APC popu-

lations from naive animals, as expected, because there was no antigen challenge in the lungs. However, after aerosol challenge with ovalbumin, lung APC populations increased dramatically. In contrast with our findings in the lymph nodes after sensitization, where the APC expansion was due almost entirely to an expansion of the B-cell compartment, the predominant APC populations to expand in the lung were macrophages and DCs. The costimulatory repertoire of the various APC types in the lung was notably different from those observed in the lymph node after aerosol challenge. Both B7.1 and B7.2 were expressed to a greater degree on lung macrophages after the challenge, whereas only B7.2 was upregulated on lung DCs. Lastly, the expression of neither B7.1 nor B7.2 was changed on lung B cells.

It is clear from the literature that APC expression of these molecules is critical for T-cell activation. Functional studies that use blocking strategies have demonstrated that B7 is essential for the development of pulmonary eosinophilia, the production of IgE and T_H2 cytokines, and airway hyperreactivity.^{4,5} These results were recently confirmed in studies with B7.1 and/or B7.2 knockout mice, demonstrating that, although B7.2 appears to play a somewhat larger role, both molecules have important complementary roles to play.⁶ The apparently differential effects of B7.1 and B7.2 implies that the molecules are not interchangeable and that there are discrete requirements for both molecules during sensitization and challenge, depending on the time, place, and context of their expression.

CELLULAR ACTIVATION

It has been firmly established that allergic inflammatory responses depend on the presence of T_H cells.¹ As such, we examined changes in T-cell surface marker expression and evidence of cytokine messenger RNA (mRNA) expression indicative of T-cell effector function (unpublished data). Flow cytometric analysis of T-cell subsets in the thoracic lymph nodes revealed that there

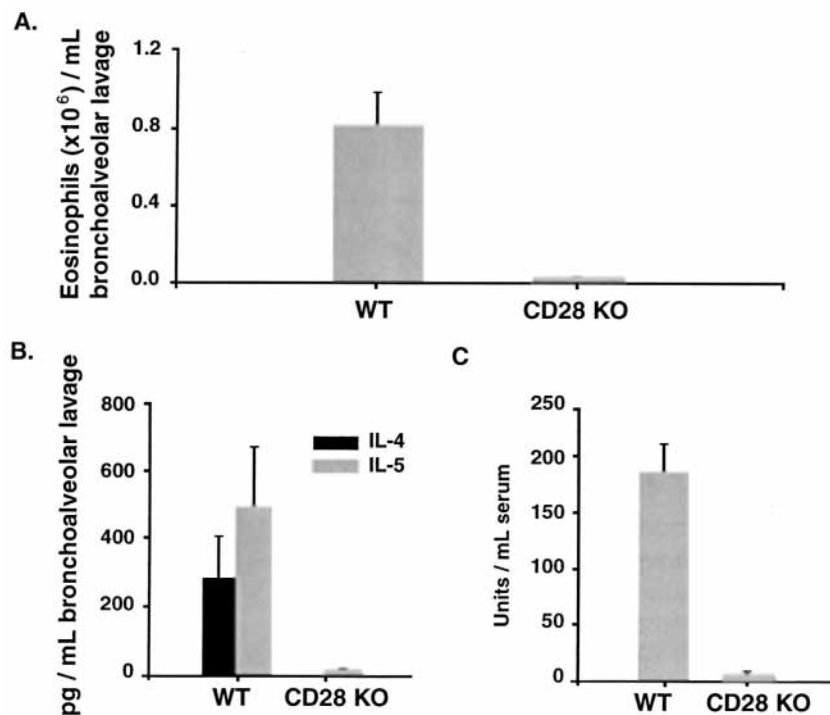


FIG 2. Characteristic allergic airway inflammation failed to develop in CD28 knockout (KO) mice. CD28 knockout mice were sensitized to ovalbumin by an intraperitoneal injection with aluminum hydroxide as an adjuvant, followed 7 days later by an aerosol challenge with ovalbumin. No recall inflammatory response to ovalbumin was observed in the CD28 knockout mice (data not shown). **A**, An assessment of BAL eosinophilia revealed a dearth of eosinophils in the airways of CD28 knockout mice 72 hours after challenge. **B**, Prototypical type 2 helper T-cell cytokines, IL-4 and IL-5, were absent in the BAL of CD28 knockout mice 24 hours after challenge. **C**, The induction of ovalbumin-specific IgE was deficient in CD28 knockout mice (unpublished data). WT, Wild type.

was a substantial expansion of both CD4⁺ and CD8⁺ T cells after the second intraperitoneal injection; evidence of T-cell activation was assessed by expression of CD69. However, RNase protection assays failed to detect appreciable cytokine mRNA expression in the lymph nodes at any time during sensitization or challenge, and expression of the putative T_H2 marker T1/ST2⁷ was not increased above background levels. Hence, it appears that, although T-cell expansion undoubtedly occurs, T-cell effector activity in the lymph nodes during sensitization is limited, at best. Furthermore, antigen challenge did not result in the further expansion of T cells or the induction of cytokine mRNA expression in the thoracic lymph nodes.

Similar to our observations in APCs, T-cell populations in the lungs did not change in number or phenotype during the sensitization phase of the model. After aerosol challenge, however, T-cell populations in the lung increased over several days, with the bulk of the expansion accounted for by CD4⁺ T_H cells. Additionally, there was an increase in T1/ST2 expression, which indicated the presence of committed T_H2 cells. In contrast to the lymph nodes, there was ample evidence of T-cell effector function, with robust

increases in mRNA for IL-4, IL-5, IL-6, and IL-13 after the challenge. Thus, the immune system appears to exhibit a practical logic in this case, in that T-cell effector activity is limited to the target tissues, thereby minimizing nonproductive effector activity in the lymph nodes.

Studies with various knockout mice have revealed the functional requirements for molecules expressed by T cells, those required for both the T-cell activation itself (ie, CD28) and the T-cell signal provision to other cells for the full expression of allergic airway inflammation (ie, CD40 ligand). Indeed, CD28 knockout mice, unable to receive T-cell stimulation from B7 molecules, completely fail to develop eosinophilic airway inflammation or peripheral blood eosinophilia or to express T_H2 cytokines or ovalbumin-specific IgE (Fig 2). In CD40 ligand knockout mice, we have shown that ovalbumin-specific IgE and airway eosinophilia were absent after aerosol challenge, despite normal IL-5 levels in the bronchoalveolar lavage (BAL) and increased peripheral blood eosinophilia.⁸ The defective recruitment of eosinophils to the airway was attributable to the absence of IL-4 and TNF- α expression and, as a consequence, lack of endothelial vascular cell adhesion molecule 1 expression.

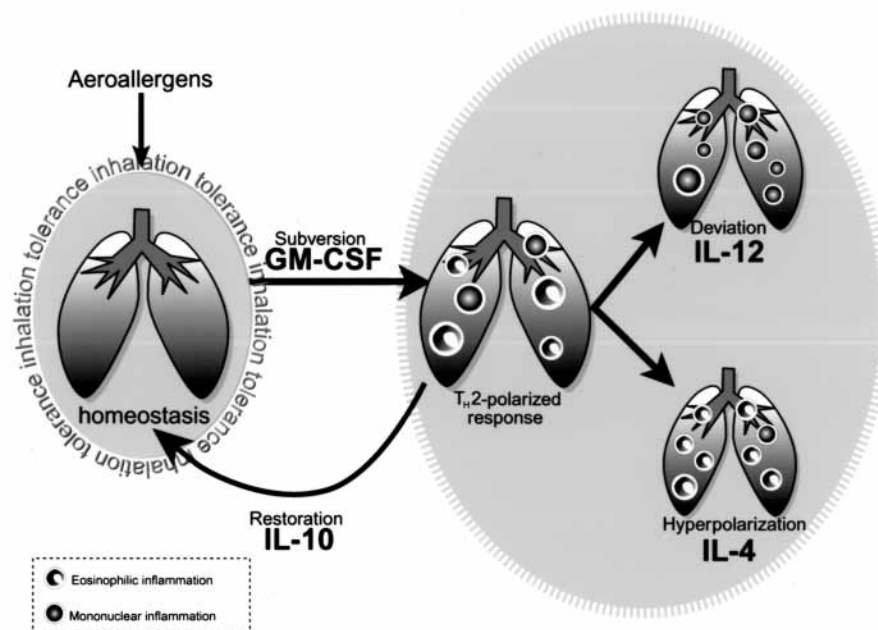


FIG 3. Influence of the cytokine microenvironment on the regulation of immune-inflammatory responses in the lung. Exposure to aeroallergens does not normally lead to allergic inflammation. Homeostatic mechanisms in the lung tend to induce a “willful ignorance” of the antigen, the immunologic state of tolerance. However, the cytokine microenvironment in the airways profoundly affects the outcome of antigen exposure. The expression of GM-CSF in the airways during antigen aerosolization subverts the homeostatic mechanisms, leading to a T_H2 -polarized immune-inflammatory response. The additional presence of IL-4 with GM-CSF further polarizes this response toward eosinophilic inflammation characteristic of T_H2 responses. In contrast, the expression of IL-12 with GM-CSF diverts the response toward the T_H1 -associated mononuclear inflammatory response. Furthermore, IL-10 expression is able to block the inflammatory responses promoted by GM-CSF and sustains the lung in homeostasis.

Notably, reconstitution of IL-4 and TNF- α at challenge with the use of adenoviral vector-mediated gene transfer restored vascular cell adhesion molecule 1 expression and eosinophilic inflammation. Thus, the T-cell arm of the specific immune response to ovalbumin is apparently nonexistent in CD28 knockout mice but remains largely intact in CD40 ligand knockout mice. However, because isotype switching and antibody secretion in B cells require help from activated T cells, the absence of either CD28 or CD40 ligand severely impairs B-cell responses.

The traditional models of allergic airway inflammation have been extraordinarily fruitful in yielding insights into the immunologic basis for allergic diseases. However, they are limited in their capacity to explain the events taking place during genuine allergic sensitization in humans. First, the route of sensitization in humans is most likely through the respiratory mucosae rather than the peritoneal cavity. Second, aluminum hydroxide and other adjuvants are used because they are efficacious in eliciting immune responses; hence, these models are restricted in their ability to reveal molecular and cellular requirements for allergic sensitization. Thus, we sought to develop an experimental model to study the effect of antigen exposure exclusively through the respiratory tract and to assess the effect of altering the airway environment on the outcome of antigen exposure.

THE CONUNDRUM FACING THE LUNG

To perform its life-sustaining function of gas exchange, the lung must be constantly in contact with the external environment. This provides access to vital oxygen but simultaneously exposes a very large surface area through which pathogenic organisms might gain access to the host. This perilous situation is further complicated by the fact that many inhaled particles are innocuous and do not warrant a defensive immune response. The dilemma facing the lung is that inflammatory responses against harmless antigens may compromise the primary function of the lung (ie, gas exchange), although failure to mount an adequate defense against a pathogenic organism could be harmful and potentially fatal for the host. A similar predicament confronts the other mucosal organs, including the gut and the genitourinary tract.

It is no coincidence that these mucosae are heavily populated with cells of the innate immune system (such as natural killer cells and macrophages) equipped with receptors for the detection of molecular patterns that are common to important pathogenic organisms that are encountered in the evolution of the species.⁹ These systems allow the immune system to distinguish between pathogenic and nonpathogenic antigens and engender a

TABLE I. Effects of cytokine expression in the airways during repeated exposures to aerosolized ovalbumin

Cytokine expressed	Outcome		Comments
	Inflammation	Eosinophils	
None	—	—	—
Control vector	±	—	—
IL-2	±	—	—
IL-4	±	—	Notably, the prominent helper T-cell–polarizing cytokines alone were unable to induce an ovalbumin-specific immune response
IL-12	±	—	—
TNF- α	+	—	No ovalbumin-specific response detectable in vivo or in vitro; inflammation due solely to TNF- α expression (unpublished data)
IL-6	±	—	—
GM-CSF	++	++	Extensively characterized this model of respiratory mucosal sensitization ¹¹

Adenoviral-mediated gene transfer techniques were used to establish the exogenous expression of a number of cytokines in the airways of mice during repeated exposures to aerosolized ovalbumin. GM-CSF was the only cytokine tested that was able to induce an ovalbumin-specific immune-inflammatory response.

mechanism for the prudent initiation of immune responses. However, in 5% to 10% of the human population, this mechanism fails to identify aeroallergens as innocuous antigens. As a consequence, these individuals experience the development of allergic airway disease.¹⁰

Given the apparent constraints on the development of immunoreactivity in the lung, we have examined the cellular and molecular requirements for allergic sensitization through the respiratory tract, with particular emphasis on the impact of the cytokine microenvironment. We have shown, using an adenoviral vector-mediated gene transfer approach, that airway expression of a single cytokine, GM-CSF, allows allergic mucosal sensitization to ovalbumin to take place under conditions that would otherwise lead to the induction of inhalation tolerance.¹¹ In addition, we have investigated the impact of the exogenous expression of IL-4, IL-10,¹² and IL-12¹³ in the airway environment during GM-CSF-driven ovalbumin sensitization. The results of these studies are summarized in Fig 3.

HOMEOSTASIS: INHALATION TOLERANCE

To prevent inflammatory responses to harmless antigens (eg, aeroallergens), it is likely that the immune system learns to “ignore” ubiquitous airborne particles by means of inhalation tolerance. Others have shown that repeated passive exposure to aerosol ovalbumin, without adjuvant, induces a state wherein subsequent sensitization of mice with intraperitoneal ovalbumin and aluminum hydroxide fails to generate a robust ovalbumin-specific IgE response, without affecting immunoglobulin responses to other antigens.^{14–16} Our recent investigations that used the traditional model of allergic airway inflammation reveal that the regulatory mechanisms generated during passive exposure to ovalbumin inhibit not only IgE responses, but also other effector arms of allergic airway inflammation, including eosinophilic inflammation, airway hyperreactivity, and T_{H2} cytokine expression (unpublished data). Although the phenomenon of

inhalation tolerance has been well established, the mechanistic and molecular basis for its establishment remains an area of intense controversy and investigation. Unpublished observations from our laboratory indicate that tolerance is established independently of IL-10 and IFN- γ .

SUBVERSION: GM-CSF-DRIVEN AIRWAY INFLAMMATION IN RESPONSE TO INNOCUOUS AIRBORNE PARTICLES

Because repeated exposure to ovalbumin failed to induce an inflammatory response in the airways, we examined the conditions under which this alleged homeostatic state could be subverted. In particular, we surveyed a number of different cytokines that were potentially capable of triggering immune responses. Some of these cytokines were associated with T-cell activation (IL-2, IL-4, IL-12), whereas others were associated with inflammation (TNF- α , IL-6) or had pleiotropic effects (GM-CSF). To induce airway expression of these cytokines, adenoviral vectors carrying the corresponding genes were introduced intranasally into mice. One day after the gene transfer, ovalbumin aerosolizations were initiated. Of these cytokines, ovalbumin-specific responses were generated only when GM-CSF was expressed in the airways (Table I).¹¹ We performed an extensive characterization of this model and demonstrated the expression of ovalbumin-specific IgE, T_{H2} cytokines, eosinophilic airway inflammation, and long-term antigen-specific memory.¹¹ With the use of knock-out mice, it was shown that T_H cells and IL-5 were required for the immune-inflammatory response. None of the other cytokines that were tested were able to create conditions under which an immune-inflammatory response against ovalbumin could be generated. Notably, although it has been suggested that expression of IL-4 in the airways may be responsible for the generation of T_{H2} responses to aeroallergens, our data clearly show that the expression of IL-4 alone is insufficient.

POLARIZATION: IL-4 AND IL-12 AND THE IMPORTANCE OF THE AIRWAY MICROENVIRONMENT

The expression of GM-CSF allowed for the development of sensitization to ovalbumin, which appeared to be T_H2 -like in character. Hence, we investigated whether concurrent expression of IL-4 in the airways could further polarize the immune-inflammatory response in the T_H2 direction and whether IL-12 expression could divert this response to the T_H1 phenotype. Adenoviral vectors carrying the IL-4 or IL-12 genes were delivered concurrently with the GM-CSF vector to achieve airway expression of these cytokines during sensitization to ovalbumin. Coexpression of IL-4 and GM-CSF in the airway had the predicted effect of enhancing the eosinophilic inflammatory response (personal observation). This finding was particularly informative, given that IL-4 per se failed to signal the development of immune responses to ovalbumin. The addition of IL-12 to the airway milieu during sensitization had a dramatic effect on the immune-inflammatory response. Although the total level of inflammation was the same in groups that expressed GM-CSF and GM-CSF with IL-12, the nature of the response was very different. Whereas eosinophilia was manifest in the GM-CSF group, eosinophils were completely abrogated in the group that coexpressed IL-12 and GM-CSF. Further, in the group expressing IL-12 and GM-CSF, eosinophilia was replaced with a mononuclear-neutrophilic inflammatory response.¹³ Also, the addition of IL-12 abolished expression of the T_H2 cytokines. The effects of IL-12 and IL-4 persisted long after adenovirally mediated cytokine secretion had ceased, as evidenced by recapitulation of the altered phenotype in IL-4- and IL-12-treated mice 40 days after the initial sensitization.

RESTORATION: IL-10 AND REGULATION OF INFLAMMATION

Because IL-10 demonstrates potent antiinflammatory and immunoregulatory activity,¹⁷⁻²⁰ we investigated the ability of this cytokine to counter the proallergic effects of GM-CSF and restore homeostasis in the lung. Compared with mice that expressed GM-CSF alone, mice that also expressed IL-10 in their airways during ovalbumin exposure demonstrated approximately 80% reduction in eosinophils in the BAL, with concurrent reductions in the BAL levels of IL-4, IL-5, and TNF- α .¹² Moreover, the effect of IL-10 was long term; when rechallenged 40 days after sensitization (ie, after IL-10 expression had ceased), the secondary inflammatory response was considerably attenuated compared with mice that had not received IL-10 during sensitization. It must be emphasized that unlike IL-12, which diverted the inflammatory response away from eosinophilia but maintained the same level of total inflammation, IL-10 inhibited the overall inflammatory response as well as eosinophilia, which may be an important consideration in the development of cytokine-

mediated therapy for allergic diseases such as asthma. An examination of T cells revealed that their expansion and expression of activation markers was not changed by IL-10 treatment. Groux et al²¹ have shown that activation of human and murine CD4⁺ T cells in the presence of IL-10 in vitro leads to the generation of a regulatory T-cell subset that actively suppresses antigen-specific immune responses in vivo. In this context, it is plausible that the expression of IL-10 in vivo during GM-CSF-driven allergic mucosal sensitization promoted the development of a T-cell subset that has regulatory/suppressive activities.

SUMMARY

It is clear that the status of the immune system determines whether an immune-inflammatory response will be elicited against a given antigen and, if so, the nature of the ensuing response. Although we can continue to regard the development of allergy as the outcome of an interaction between a single antigen and the immune system, attempting to understand exposure to an aeroallergen in isolation may be a misleading simplification. It is not only the antigens to which we are exposed, but also the conditions of the airways under which that exposure takes place, that ultimately determine the immunologic outcome of aeroallergen exposure. Moreover, with every inhalation, we are exposed to a complex mix of aeroallergens, chemicals, pathogens, pollutants, and innumerable other particles. The immune system confronts this eclectic collection of entities in concert, and the signals evoked by each element potentially influence the immune response to all of the others, directly or indirectly. Our experimental work on the cytokine microenvironment of the airways borrows from and elaborates on these concepts. Because we have used adenovirally-mediated gene transfer to directly initiate the expression of cytokines in the airway, we speculate that the natural triggers of cytokine expression would have comparable repercussions. For example, the induction of GM-CSF expression by diesel exhaust particles^{22,23} could precipitate immune responses to house dust mite that would have otherwise elicited tolerance. Additionally, IL-12 production elicited by *Mycobacterium tuberculosis* could generate T_H1 responses that would prevail over a T_H2 response against cat dander.

We have shown that airway expression of GM-CSF, alone or in conjunction with IL-12 or IL-10, elicits divergent immune-inflammatory responses to the same antigen. Whether these immune responses are the consequence of these cytokines providing discrete instructions to APCs is a likely, but still unproved, hypothesis; one in which our laboratory and others are actively investigating. We suggest that if we are to fully grasp the development and perpetuation of allergic disease, we must mechanistically dissect its cellular and molecular basis, the crux of which is the tripartite interaction between antigen, APC, and T cell. Fortunately, extensive experimental evidence for the fundamental roles played by various cells, molecules, and cytokines has been

accumulated over the past decade. More recently, there has been increasing focus on the interactions between the innate and adaptive arms of the immune response in the handling of foreign antigens. A further challenge before us is to translate this knowledge into novel therapeutic practice.

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