

Th2-Cell-Mediated Chemokine Synthesis Is Involved in Allergic Airway Inflammation in Mice

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Key Words

Bronchial asthma · Chemokine · Eosinophil · Th2 cells

Abstract

Eosinophilic inflammation in the bronchial mucosa has been recognized as a prominent pathological feature of bronchial asthma. Th2 cells have been implicated in the local infiltration and activation of eosinophils. The migration of eosinophils as well as Th2 cells is controlled by chemokines, suggesting a crucial role of chemokines in the pathogenesis of bronchial asthma. To elucidate the mechanism by which Th2 cells induce eosinophilic inflammation, a Th2-cell-dependent murine model of asthma was employed in this study. Along with the infiltration of eosinophils and antigen-specific Th2 cells, CC chemokine receptor-3-active eotaxin, monocyte chemoattractant protein (MCP)-3 and RANTES, as well as CC chemokine receptor-3-inactive MCP-1 were produced in the lungs of Th2-cell-transferred mice after antigen provocation in vivo. On the other hand, differentiated antigen-specific Th2 cells produced MCP-3 and RANTES but not eotaxin or MCP-1 upon stimulation in vitro. Chemokines synthesized by Th2 cells and other cell types are involved in the development of eosinophilic inflammation in bronchial asthma.

Introduction

The importance of chronic eosinophilic inflammation in the pathogenesis of bronchial asthma has been established [1, 2]. Upon activation by cytokines and other inflammatory mediators, eosinophils induce tissue injury by releasing toxic granule proteins and oxygen metabolites [3]. The recruitment of eosinophils into inflamed tissues is largely regulated by structurally related chemotactic cytokines, i.e. chemokines. Chemokines such as eotaxin, monocyte chemoattractant protein (MCP)-3 and RANTES regulate eosinophil recruitment and degranulation through the CC chemokine receptor 3 (CCR3), which is highly expressed on eosinophils [4]. The expression of these CCR3 ligands as well as CCR3 itself is upregulated in the lungs of asthmatic patients compared with normal subjects. Not only is the concentration of eotaxin in bronchoalveolar lavage (BAL) fluid increased after allergen challenge, but the levels of eotaxin and RANTES are also correlated with pulmonary eosinophilia [5]. Gene disruption and/or blockade of CCR3 as well as the CCR3 ligand eotaxin result in a reduction in eosinophilic infiltration in animal models of asthma [6–9], suggesting that CCR3 and its ligands play crucial roles in the local accumulation of eosinophils in bronchial asthma.

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Accumulating evidence suggests that activated CD4⁺ T cells and T cell cytokines are involved in the migration and activation of eosinophils [1]. CD4⁺ T cells can be classified into two major subsets, Th1 and Th2, based on their cytokine production profile [10, 11]. CD4⁺ T cells infiltrating inflamed tissues of allergic patients appear to have a Th2 phenotype [11]. Accordingly, interleukin (IL)-4, IL-5 and IL-13, which are predominantly produced by Th2 cells, have been implicated in the pathogenesis of bronchial asthma [1, 11]. IL-5 is crucially involved in the activation of eosinophils by promoting growth, differentiation and chemotaxis of this cell type [1]. IL-4 and IL-13 also participate in the migration of eosinophils by up-regulating cell adhesion molecule expression in vascular endothelial cells [12]. Nevertheless, the contribution of chemokines to Th2-cell-mediated eosinophilic inflammation has not been fully elucidated.

An immunoglobulin E/mast cell-dependent cascade has been implicated in allergic diseases [13], whereas our recent investigations clearly demonstrated that airway eosinophilic inflammation can be reconstituted in unprimed mice by the adoptive transfer of antigen-specific Th2 cells, independently of immunoglobulin E and mast cells [14, 15]. Antigen-induced lung eosinophilia in Th2-cell-transferred mice was dependent on IL-5 produced by the infused T cells [14]. Furthermore, the number of accumulated eosinophils correlated well with bronchial responsiveness to nonspecific stimuli [15]. Therefore, this experimental system seems to be useful to analyze whether or how Th2 cells utilize chemokine/chemokine receptor interactions to induce eosinophilic inflammation.

To delineate the mechanisms by which Th2 cells facilitate allergic inflammation, we analyzed the synthesis of chemokines by Th2 cells in vitro and that in the lungs of antigen-specific Th2-cell-transferred mice in vivo.

Materials and Methods

Materials

Mice expressing the transgene for ovalbumin (OVA)-reactive DO11.10 T cell receptor- α/β were obtained from the Jackson Laboratory (Bar Harbor, Me., USA). Specific pathogen-free male BALB/c mice, 6–8 weeks of age, were obtained from Japan SLC (Shizuoka, Japan). A hybridoma that generates anti-CD3 monoclonal antibody (145-2C11) was obtained from ATCC (Rockville, Md., USA). AIM-V medium was from Gibco BRL (Gaithersburg, Md., USA). All other materials were obtained from Sigma (St. Louis, Mo., USA).

Th2 Cell Preparation

OVA-specific T cell receptor-transgenic CD4⁺ T cells were isolated from the spleen using mouse CD4⁺ T cell enrichment columns (R&D Systems, Minneapolis, Minn., USA) and cultured in AIM-V medium supplemented with 10% fetal bovine serum in the presence of 100 μ g/ml OVA, 10 U/ml recombinant IL-2 (Shionogi Co. Ltd., Osaka, Japan), 10 U/ml recombinant murine IL-4 (PeproTech, London, UK), 1 μ g/ml anti-murine IL-12 monoclonal antibody (mAb; PeproTech) and X-ray-irradiated splenocytes of BALB/c mice. Seven to 10 days after stimulation, successful polarization of the resulting Th2 cells was confirmed by ELISA for IL-4, IL-5 and interferon- γ (data not shown). These cells were used for stimulation culture in vitro and for adoptive transfer in vivo.

Chemokine Production by Th2 Cells in vitro

The established Th2 cells (5×10^5 /ml) were cultured in 24-well culture plates immobilized with anti-CD3 antibody for 24 h. The resulting supernatants were assayed for chemokine production by ELISA. For quantification of MCP-3, purified rat anti-mouse MCP-3 Ab (PeproTech) and biotinylated rat anti-mouse MCP-3 Ab (R&D Systems) were used as the capture and detecting antibodies, respectively. Eotaxin was assayed using a Quantikine® M mouse eotaxin immunoassay kit (R&D Systems), MCP-1 using an OptEIA™ mouse MCP-1 set (Pharmingen, San Jose, Calif., USA) and RANTES using an AN'ALYZA™ mouse RANTES immunoassay kit (Genzyme, Cambridge, Mass., USA), according to the manufacturers' instructions. The minimum detectable concentrations were 5, 5, 20 and 2 pg/ml for eotaxin, MCP-1, MCP-3 and RANTES, respectively.

Cell Transfer, Antigen Challenge and BAL

Th2 cells were injected into the tail vein of unprimed mice (3×10^7 /head). 24 h after cell transfer, mice were challenged with aerosolized 10% OVA or bovine serum albumin (BSA), and another 24 h later, BAL was performed as described previously [15]. The chemokine production in BAL fluid was analyzed by ELISA.

Results and Discussion

In order to elucidate the mechanism by which Th2 cells induce eosinophilic inflammation, we analyzed the synthesis of CCR3-active and CCR3-inactive chemokines in the lungs of antigen-specific Th2-cell-transferred mice. In this system, we have demonstrated that massive eosinophilic inflammation is developed in the lungs, accompanied by the bronchial hyperresponsiveness, upon provocation with a specific antigen [14, 15].

The first experiment was performed to investigate the potential of antigen-specific Th2 cells to produce chemokines. In vitro differentiated Th2 cells of DO11.10 mice were stimulated and the concentrations of CCR3-active chemokines, eotaxin, MCP-3 and RANTES, as well as CCR2-active but CCR3-inactive MCP-1 in the culture supernatant were measured by ELISA. As shown in table 1, detectable amounts of MCP-3 and RANTES were

Table 1. Th2-cell-mediated chemokine production in vitro and in vivo

Stimulation/ challenge	Chemokine production, pg/ml			
	in vitro		in vivo	
	–	anti-CD3	BSA	OVA
Eotaxin	<5	<5	<5	110
MCP-1	<5	<5	10	140
MCP-3	130	700	<20	400
RANTES	140	960	<2	21

Differentiated Th2 cells (5×10^5 /ml) were stimulated with plate-bound anti-CD3 antibody for 24 h in vitro. In vivo, Th2 cells (3×10^7) were transferred to normal mice by intravenous injection. After 24 h, these mice were challenged with inhaled 10% OVA or BSA. BAL was performed 24 h after challenge. The concentrations of eotaxin, MCP-1, MCP-3 and RANTES in the culture supernatant and BAL fluid were measured by ELISA. Data are the means of quadruplicate cultures (SEM <10%).

spontaneously produced by Th2 cells, and this production was clearly upregulated by stimulation with anti-CD3 antibody. The concentrations of eotaxin and MCP-1 were below the detection limits of their assays even upon stimulation (table 1). These findings suggest that Th2 cells produce several but not all CCR3-active chemokines.

Next, the levels of chemokines in the BAL fluid of Th2-cell-transferred mice were examined. As shown in table 1, the concentrations of eotaxin, MCP-1, MCP-3 and RANTES were elevated in BAL fluid upon challenge with a specific antigen, compared with those with control BSA, suggesting that these chemokines were synthesized in the lung, dependent on infused antigen-specific T cells. The contribution of eotaxin and MCP-3 of these CCR3-active chemokines to the development of eosinophilic inflam-

mation in this model has been confirmed by the fact that neutralizing antibodies against eotaxin and MCP-3 effectively suppressed antigen-induced lung accumulation of eosinophils [Mori et al., manuscript in preparation].

The source of these chemokines, especially eotaxin and MCP-1 which were not produced by the infused Th2 cells themselves, was not identified in the present study; however, mRNA and protein expression of these chemokines have been demonstrated in many different cell types at sites of inflammation. Notably, resident cells such as epithelial cells, fibroblasts and endothelial cells produce significant levels of such chemokines upon activation [16–18]. In addition, Th2 cytokines initiate the synthesis of chemokines from these cell types. For instance, lung epithelial cells as well as fibroblasts produce eotaxin in response to IL-4 and/or IL-13 [19–21]. In addition, antigen-induced production of these cytokines in the lungs of Th2-cell-transferred mice has been demonstrated in our previous report [14]. Therefore, it is likely that chemokines, at least eotaxin and MCP-1, were released from these resident cells in the lungs in response to Th2 cytokines released from infused T cells.

In conclusion, the present study suggests that Th2-cell-mediated production of CCR3-active chemokines in the lung is involved in the pathogenesis of eosinophilic inflammation in bronchial asthma. Further details of the exact source of each chemokine in the lung during allergic inflammation remain to be determined.

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