[Frontiers in Bioscience 8, e147-156, January 1, 2003]

CYTOKINES AND CHEMOKINES IN ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS (ABPA) AND EXPERIMENTAL ASPERGILLUS-INDUCED ALLERGIC AIRWAY OR ASTHMATIC DISEASE

Cory M. Hogaboam, Kate Blease, and Jane M. Schuh

Department of Pathology, University of Michigan Medical School, 1301 Catherine Road, Ann Arbor, MI 48109-0602

TABLE OF CONTENTS

1. ABSTRACT

Allergic bronchopulmonary aspergillosis (ABPA) is a devastating clinical disease that results from an aggressive pulmonary allergic response to the antigens released by colonizing *Aspergillus fumigatus* (*A. fumigatus*) in the respiratory system. Many of the allergic features of clinical ABPA have been reproduced in murine models, thereby facilitating a detailed analysis of the inflammatory and immune events that surround the initiation and maintenance of this disease. Herein, we describe the involvement of cytokines and chemokines in murine allergic pulmonary disease elicited by *A. fumigatus* antigens and spores (or conidia). More importantly, data derived from murine models of Aspergillus-induced allergic airway disease or asthma also suggest that the specific targeting of cytokines and/or chemokines may provide a novel therapeutic strategy in the treatment of clinical ABPA.

2. INTRODUCTION

2.1. Allergic bronchopulmonary aspergillosis

Aspergillus fumigatus is unique among the members of its genus because this ubiquitous fungus can cause multiple pulmonary diseases that reflect both the immunological status of the patient and the pre-existing integrity of the lung (1). *A. fumigatus* represents a relatively minor threat to non-allergic or immunocompetent individuals, but it poses significant problems in people that are atopic or immunocompromised. Several clinical disorders associated with this fungus have been described including: allergic disease, airway colonization, invasive or infective aspergillosis, and pneumonitis. These various manifestations of Aspergillus-induced lung disease are demarcated based on unique clinical, radiological, and immunological characteristics; and therapies are administered accordingly (1-4). This review focuses on the allergic diseases due to *A. fumigatus*. These diseases are often collectively referred to as ABPA in humans (1) and allergic aspergillosis (5) or fungal asthma in rodents (6). In patients with asthma or cystic fibrosis, ABPA is a debilitating pulmonary complication with major ramifications on pulmonary function and can ultimately be fatal (7). ABPA is routinely under-diagnosed particularly in asthmatics; the prevalence of ABPA has been estimated at 25% in this population (8). The underlying mechanism through which *A. fumigatus* induces allergic airway disease is not presently known, but it is speculated that conidia and mycelium from this fungus persist long enough in the airways to release potent antigens and/or disrupt the lung architecture such that an immune response is invoked (9). Sensitization to fungi is relatively high among children and declines rapidly with increasing age presumably reflecting the fact that children may lack or have inefficient immune

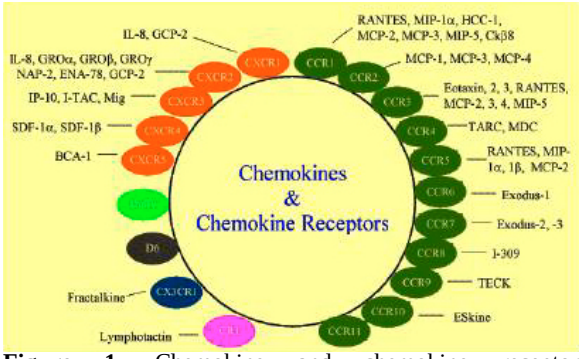

Figure 1. Chemokine and chemokine receptor nomenclature.

mechanisms necessary for clearing fungi from the airways (9). The systemic immune response associated with ABPA is characterized by systemic changes in IgE, IgG, and T helper 2 (Th2) cell cytokine profiles. In the lung, ABPA is distinguished by an intense eosinophilic airway inflammation and the formation of mucus plugs. Without early diagnosis and treatment, ABPA can progress to bronchiectasis and pulmonary fibrosis (10).

2.2. Cytokines and chemokines

Cytokines are small, mainly soluble proteins that have potent effects on the activation of leukocytes and structural cells during inflammatory and immune events. Examples of immunomodulatory cytokines include interleukin-1beta (IL-1beta), tumor necrosis factor alpha (TNF-alpha), IL-6, IL-12, IL-18, and IL-10. The discussion of these immunomodulatory cytokines is germane to the discussion of ABPA given that they elicit a number of critical cellular events pertinent to the initiation of inflammatory and immune events directed against *A. fumigatus*. Most of the immunomodulatory cytokines have global effects on immune cells including the induction of selectins and addressins necessary for leukocyte extravasation into sites of injury or pathogen challenge. These cytokines also elicit or regulate the potent cellular events that facilitate the destruction of inciting pathogens. These events include free radical generation, protease release, vasoactive peptide, and arachidonic acid metabolite generation, and phagocytic activation. More recent studies have shown that these cytokines also affect the expression of leukocyte pathogen recognition molecules that are necessary for detecting the presence of pathogens and responding accordingly. Within the cytokine superfamily, a unique family of small proteins (the majority of which are 8-10 kDa in size) has come to be known as chemotactic cytokines or chemokines. The distinctive feature of chemokines is their ability to elicit the directed movement of leukocytes from the vasculature to sites of inflammation or infection. However, it is now appreciated that chemokines, like other cytokines, provoke a plethora of effects including angiogenesis, hematopoiesis, and organogenesis (11). The nomenclature for chemokines differs from that used for most other cytokines and reflects the unique positioning of cysteine residues at the amino terminus of these molecules. The two largest chemokine groups encompass the C-C and the C-X-C chemokines. This review will focus on the relative importance of C-C chemokines such as monocyte chemoattractant protein-1 (MCP-1/CCL2); regulated on T-cell activation, normal T cell expressed and secreted (RANTES/CCL5); and C-X-C chemokines such as IL-8/CXCL8 in the pulmonary allergic response to *A. fumigatus*. Two other groups of chemokines have been identified but only a single member has been identified in each. These two groups include lymphotactin, a C chemokine lacking the first and third cysteine but structurally homologous to the C-C chemokines, and fractalkine (12), a membrane-bound C-X_3-C chemokine(13). Chemokine nomenclature has been standardized to mirror the sequential classification scheme presently used for the chemokine receptors. C-C ligands are CCLs, C-X-C ligands are CXCLs, the C ligand is XCL, and the C-X_3-C ligand is CX3CL thereby eliminating the confusion that surrounds the use of multiple names for a single chemokine (14). C-C chemokines interact with at least 11 distinct receptors designated CCR1 through CCR11, whereas C-X-C chemokines interact with a minimum of 5 receptors (15) (see Figure 1). The following chemokine ligand-receptor interactions are relevant to the discourse of ABPA: MCP-1/CCL2 binds CCR2 (16); RANTES/CCL5 binds CCR1, CCR3, and CCR5 (17); and IL-8/CXCL8 binds CXCR2. Although the promiscuity of chemokine receptors appears to obscure the relative importance of each chemokine in immune responses (Figure 1), the selectivity of chemokine effects in these responses is presumably strictly regulated at the level of chemokine receptor expression and the cell distribution of these receptors. Whereas this review will focus primarily on the role of cytokines and chemokines on the development and maintenance of allergic responses to *A. fumigatus*, it should be noted that these factors have also been examined in the context of immunocompetent and immunocompromised hosts (i.e. invasive aspergillosis) that lack allergic sensitivity to Aspergillus. Discussion of these aspects of cytokine and chemokine biology during immune responses to Aspergillus may be assessed elsewhere in this issue.

2.3. Allergic inflammation: The aggressive Th2-type response

The development of allergic responses to antigenic stimuli involves a concerted cascade of events in which cytokines play a major role. Early examination of the cytokines generated during T cell responses to distinct antigens revealed that T cells could be divided into subsets based upon production of interferon-gamma (Th1) or IL-4 (Th2) (18). It is now appreciated that Th1 and Th2 responses involve the cytokine-generating capacities of other immune cells besides T cells. For example, natural killer cells are an important source of IFN-gamma while mast cells can generate IL-4 (19). In addition, macrophage-derived IL-12 and IL-10 have recently been shown to exert prominent cross-regulatory effects on Th cell cytokine profiles, and thus have also been included in the Th cytokine profiles of Th1-type and Th2-type, respectively. Considerable research has been directed towards elucidating the manner in which the allergic Th2-type response develops in deference to the innate Th1-type response. Numerous studies have shown that a deficiency

in cytokines such as IL-4 and IL-13 severely impair the development of Th2-type responses and favor the development or maintenance of a Th1-type response (20). The relative importance of the Th2-type response in protecting the host from pathogens has been debated, but it is clear that the production of mucus can serve as an effective way to clear some pathogens from mucosal surfaces (21). In addition, the tissue repair process, characterized by cell proliferation and matrix deposition, is absolutely required to counteract the frequently destructive effects of a Th1-type response in the tissues. Given the importance of both immune responses to the host, it is not surprising that Th1- and Th2-type cytokines possess cross-regulatory properties, which appear to ensure that an immune response is appropriately balanced to prevent excess tissue injury or tissue repair (22).

3. CYTOKINES IN ABPA

3.1. Cellular sources of cytokines and chemokines in ABPA

The manner in which cytokines participate in the inflammatory response elicited by *A. fumigatus* remains the focus of both clinical and basic research. The airway inflammation initially elicited by *A. fumigatus* is characterized by a dramatic influx of blood-borne neutrophils, eosinophils, and T cells, suggesting that this fungus elicits an inflammatory response in the lung that subsequently promotes the recruitment of these immune cells (6). *In vitro* studies have shown that the pulmonary epithelium plays an integral role in obtaining leukocyte recruitment following *A. fumigatus* exposure. These studies revealed that fungal proteases promote the release of proinflammatory cytokines, such as IL-6, and chemokines, such as IL-8/CXCL8 and MCP-1/CCL2 (23) (24) (25). The effect of *A. fumigatus* proteases on the production of IL-8/CXCL8 by epithelial cells has clear implications on the movement of neutrophils into the lung, whereas increased generation of MCP-1/CCL2 presumably promotes the directed migration of mononuclear and lymphocytic cells. In addition, proteases released by this fungus are directly toxic to pulmonary epithelial cells causing cell detachment and death (23) (25), events that further promote the influx of inflammatory cells. Other potential cellular sources of cytokines and chemokines in the lung during immune responses to Aspergillus are the alveolar macrophages. These cells appear to be an excellent source of cytokines, such as TNF-α, and chemokines, such as IL-8/CXCL8, C10/CCL6, and MCP-1/CCL2. The manner in which *A. fumigatus* stimulates cytokine and chemokine production by alveolar macrophages during allergic disease is not presently known, but we are currently exploring the relative contribution of pattern recognition molecules such as toll-like receptor-2 (TLR2) in this response (26). TLR2 mRNA levels are negligible in lung tissue from *A. fumigatus*-sensitized mice, but present in abundance in lungs from non-sensitized controls. This discrepancy appears to be specific to TLR2 since another toll-receptor, TLR4, is not affected by the allergic sensitization to Aspergillus or the development of chronic allergic disease. Thus, the activation of epithelial cells and alveolar macrophages by fungal proteases and antigens appears to be a critical initial event during the early development of allergic responses to *A. fumigatus.*

3.2. The Th1-type vs. Th2-type paradigm in ABPA

While it is appreciated that lung resident cells and recruited neutrophils respond to *A. fumigatus* and initiate an inflammatory response that is necessary for containing and killing this fungus, the predominant culprits in the maintenance, or chronicity, of *A. fumigatus*-induced allergic disease are eosinophils and T cells. Unlike individuals that develop invasive Aspergillosis, the immune response invoked in ABPA patients appears to control, but not entirely eliminate, this fungus. The nature of the Th2-type response exhibited in patients with ABPA is believed to explain this dichotomy (27). Independent studies have shown that T cells from patients with ABPA secreted significantly greater amounts of IL-4 and IL-5 than IFN-gamma and IL-2 in response to *A. fumigatus* antigenic stimulation (28,29). Similar findings were shown in cystic fibrosis patients with ABPA (30). The imbalance in cytokine generation by T cells from ABPA patients appears to stem from their responsiveness to major *A. fumigatus* antigens such as Asp f1 and Asp f2. In Asp f1-specific T cell lines, proliferation was IL-4-, but not IL-2, -dependent; and the predominant cytokine generated by these cells was IL-4 (28). T cell clones from ABPA patients robustly proliferate when challenged with Asp f2 or an epitope from the N-terminal region of Asp f2 and generate large quantities of IL-5 (31). B cells from ABPA patients are also more sensitive to IL-4 compared with atopic and non-atopic patient controls as evidenced by the upregulation of CD23 and CD86 (32). Together, these clinical studies have shown that the excessive production of and response to Th2-type cytokines is a major immune defect in ABPA, but the relative importance of this immune deviation in the clinical progression of ABPA has yet to be elucidated.

4. CYTOKINES AND CHEMOKINES IN ASPERGILLUS-INDUCED EXPERIMENTAL ALLERGIC AIRWAY OR ASTHMATIC DISEASE

The experimental investigation of the relative importance of cytokines in the lung pathology caused by *A. fumigatus* has relied upon the development of a number of mouse models. Various forms of *A. fumigatus*-induced lung disease can be partly recapitulated in mice, however, no mouse model precisely mirrors the various clinical conditions associated with this fungus (33). Three major caveats of *A. fumigatus*-induced allergic airway or asthmatic disease models are as follows: 1) murine allergic response to Aspergillus may differ from that observed in humans, 2) murine models of allergic lung disease are initiated by short-term exposure to relatively high amounts of *A. fumigatus* allergens or conidia in contrast to patients who are chronically exposed to relatively small numbers of conidia, and 3) allergic murine models fail to show the colonization of Aspergillus in the bronchioles typically observed clinically (33). Nonetheless, mice have proved to be exceptional tools in the study of cytokine and chemokine involvement of experimental invasive aspergillosis (34) as well as acute (5, 35) and chronic (6) forms of allergic or asthmatic airway disease.

4.1. Immunomodulatory cytokines

Through the study of various models of *A. fumigatus*-induced lung injury, a temporal picture of cytokine networking in the lung following exposure to *A. fumigatus* has emerged. First, in the context of allergic responses to instilled *A. fumigatus* antigens or conidia, it is now apparent that Th1-type cytokines, such as IL-12 and IL-18, are induced quickly in the allergic lung (6, 26). Immunoneutralization of IL-18 alone (26) clearly resulted in a marked increase in both the growth and persistence of *A. fumigatus* in the lungs of allergic mice. Second, it is apparent that endogenous IL-10 is absolutely required for regulating both the innate Th1-type response against *A. fumigatus* and the development of allergic disease. Interestingly, IL-10 levels were significantly decreased early (i.e. at day 3) but not at later time points after the introduction of conidia into the lungs of *A. fumigatus*-sensitized mice (6). Additional studies have revealed that mice lacking IL-10 due to gene deletion exhibited the exaggerated production of Th1-type (IFN-gamma) and Th2-type (IL-4 and IL-5) cytokines compared with wild-type mice during allergic responses to *A. fumigatus* (36). Pre-sensitized IL-10-/- mice exposed to *A. fumigatus* also exhibited a 50-60% mortality rate contrasting sharply with similarly sensitized and challenged wild-type mice (36). These studies showed that IL-10 dampens both the Th1-type and Th2-type responses associated with allergic responses to *A. fumigatus*. Thus, immunomodulatory cytokines such as IL-12, IL-18, and IL-10 have marked effects on the course and severity of experimental *A. fumigatus*-induced allergic airway disease. Given the major contribution that these mediators exert on the allergic response to *A. fumigatus* in experimental models, it is possible that modulation of one or all of these cytokines may represent a viable treatment option for clinical ABPA.

4.2. Balancing Th1-type and Th2-type cytokines

The overall balance of Th1-type and Th2-type cytokines is critical for mounting an appropriate anti-Aspergillus immune response without promoting debilitating allergic responses to the fungus. As discussed previously, attenuation or modulation of IL-12, IL-18, or IL-10 is problematic because of the global effects of these cytokines on the immune response (36, 37). However, the specific manipulation of the Th2-type response during allergic disease due to *A. fumigatus* has produced promising results, particularly as it relates to attenuating acute and chronic allergic airway disease without affecting innate immune responses. For example, the treatment of *A. fumigatus*-sensitized mice with neutralizing anti-IL-4 antibody prior to and after *A. fumigatus* antigen (5,38,39) or conidia (40) challenge or the sensitization and exposure of IL-4-/- mice (38,41) to *A. fumigatus* antigens revealed a critical role for IL-4 in the development and maintenance of IgE, eosinophilia, and airway hyperresponsiveness in these models. In contrast, anti-IL-5 antibody therapy in *A. fumigatus*-sensitized and challenged mice (5, 42,43) or sensitization and exposure of IL-5-/- mice (38) to *A. fumigatus* antigens revealed a more limited role for IL-5 in the eosinophilic response to *A. fumigatus.* Another Th2-type cytokine, IL-13, has been more recently studied in the context of chronic *A. fumigatus*-induced allergic airway disease and has been shown to be the major instigator of airway inflammatory responses (characterized by eosinophils and T cells), airway hyperreactivity, and airway remodeling (i.e. goblet cell hyperplasia and peribronchial fibrosis) (40, 44, 45). Further, neutralizing IL-13 with polyclonal antibodies (40) or killing IL-13-responsive cells with a chimeric protein comprised of human IL-13 and Pseudomonas exotoxin A (IL13-PE) (44, 45) did not prevent the clearance of *A. fumigatus* conidia from the airways of *A. fumigatus*-sensitized mice. Continued discussion of this chronic model of fungal asthma and the therapeutic efficacy of IL13-PE is provided below.

4.3. Chemokines and chemokine receptors

The relative role of chemokines in the initiation and maintenance of clinical ABPA has yet to be explored, but emerging experimental data illustrates the major roles these proteins perform in several aspects of the allergic response to *A. fumigatus*. Acute models of Aspergillus-induced allergic airway disease have revealed the relative importance of chemokines such as RANTES/CCL5 (46), C10/CCL6 (35), eotaxin/CCL11 (47), and eotaxin-2/CCL24 (48) in the recruitment of eosinophils and the development of airway hyperreactivity. With the development of a chronic model of fungal asthma that continues over days after the introduction of conidia into *A. fumigatus*-sensitized mice (6), we have characterized the role of chemokines and chemokine receptors in various aspects of the associated airway disease. This chronic model of *A. fumigatus*-induced airway inflammation exhibits the characteristic pulmonary phenotype of asthmatics, incorporating local and systemic allergic inflammation associated with a chronic pulmonary eosinophilia, elevated IgE levels, reversible airway obstruction, goblet cell hyperplasia, and peribronchial fibrosis (49). Given that live fungal conidia initiate the allergic disease, this model further lends itself to the exploration of aspects of chemokine biology that are required for an efficient anti-fungal response while avoiding the devastating consequences of acquired allergic responsiveness. At present, we have examined the contribution of CC chemokine receptors-1 (CCR1) (50), CCR2 (51), CXCR2 (52), and CCR5 (53) in the development and maintenance of chronic fungal asthma. The individual contribution of each receptor has been explored in the context of genetically altered mice that lack the appropriate chemokine receptor due to homologous recombination or gene knockout. These studies have presented several unique findings including the fact that, at least in vivo, chemokine receptors have defined, non-redundant roles in the innate, effector, and remodeling responses associated with allergic airway disease. For example, CCR1 through its interactions with macrophage inflammatory protein-1alpha (MIP-1alpha/CCL3) contributes to remodeling responses (i.e. goblet cell hyperplasia and peribronchial fibrosis) during chronic fungal asthma without any impact on the innate immune response and airway hyperresponsiveness (50). CCR2 and its major ligand monocyte chemoattractant protein-1 (MCP-1/CCL2) are critical in the clearance of fungal spores from the lungs of mice, and the absence of either ligand or receptor leads to exacerbated allergic disease (51,

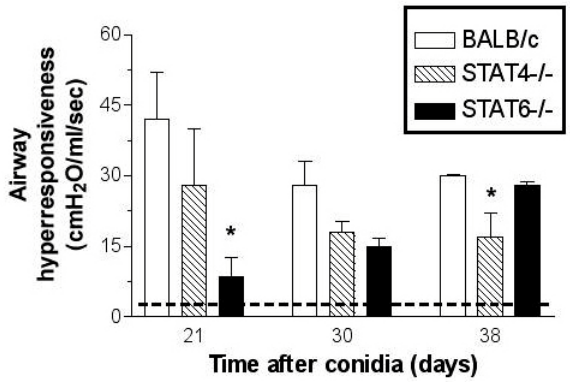

Figure 2. Airway hyperresponsiveness in A. fumigatus-sensitized BALB/c, Stat4-deficient (-/-) and Stat6$^{-/-}$ mice at various times after an A. fumigatus conidia challenge. Airway resistance (units = cm H2O/ml/sec) was calculated at each time point prior to (dashed line) and after methacholine (5µg; i.v.). Values are expressed as mean ± SE; n = 5/group/time point. *, P<0.05 demonstrates a significant difference in airway resistance between the wildtype and appropriate Stat-deficient groups.

54). However, once the fungal spores are eliminated, MCP-1/CCL2 and CCR2 are major contributors to airway hyperresponsiveness and airway remodeling associated with this model (54). CCR5 modulates the movement of eosinophils and T cells into the airways of *A. fumigatus*-sensitized mice challenged with conidia (53). Furthermore, CCR5 and its major ligand RANTES/CCL5 appear to inhibit the innate response by alveolar macrophages towards *A. fumigatus* conidia, to contribute to airway hyperresponsiveness, and to promote airway remodeling during chronic fungal asthma (53). Taken together, these studies have revealed the complex and specific roles of chemokines and chemokine receptors during fungal asthma and should lead to the identification of anti-chemokine strategies that ameliorate all features of chronic fungal asthma without compromising the innate immune response against *A. fumigatus* conidia.

5. PUTATIVE NOVEL CYTOKINE- AND CHEMOKINE-BASED THERAPIES FOR ABPA

Treatment for ABPA relies on corticosteroids to suppress eosinophilic airway inflammation and azole antifungal agents (i.e. itraconazole) to contain the growth of Aspergillus (8,55). These therapies, while not assessed adequately in well-designed clinical trials, provide marked beneficial effects but also present challenges that require constant patient monitoring due to potential toxic side effects (56). Given the shortfalls in current therapies, it is certainly appropriate to consider novel treatments for clinical ABPA, and it has been proposed that cytokines should be considered in the development of novel therapies for Aspergillus-induced lung diseases (57). Considerable progress has been made in the development of small molecule antagonists/inhibitors of cytokines and chemokines. Many small, orally bioactive molecules and specific antibodies are presently being tested in the treatment of ulcers, allergies, migraines, kidney disease, and schizophrenia (58,59). It is anticipated that these drugs will form the future foundation of therapeutics marketed by many pharmaceutical companies (60). What follows is a brief synopsis of three strategies that we are presently investigating as potential therapies for chronic allergic disease induced by *A. fumigatus.*

5.1. Targeting cytokine transcription factors: Stat6 and Stat4

Proteases secreted by *A. fumigatus* induce the production of cytokines (i.e. IL-6) and chemokines (i.e. IL-8) by epithelial cells through transcriptional induction of the respective genes (24). These findings and others highlighting the importance of transcription factors in cytokine biology were the impetus to examine in greater detail the manner in which transcriptional factors modulate the allergic response due to *A. fumigatus* conidia. We focused our initial efforts on the Stat (signal transducers and activators of transcription) family, which is comprised of seven distinct members that regulate signal transduction associated with distinct cytokines (61). Stats have also been shown to regulate chemokine production by cloned Th1 and Th2 cells (62). Employing mice with Stat4 or Stat6 gene deletions, we have been able to explore the impact of defects in IL-12 (63) and IL-4/IL-13 (64) signaling , respectively, during the course of chronic fungal asthma. When examined at day 21 after conidia challenge in *A. fumigatus*-sensitized wildtype (BALB/c), Stat4-/-, and Stat6-/- mice, it was apparent that the Stat6-/- mice had significantly decreased airway hyperreactivity compared with the other two groups of mice (45)(Figure 2). Subsequent to day 21, Stat6-/- mice appeared to gain airway hyperresponsiveness whereas this response in Stat4-/- mice declined (Figure 2). Although further investigation is warranted, these studies emphasize the complexity of transcriptional factor involvement in chronic fungal allergic responses, but it would appear that targeting of either Stat4 or Stat6 might provide a temporally dependent benefit in the treatment of allergic airway responses to *A. fumigatus*.

5.2. Targeting Th2-type cytokines and Th2-type cytokine-responsive cells with chimeric proteins

Clinical (65, 66) and experimental (67, 68) studies have revealed the prominent expression of the type 2 cytokine IL-13 by T cells, eosinophils, and activated mast cells in asthma and allergic airway disease. IL-13 shares a receptor component and signaling pathways with IL-4, including the alpha chain of the IL-4 receptor (69). The classical IL-4R is found on hematopoietic cells and consists of IL-4R (or alpha chain) and IL-2R (or gamma chain), whereas the alternative form of IL-4R is predominantly found on nonhematopoietic cells and consists of IL-4Ralpha and IL-13Ralpha1 chains. The alternative IL-4R can also recognize IL-13 and appears to be the major IL-13R in hematopoietic and nonhematopoietic cells (70). An additional receptor, IL-13Ralpha2, binds to IL-13 with 100-fold higher affinity than IL-13Ralpha1, but lacks the cytoplasmic domain for intracellular signaling (71). Furthermore, cells that respond to IL-13 are recognized as excellent sources of that same cytokine. These cells include activated B cells, basophils, and alveolar macrophages (72). The targeted pulmonary over-

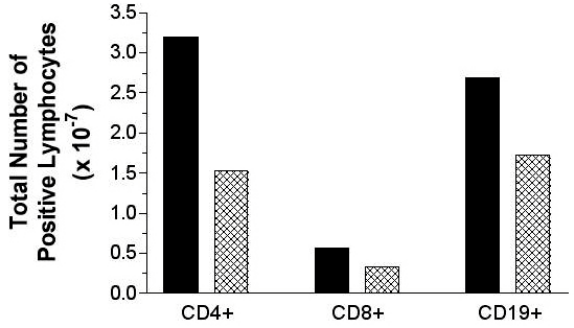

Figure 3. Flow cytometric analysis of CD^{4+}, CD^{8+}, and CD^{19+} cells at day 28 after conidia challenge. A. fumigatus-sensitized mice received diluent or 200 ng of IL13-PE every other day starting at day 14 and concluding at day 28 after a conidia challenge. Whole lungs from both groups were dispersed, stained, and analyzed by flow cytometry.

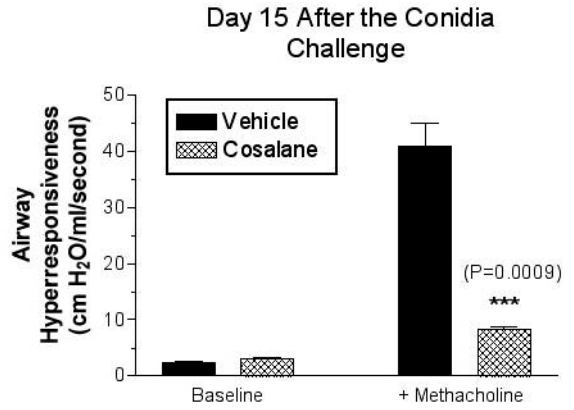

Figure 4. Airway hyperresponsiveness in untreated and Cosalane-treated A. fumigatus mice at day 15 after an A. fumigatus conidia challenge. In the Cosalane-treatment group, mice received 600 µg/kg of Cosalane orally every day beginning immediately after the conidia challenge and continuing until day 15. Airway resistance (units = cm H_2O/ml/sec) was calculated at each time point prior to (baseline) and after methacholine (5µg; i.v.). Values are expressed as mean ± SE; n = 5/group/time point. ***, P<0.001 demonstrates a significant difference in airway resistance between the control and Cosalane groups.

expression of IL-13 in mice promotes severe inflammatory and airway remodeling responses reminiscent of asthma and allergic airway disease such as a mononuclear and eosinophil inflammatory response, goblet cell hyperplasia, subepithelial fibrosis, and non-specific airway hyperresponsiveness (73). These observations have prompted the employment of treatment strategies that inhibit IL-13-mediated events during allergic airway diseases (74). Experimental studies have shown that the systemic administration of anti-IL-13 antibody (67, 75) or the IL-13 inhibitor, soluble IL-13 receptor alpha2-Fc (68), successfully abolishes the airway hyperresponsiveness and remodeling associated with allergic airway disease. Given that the number of IL-13 producing cells are markedly increased during the course of airway inflammation associated with asthma and allergy, maintenance of adequate IL-13 immunoneutralization may be problematic in these chronic diseases, as we have observed in mice during chronic fungal asthma (40). Our more recent approach to targeting IL-13 in this model of fungal asthma has involved the use of a chimeric protein comprised of IL-13 and a mutated form of *Pseudomonas* exotoxin (IL-13-PE38QQR or IL13-PE). Dr. Raj Puri (Laboratory of Molecular Tumor Biology, FDA) and his group created IL13-PE and have used this fusion protein to selectively target and eradicate solid tumor cells with endogenous (76, 77) and induced (78) IL-13 receptor expression. IL13-PE binds specifically to IL-13-responsive cells, and the incorporation of cytokine-exotoxin then leads to cell death. In our studies with this chimeric protein, we have observed that its intranasal delivery after the establishment of chronic fungal asthma significantly attenuated all the Th2-mediated features of this model (44, 45). Flow-cytometric analysis of whole lungs from IL13-PE-treated mice showed that this protein significantly reduced the numbers of CD4+, CD8+, and CD19+ cells compared with untreated controls (Figure 3). Thus, the targeting of IL-13-responsive cells during the course of chronic fungal asthma provided a marked therapeutic effect that warrants additional studies.

5.3. Targeting Chemokines: The anti-RANTES/CCL5 drug cosalane has protective and therapeutic effects in chronic fungal asthma

Since the discovery that RANTES/CCL5 and other CCR5 agonists inhibit HIV infection (79), the identification of novel inhibitors and antagonists of various chemokines and their receptors has accelerated tremendously. For example, the anti-HIV agent Cosalane (developed by Dr. Mark Cushman at Purdue University) is a novel drug comprised of aurintricarboxylic acid (ATA) and a steroid molecule. Howard and colleagues (80) have recently shown that Cosalane inhibited RANTES/CCL5-induced migration of human monocytes, but it did not inhibit monocyte migration induced by MIP-1alpha/CCL3 or MIP-1beta/CCL4. Cosalane also modulated RANTES/CCL5-induced migration of single receptor CCR1- and CCR5-HEK transfectants. Howard et al. (80) also showed that the acetylation of the reactive amino groups of RANTES/CCL5 abrogated the inhibitory activity of Cosalane signifying that this compound binds directly to RANTES/CCL5, thereby inhibiting its ability to bind to its receptors. In preliminary studies, we have found that orally delivered Cosalane has protective and therapeutic effects in the chronic fungal asthma model. In these studies, we examined the effects of Cosalane delivered orally at 600 µg/kg daily to *A. fumigatus*-sensitized mice for the first 15 days of the conidia challenge or from days 15 to 30 after the conidia challenge. Control groups received the vehicle (3.5% DMSO in PBS) for Cosalane. Airway hyperresponsiveness in *A. fumigatus*-sensitized mice at day 15 after the conidia challenge is shown in Figure 4. A marked and significant decrease in airway hyperresponsiveness was observed in the Cosalane-treated group compared with the controls. When the Cosalane treatment was given from days 15-30 after the conidia challenge, airway hyperresponsiveness was reduced by approximately 45%. Significant reductions in eosinophil numbers were observed in both early and late Cosalane

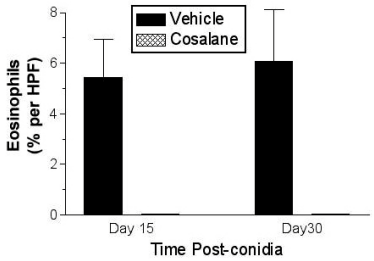

Figure 5. Airway eosinophil counts in untreated and Cosalane-treated A. fumigatus mice at day 15 after an A. fumigatus conidia challenge. In the Cosalane-treatment group, mice received 600 μg/kg of Cosalane orally every day beginning immediately after the conidia challenge and continuing until day 15, or from day 15 until day 30 after conidia.

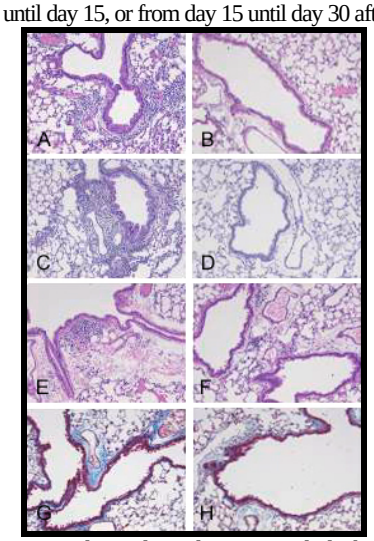

Figure 6. Histological analysis revealed that Cosalane treatment (600 μg/kg p.o.) from days 0-15 reduced the peribronchial inflammation (Fig. 6B) and goblet cell hyperplasia (Fig. 6D; magenta-stained cells) compared with the vehicle (shown in Fig. 6A & C, respectively). Cosalane treatment from days15-30 reduced peribronchial inflammation (Fig. 6F) and fibrosis (Fig. 6D; blue material) compared with the vehicle group (Fig 6E & G, respectively).

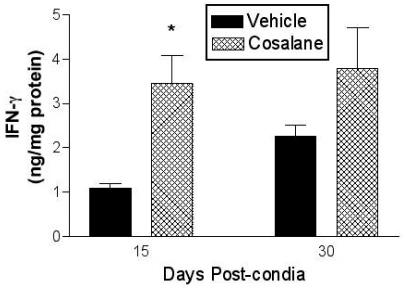

Figure 7. Both Cosalane treatment protocols (days 0-15 and days 15-30) showed increased whole lung IFN-γ levels. In the Cosalane-treatment groups, mice received 600 μg/kg of Cosalane orally every day beginning immediately after the conidia challenge and continuing until day 15, or from day 15 to 30 after conidia. *, $P<0.05$ demonstrates a significant difference in IFN-γ levels between the untreated and Cosalane-treated groups.

treatment groups (Figure 5). Histological analysis revealed that Cosalane treatment from days 0-15 reduced peribronchial inflammation (Figure 6B) and goblet cell hyperplasia (Figure 6D; magenta-stained cells) compared with the vehicle (shown in Figure 6A & C, respectively). Cosalane treatment from days 15-30 reduced peribronchial inflammation (Figure 6B) and fibrosis (Figure 6D; blue material) compared with the vehicle group (Fig 6A & C, respectively). Whole lung levels of IFN-gamma are shown in Figure 7. It was apparent that the oral Cosalane treatment significantly increased whole lung levels of IFN-gamma at day 15 and 30. Thus, Cosalane had protective and therapeutic effects during chronic fungal asthma, which suggest that RANTES/CCL5 has a major role in this disease model.

6. CONCLUDING REMARKS

Both anecdotal and clinical data indicate that individuals in industrialized countries are at growing risk of developing fungus-related allergic diseases like ABPA. The reasons for this trend are unknown at present, but it provides incentive to explore in greater detail the immune and inflammatory events that develop in the pulmonary system due to *A. fumigatus*. The recent discovery that cytokines and chemokines are major participants in the allergic events elicited by *A. fumigatus* indicates that clinical therapies for ABPA may best be directed at regulating the production and/or actions of these soluble proteins in the lung.

7. ACKNOWLEDGEMENTS

We thank Dr. Puri for his ongoing collaboration in studies assessing the effects of IL13-PE on Th2-type lung diseases and Drs. Howard and Cushman for their collaboration in the Cosalane therapy studies.

8. REFERENCES

1. Denning D. W.: Chronic forms of pulmonary aspergillosis. *Clin Microbiol Infect* 7 Suppl 2, 25-31 (2001)
2. Sharma O. P., R. Chwogule: Many faces of pulmonary aspergillosis. *Eur Respir J* 12, 705-715 (1998)
3. Franquet T., N. L. Muller, A. Gimenez, P. Guembe, J. de La Torre, S. Bague: Spectrum of pulmonary aspergillosis: histologic, clinical, and radiologic findings. *Radiographics* 21, 825-837 (2001)
4. Daly P., K. Kavanagh: Pulmonary aspergillosis: clinical presentation, diagnosis and therapy. *Br J Biomed Sci* 58, 197-205 (2001)
5. Kurup V. P., H. Choi, P. S. Murali, R. L. Coffman: IgE and eosinophil regulation in a murine model of allergic aspergillosis. *J Leukoc Biol* 56, 593-598 (1994)
6. Hogaboam C. M., K. Blease, B. Mehrad, M. L. Steinhauser, T. J. Standiford, S. L. Kunkel, N. W. Lukacs: Chronic airway hyperreactivity, goblet cell hyperplasia, and peribronchial fibrosis during allergic airway disease induced by Aspergillus fumigatus. *Am J Pathol* 156, 723-732 (2000)
7. Hanley-Lopez J., L. T. Clement: Allergic bronchopulmonary aspergillosis in cystic fibrosis. *Curr Opin Pulm Med* 6, 540-544 (2000)

8. Wark P. A., P. G. Gibson: Allergic bronchopulmonary aspergillosis: new concepts of pathogenesis and treatment. *Respirology* 6, 1-7 (2001)
9. Kauffman H. F., J. F. Tomee, T. S. van der Werf, J. G. de Monchy, G. K. Koeter: Review of fungus-induced asthmatic reactions. *Am J Respir Crit Care Med* 151, 2109-2115; discussion 2116 (1995)
10. Vlahakis N. E., T. R. Aksamit: Diagnosis and treatment of allergic bronchopulmonary aspergillosis. *Mayo Clin Proc* 76, 930-938 (2001)
11. Taub D. D.: Chemokine-leukocyte interactions. The voodoo that they do so well. *Cytokine Growth Factor Rev* 7, 355-376 (1996)
12. Kelner G. S., J. Kennedy, K. B. Bacon, S. Kleyensteuber, D. A. Lagaespada, N. A. Jenkins, N. G. Copeland, J. F. Bazan, K. W. Moore, T. J. Schall, A. Zlotnik: Lymphotactin: a cytokine that represents a new class of chemokine. *Science* 266, 1395. (1994)
13. Pan Y., C. Lloyd, H. Zhou, S. Dolich, J. Deeds, J. A. Gonzalo, J. Vath, M. Gosselin, J. Ma, B. Dussault, E. Woolf, G. Alperin, J. Culpepper, J. C. Gutierrez-Ramos, D. Gearing: Neurotactin, a membrane-anchored chemokine upregulated in brain inflammation. *Nature* 387, 611-617. (1997)
14. Zlotnik A., O. Yoshie: Chemokines: a new classification system and their role in immunity. *Immunity* 12, 121-127 (2000)
15. Premack B. A., T. J. Schall: Chemokine receptors: gateways to inflammation and infection. *Nature Med.* 2, 1174-1178. (1996)
16. Frade J. M., M. Mellado, G. del Real, J. C. Gutierrez-Ramos, P. Lind, A. C. Martinez: Characterization of the CCR2 chemokine receptor: functional CCR2 receptor expression in B cells. *J Immunol* 159, 5576-5584 (1997)
17. Raport C. J., J. Gosling, V. L. Schweickart, P. W. Gray, I. F. Charo: Molecular cloning and functional characterization of a novel human CC chemokine receptor (CCR5) for RANTES, MIP-1beta, and MIP-1alpha. *J Biol Chem* 271, 17161-17166 (1996)
18. Mosmann T. R., H. Cherwinski, e. a. M.W. Bond MW: Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 136, 2348-2357 (1986)
19. Plaut M., J. H. Pierce, C. J. Watson, J. Hanley-Hyde, R. P. Nordan, W. E. Paul: Mast cell lines produce lymphokines in response to crosslinking FcER1 or to calcium ionophores. *Nature* 339, 64 (1989)
20. McKenzie G. J., A. Bancroft, R. K. Grencis, A. N. McKenzie: A distinct role for interleukin-13 in Th2-cell-mediated immune responses. *Curr Biol* 8, 339-342 (1998)
21. Finkelman F. D., J. F. Urban, Jr.: The other side of the coin: the protective role of the TH2 cytokines. *J Allergy Clin Immunol* 107, 772-780. (2001)
22. Mosmann T. R., K. W. Moore: The role of IL-10 in crossregulation of Th1 and Th2 responses. *Immunol Today* 12, 49-53. (1991)
23. Tomee J. F., A. T. Wierenga, P. S. Hiemstra, H. K. Kauffman: Proteases from Aspergillus fumigatus induce release of proinflammatory cytokines and cell detachment in airway epithelial cell lines. *J Infect Dis* 176, 300-303 (1997)
24. Borger P., G. H. Koeter, J. A. Timmerman, E. Vellenga, J. F. Tomee, H. F. Kauffman: Proteases from Aspergillus fumigatus induce interleukin (IL)-6 and IL-8 production in airway epithelial cell lines by transcriptional mechanisms. *J Infect Dis* 180, 1267-1274 (1999)
25. Kauffman H. F., J. F. Tomee, M. A. van de Riet, A. J. Timmerman, P. Borger: Protease-dependent activation of epithelial cells by fungal allergens leads to morphologic changes and cytokine production. *J Allergy Clin Immunol* 105, 1185-1193 (2000)
26. Blease K., S. L. Kunkel, C. M. Hogaboam: IL-18 modulates chronic fungal asthma in a murine model; putative involvement of Toll-like receptor-2. *Inflamm Res* 50, 552-560 (2001)
27. Schuyler M.: The Th1/Th2 paradigm in allergic bronchopulmonary aspergillosis. *J Lab Clin Med* 131, 194-196 (1998)
28. Knutsen A. P., K. R. Mueller, A. D. Levine, B. Chouhan, P. S. Hutcheson, R. G. Slavin: Asp f I CD4+ TH2-like T-cell lines in allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol* 94, 215-221 (1994)
29. Walker C., W. Bauer, R. K. Braun, G. Menz, P. Braun, F. Schwarz, T. T. Hansel, B. Villiger: Activated T cells and cytokines in bronchoalveolar lavages from patients with various lung diseases associated with eosinophilia. *Am J Respir Crit Care Med* 150, 1038-1048 (1994)
30. Skov M., L. K. Poulsen, C. Koch: Increased antigen-specific Th-2 response in allergic bronchopulmonary aspergillosis (ABPA) in patients with cystic fibrosis. *Pediatr Pulmonol* 27, 74-79 (1999)
31. Rathore V. B., B. Johnson, J. N. Fink, K. J. Kelly, P. A. Greenberger, V. P. Kurup: T cell proliferation and cytokine secretion to T cell epitopes of Asp f 2 in ABPA patients. *Clin Immunol* 100, 228-235 (2001)
32. Khan S., J. S. McClellan, A. P. Knutsen: Increased sensitivity to IL-4 in patients with allergic bronchopulmonary aspergillosis. *Int Arch Allergy Immunol* 123, 319-326 (2000)
33. Latge J. P.: Aspergillus fumigatus and aspergillosis. *Clin Microbiol Rev* 12, 310-350 (1999)
34. Mehrad B., R. M. Strieter, T. J. Standiford: Role of TNF-alpha in pulmonary host defense in murine invasive aspergillosis. *J Immunol* 162, 1633-1640 (1999)
35. Hogaboam C. M., C. S. Gallinat, D. D. Taub, R. M. Strieter, S. L. Kunkel, N. W. Lukacs: Immunomodulatory role of C10 chemokine in a murine model of allergic bronchopulmonary aspergillosis. *J Immunol* 162, 6071-6079 (1999)
36. Grunig G., D. B. Corry, M. W. Leach, B. W. Seymour, V. P. Kurup, D. M. Rennick: Interleukin-10 is a natural suppressor of cytokine production and inflammation in a murine model of allergic bronchopulmonary aspergillosis. *J Exp Med* 185, 1089-1099 (1997)
37. Roilides E., A. Dimitriadou, I. Kadiltsoglou, T. Sein, J. Karpouzas, P. A. Pizzo, T. J. Walsh: IL-10 exerts suppressive and enhancing effects on antifungal activity of mononuclear phagocytes against Aspergillus fumigatus. *J Immunol* 158, 322-329 (1997)
38. Corry D. B., G. Grunig, H. Hadeiba, V. P. Kurup, M. L. Warnock, D. Sheppard, D. M. Rennick, R. M. Locksley: Requirements for allergen-induced airway hyperreactivity in T and B cell-deficient mice. *Mol Med* 4, 344-355 (1998)
39. Kurup V. P., J. Q. Xia, D. A. Rickaby, C. A. Dawson, H. Choi, J. N. Fink: Aspergillus fumigatus antigen

exposure results in pulmonary airway resistance in wild-type but not in IL-4 knockout mice. *Clin Immunol* 90, 404-410 (1999)
40. Blease K., C. Jakubzick, J. Westwick, N. Lukacs, S. L. Kunkel, C. M. Hogaboam: Therapeutic effect of IL-13 immunoneutralization during chronic experimental fungal asthma. *J Immunol* 166, 5219-5224 (2001)
41. Kurup V. P., H. Y. Choi, P. S. Murali, J. Q. Xia, R. L. Coffman, J. N. Fink: Immune responses to Aspergillus antigen in IL-4-/-mice and the effect of eosinophil ablation. *Allergy* 54, 420-427 (1999)
42. Murali P. S., A. Kumar, H. Choi, N. K. Banasal, J. N. Fink, V. P. Kurup: Aspergillus fumigatus antigen induced eosinophilia in mice is abrogated by anti-IL-5 antibody. *J Leukoc Biol* 53, 264-267 (1993)
43. Kurup V. P., P. S. Murali, J. Guo, H. Choi, B. Banerjee, J. N. Fink, R. L. Coffman: Anti-interleukin (IL)-4 and -IL-5 antibodies downregulate IgE and eosinophilia in mice exposed to Aspergillus antigens. *Allergy* 52, 1215-1221 (1997)
44. Blease K., C. Jakubzick, J. M. Schuh, B. H. Joshi, R. K. Puri, C. M. Hogaboam: IL-13 fusion cytotoxin ameliorates chronic fungal-induced allergic airway disease in mice. *J Immunol* 167, 6583-6592 (2001)
45. Blease K., J. Schuh, C. Jakubzick, N. W. Lukacs, S. L. Kunkel, B. H. Joshi, R. K. Puri, M. H. Kaplan, C. M. Hogaboam: Stat6-deficient mice develop airway hyperresponsiveness and peribronchial fibrosis during chronic fungal asthma. *Am J Pathol* in press., (2001)
46. Kurup V. P., J. Guo, P. S. Murali, H. Choi, J. N. Fink: Immunopathologic responses to Aspergillus antigen in interleukin-4 knockout mice. *J Lab Clin Med* 130, 567-575 (1997)
47. Schuh J. M., K. Blease, S. L. Kunkel, C. M. Hogaboam: Eotaxin/CCL11 is involved in acute, but not chronic, allergic airway responses to Aspergillus fumigatus. *Am J Physiol Lung Cell Mol Physiol* in press, (2002)
48. Zimmermann N., S. P. Hogan, A. Mishra, E. B. Brandt, T. R. Bodette, S. M. Pope, F. D. Finkelman, M. E. Rothenberg: Murine eotaxin-2: a constitutive eosinophil chemokine induced by allergen challenge and IL-4 overexpression. *J Immunol* 165, 5839-5846 (2000)
49. Hogaboam C. M., K. Blease, B. Mehrad, M. L. Steinhauser, T. J. Standiford, S. L. Kunkel, N. W. Lukacs: Chronic airway hyperreactivity, goblet cell hyperplasia, and peribronchial fibrosis during allergic airway disease induced by Aspergillus fumigatus. *Am. J. Pathol.* 156, 723-732. (2000)
50. Blease K., B. Mehrad, T. J. Standiford, N. W. Lukacs, S. L. Kunkel, S. W. Chensue, B. Lu, C. J. Gerard, C. M. Hogaboam: Airway remodeling is absent in CCR1-/- mice during chronic fungal allergic airway disease. *J Immunol* 165, 1564-1572 (2000)
51. Blease K., B. Mehrad, T. J. Standiford, N. W. Lukacs, J. Gosling, L. Boring, I. F. Charo, S. L. Kunkel, C. M. Hogaboam: Enhanced pulmonary allergic responses to Aspergillus in CCR2-/- mice. *J Immunol* 165, 2603-2611 (2000)
52. Schuh J. M., K. Blease, C. M. Hogaboam: CXCR2 is necessary for the development and persistence of chronic fungal asthma in mice. *J Immunol* 168, 1447-1456 (2002)
53. Schuh J. M., K. Blease, C. M. Hogaboam: The role of CC chemokine receptor 5 (CCR5) and RANTES/CCL5 during chronic fungal asthma in mice1. *Faseb J*, (2001)
54. Blease K., B. Mehrad, N. W. Lukacs, S. L. Kunkel, T. J. Standiford, C. M. Hogaboam: Antifungal and airway remodeling roles for murine monocyte chemoattractant protein-1/CCL2 during pulmonary exposure to Asperigillus fumigatus conidia. *J Immunol* 166, 1832-1842. (2001)
55. Judson M. A., D. A. Stevens: Current pharmacotherapy of allergic bronchopulmonary aspergillosis. *Expert Opin Pharmacother* 2, 1065-1071 (2001)
56. Stevens D. A., H. J. Schwartz, J. Y. Lee, B. L. Moskovitz, D. C. Jerome, A. Catanzaro, D. M. Bamberger, A. J. Weinmann, C. U. Tuazon, M. A. Judson, T. A. Platts-Mills, A. C. DeGraff, Jr.: A randomized trial of itraconazole in allergic bronchopulmonary aspergillosis. *N Engl J Med* 342, 756-762 (2000)
57. Stevens D. A., B. J. Kullberg, E. Brummer, A. Casadevall, M. G. Netea, A. M. Sugar: Combined treatment: antifungal drugs with antibodies, cytokines or drugs. *Med Mycol* 38 Suppl 1, 305-315 (2000)
58. Bianchi G., S. Sozzani, A. Zlotnick, A. Mantovani, P. Allavena: Migratory response of human NK cells to lymphotactin. *Eur. J. Immunol.* 26, 3238. (1996)
59. Wells T. N., A. E. Proudfoot: Chemokine receptors and their antagonists in allergic lung disease. *Inflamm Res* 48, 353-362 (1999)
60. Proudfoot A. E., T. N. Wells, P. R. Clapham: Chemokine receptors--future therapeutic targets for HIV? *Biochem Pharmacol* 57, 451-463 (1999)
61. Takeda K., S. Akira: STAT family of transcription factors in cytokine-mediated biological responses. *Cytokine Growth Factor Rev* 11, 199-207 (2000)
62. Zhang S., N. W. Lukacs, V. A. Lawless, S. L. Kunkel, M. H. Kaplan: Cutting edge: differential expression of chemokines in Th1 and Th2 cells is dependent on Stat6 but not Stat4. *J Immunol* 165, 10-14 (2000)
63. Lawless V. A., S. Zhang, O. N. Ozes, H. A. Bruns, I. Oldham, T. Hoey, M. J. Grusby, M. H. Kaplan: Stat4 regulates multiple components of IFN-gamma-inducing signaling pathways. *J Immunol* 165, 6803-6808 (2000)
64. Takeda K., T. Kishimoto, S. Akira: STAT6: its role in interleukin 4-mediated biological functions. *J Mol Med* 75, 317-326 (1997)
65. Prieto J., C. Lensmar, A. Roquet, I. van der Ploeg, D. Gigliotti, A. Eklund, J. Grunewald: Increased interleukin-13 mRNA expression in bronchoalveolar lavage cells of atopic patients with mild asthma after repeated low-dose allergen provocations. *Respir Med* 94, 806-814 (2000)
66. Huang S. K., H. Q. Xiao, J. Kleine-Tebbe, G. Paciotti, D. G. Marsh, L. M. Lichtenstein, M. C. Liu: IL-13 expression at the sites of allergen challenge in patients with asthma. *J Immunol* 155, 2688-2694 (1995)
67. Grunig G., M. Warnock, A. E. Wakil, R. Venkayya, F. Brombacher, D. M. Rennick, D. Sheppard, M. Mohrs, D. D. Donaldson, R. M. Locksley, D. B. Corry: Requirement for IL-13 independently of IL-4 in experimental asthma [see comments]. *Science* 282, 2261-2263 (1998)
68. Wills-Karp M., J. Luyimbazi, X. Xu, B. Schofield, T. Y. Neben, C. L. Karp, D. D. Donaldson: Interleukin-13: central mediator of allergic asthma [see comments]. *Science* 282, 2258-2261 (1998)
69. Hilton D. J., J. G. Zhang, D. Metcalf, W. S. Alexander, N. A. Nicola, T. A. Willson: Cloning and characterization of a binding subunit of the interleukin 13 receptor that is

also a component of the interleukin 4 receptor. *Proc Natl Acad Sci U S A* 93, 497-501 (1996)
70. Murata T., J. Taguchi, R. K. Puri, H. Mohri: Sharing of receptor subunits and signal transduction pathway between the IL-4 and IL-13 receptor system. *Int J Hematol* 69, 13-20 (1999)
71. Donaldson D. D., M. J. Whitters, L. J. Fitz, T. Y. Neben, H. Finnerty, S. L. Henderson, R. M. O'Hara, Jr., D. R. Beier, K. J. Turner, C. R. Wood, M. Collins: The murine IL-13 receptor alpha 2: molecular cloning, characterization, and comparison with murine IL-13 receptor alpha 1. *J Immunol* 161, 2317-2324 (1998)
72. Brombacher F.: The role of interleukin-13 in infectious diseases and allergy. *Bioessays* 22, 646-656 (2000)
73. Zhu Z., R. J. Homer, Z. Wang, Q. Chen, G. P. Geba, J. Wang, Y. Zhang, J. A. Elias: Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J Clin Invest* 103, 779-788 (1999)
74. Corry D. B.: IL-13 in allergy: home at last. *Curr Opin Immunol* 11, 610-614 (1999)
75. Webb D. C., A. N. McKenzie, A. M. Koskinen, M. Yang, J. Mattes, P. S. Foster: Integrated signals between IL-13, IL-4, and IL-5 regulate airways hyperreactivity. *J Immunol* 165, 108-113 (2000)
76. Husain S. R., B. H. Joshi, R. K. Puri: IL-13 receptor directed therapy of human malignant glioma in xenograft model. *Int. J. Cancer* 92, 168-175 (2001)
77. Kawakami K., M. Kawakami, B. H. Joshi, R. K. Puri: Interleukin-13 receptor-targeted cancer therapy in an immunodeficient animal model of human head and neck cancer. *Cancer Res* 61, 6194-6200 (2001)
78. Kawakami K., B. H. Joshi, R. K. Puri: Sensitization of cancer cells to interleukin 13-pseudomonas exotoxin-induced cell death by gene transfer of interleukin 13 receptor alpha chain. *Hum Gene Ther* 11, 1829-1835. (2000)
79. Cocchi F., A. L. DeVico, G.-D. A., S. K. Arya, R. C. Gallo, P. Lusso: Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. *Science* 270, 1811-1815. (1995)
80. Howard O. M., H. F. Dong, J. J. Oppenheim, S. Insaf, K. C. Santhosh, G. Paul, M. Cushman: Inhibition of RANTES/CCR1-mediated chemotaxis by cosalane and related compounds. *Bioorg Med Chem Lett* 11, 59-62. (2001)

Key Words: Chemokines; Cytokines, Th1, Th2, Therapy, Review

Send Correspondence to: Dr. Cory M. Hogaboam, Ph.D., Department of Pathology, University of Michigan Medical School, Rm. 5214, Med Sci I, 1301 Catherine Rd., Ann Arbor, MI 48109-0602, Tel: 734-936-1020, Fax: 734-764-2397, E-mail: hogaboam@med.umich.edu