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*J Immunol* 2003; 170:4810-4817; doi: 10.4049/jimmunol.170.9.4810

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CC Chemokine Ligand 1 Promotes Recruitment of Eosinophils But Not Th2 Cells During the Development of Allergic Airways Disease¹

Benjamin Bishop and Clare M. Lloyd²

One of the characteristic features of allergic asthma is recruitment of large numbers of inflammatory cells including eosinophils and Th2 lymphocytes to the lung. This influx of inflammatory cells is thought to be a controlled and coordinated process mediated by chemokines and their receptors. It is thought that distinct, differential expression of chemokine receptors allows selective migration of Th cell subsets in response to the chemokines that bind these receptors. Th2 cells preferentially express CCR8 and migrate selectively to its ligand, CC chemokine ligand (CCL)1. We studied the role of the CCR8 ligand, CCL1, in the specific recruitment of Th2 cells and eosinophils to the lung in a murine model of allergic airway disease. We have demonstrated for the first time that CCL1 is up-regulated in the lung following allergen challenge. Moreover, a neutralizing Ab to CCL1 reduced eosinophil migration to the lung, but had no effect on recruitment of Th2 cells following allergen challenge. In addition, there was no change in airway hyperresponsiveness or levels of Th2 cytokines. In a Th2 cell transfer system of pulmonary inflammation, anti-CCL1 also failed to affect recruitment of Th2 cells to the lung following allergen challenge. Significantly, intratracheal instillation of rCCL1 increased recruitment of eosinophils but not Th2 cells to the lung in allergen-sensitized and -challenged mice.

In summary, our results indicate that CCL1 is important for the pulmonary recruitment of eosinophils, rather than allergen-specific Th2 cells, following allergen challenge. The Journal of Immunology, 2003, 170: 4810–4817.

Abbreviations used in this paper: AAD, allergic airway disease; AHR, airway hyperreactivity; CCL, CC chemokine ligand; BAL, bronchoalveolar lavage; PENh, enhanced Penh; Th2, T helper 2 cell; Th1, T helper 1 cell; Th0, T helper 0 cell; CCR3, CCR4, CCR8, CC chemokine receptor 3, 4, 8; CCL1, CCL2, CCL7, CC chemokine ligand 1, 2, 7.

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3 Received for publication December 2, 2002. Accepted for publication February 24, 2003.

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0022-1767/03/$02.00
following allergen challenge and is directly involved in the recruitment of eosinophils, rather than Th2 cells, to the lung.

**Materials and Methods**

**Mice**

Female BALB/c mice were purchased from Harlan Olac (Bicester, U.K.) at 6–8 wk of age and housed for at least 1 wk before being sensitized. Food and water were supplied ad libitum. U.K. Home Office guidelines for animal welfare, based on the Animals (Scientific Procedures) Act 1986 were strictly observed.

**Induction of AAD by allergen sensitization**

Mice were sensitized using OVA (grade V; Sigma-Aldrich, Dorset, U.K.) at a concentration of 0.015 mg/mouse in 0.2 ml of alum (Au-Gel-S; Serva Elektrophoresis, Heidelberg, Germany) i.p. on days 0 and 12. Control mice received the same volume of PBS in alum. All groups of mice were challenged daily with 5% OVA (aerosolized for 30 min) via the airways between days 19 and 24. Mice were sacrificed by exsanguination under terminal anesthesia at 24 h after OVA administration, and the following parameters were analyzed: AHR, airway lumen and lung tissue inflammation, IgE, and Th2 cytokine production.

**Induction of AAD by adoptive transfer of Th2 cells**

Allergen-specific Th2 cells were generated in vitro and transferred to naive recipient mice as described previously (18). Briefly, CD4+ T cells were isolated from the spleens of mice expressing the transgene for the D01.10 μg-TCR, which recognizes residues 323–339 of chicken OVA in association with I-A^d. These mice were originally generated by Dr. D. Loh (Washington University, St. Louis, MO) and were kindly provided by Dr. J. Skok (Imperial College). Cells were cultured in complete RPMI 1640 medium with OVA peptide (1 μg/ml) and mitomycin C-treated splenocytes, with recombiant murine IL-4 (50 ng/ml) and anti-IL-12 (11B11; 10 μg/ml), Endogen, Buckingham, U.K.). Cells were cultured for three rounds of antigenic stimulation under polarizing conditions. At this point, the cells were divided into two portions with the majority being used to induce pulmonary inflammation as described in the next paragraph. A small sample (2 × 10^6 cells) from each culture was activated on immobilized anti-CD3 mAb (2C11; 10 μg/ml, BD Pharmingen, Abingdon, U.K.) in the presence of murine IL-2 (10 U/ml) (R&D Systems, Abingdon, U.K.) for 48 h to determine the integrity of the polarization. Culture supernatants were collected for measurement of IL-4, IL-5, and IFN-γ levels by ELISA (as described below in Cytokine analysis), and cell pellets were collected for RNA extraction and PCR analysis. Th2 cells produced high IL-4 and IL-5 but little IFN-γ. Similarly, RNA expression analysis revealed that Th2 cells expressed predominantly IL-4 and IL-5 but little, if any, IFN-γ.

In preparation for induction of allergic inflammation, Th2 cells were rested in murine IL-2 (10 U/ml) for 48 h before being washed in tissue culture medium. Recipient BALB/c mice were given 5 × 10^6 cells i.v. Twenty-four hours later, mice were exposed to an aerosol of 5% OVA for 30 min. Thereafter, mice were challenged daily and were sacrificed 24 h after the last allergen challenge on day 7. Control mice received cells but were challenged with aerosolized PBS.

**Blocking experiments with anti-CCL1**

To disrupt CCR8/CCL1 interactions during allergic inflammation, goat polyclonal anti-mouse CCL1 Abs (R&D Systems) were administered i.v. at a dose of 10 μg/g body weight 30 min before each allergen challenge. Littermate controls were given the same dose of purified goat Ig (Stratech Scientific, Luton, U.K.). Mice were sacrificed by exsanguination under terminal anesthesia at 24 h after OVA administration on day 24, and the following parameters were analyzed: AHR, inflammation, IgE, and Th2 cytokine production.

**Instillation of CCL1 in vivo**

To directly characterize the phenotype of cells that are recruited by CCL1 in vivo, rCCL1 was instilled via the trachea in OVA-sensitized mice following airway challenge with OVA. Mice were sensitized with OVA in alum, as described above in Induction of AAD, and on day 18 were given one 30-min airway challenge with 5% OVA solution. After 24 h, 2.5 μg of rCCL1 (R&D Systems; a gift from Novartis (East Hanover, NJ)) in a volume of 25 μl was instilled directly into the lungs. Three hours later, AHR was measured, and mice were then sacrificed, and cellular infiltration in the BAL and lung were analyzed as described below in Cell recovery.

**Airway hyperreactivity**

Airway responsiveness was measured in mice 24 h after the final OVA challenge by recording respiratory pressure curves by whole-body plethysmography (Buxco Technologies, Troy, NY) in response to inhaled methacholine (Sigma-Aldrich) at concentrations of 3–100 mg/ml for 1 min, as described previously (21).

**Cell recovery**

**Airway lumen.** Bronchoalveolar lavage (BAL) was performed as described previously. Briefly, the lungs of the mice were lavaged three times with 0.4 ml of PBS via a tracheal cannula. BAL fluid was centrifuged (700 × g, 5 min; 4°C); cells were counted, then pelleted onto glass slides by cytocentrifugation (5 × 10^4 cells/slide). Differential cell counts (×40 magnification; total area of 0.5 mm² area randomly selected) were performed on Giemsa (Shandon, Runcorn, U.K.)-stained cytospins. All differential counts were performed blind and in a randomized order at the end of the study.

**Lung parenchyma.** To disaggregate the cells from the lung tissue, one lobe (100 mg) of lung was incubated (37°C) for 1 h in digest reagent: 75 U/ml collagenase (type D; Boehringer Mannheim, Lewes, U.K.), 50 U/ml DNase (type 1; Boehringer Mannheim), 100 U penicillin, and 100 mg/ml streptomycin (Life Technologies, U.K.) in 100 ml of RPMI 1640/FCS. The recovered cells were filtered through a 70-μm nylon sieve (Falcon; Mar- athon Lab Supplies, London, U.K.), washed twice, and resuspended in 1 ml of RPMI 1640/FCS. Cytocentrifuge preparations were stained and counted as for BAL. A proportion of cells were analyzed for T1/ST2 expression by FACS as described previously (2).

**Cytokine analysis**

Cytokines were analyzed in BAL samples and lung tissue homogenates. Lung tissue (100 mg) was homogenized in 2 ml of HBSS and centrifuged (19,000 g, 10 min), and the supernatant was collected. Paired Abs for murine IFN-γ and IL-4 (BD Pharmingen) and IL-5 (Endogen) were used in standardized sandwich ELISAs according to the manufacturer’s protocol. Kits to measure IL-13 were purchased from R&D Systems.

**IgE levels**

Levels of total IgE were measured in serum by ELISA using paired Abs according to the manufacturer’s instructions (BD Pharmingen). Levels of anti-OVA IgE were measured in serum by ELISA as described previously (22), and Ab titers were then related to pooled standards generated in the laboratory, and were assigned the arbitrary values U/ml.

**RNase protection analysis**

Lung digest RNA for CCL1 was quantified by RNase protection assay according to the manufacturer’s instructions (Riboquant; BD Pharmingen). Briefly, 5-μg aliquots of RNA were hybridized with [α-^32P]-UTP-labeled riboprobes complementary to the transcripts and the housekeeping genes L32 and GAPDH. After overnight hybridization, samples were digested with RNase A/T1 mix and separated on a urea gel and analyzed by autoradiography. To measure the relative abundance of CCL1 mRNA, the Scion densitometry program (Scion Java package 1.0 for ImageJ, Windows version; Frederick, MD) was used to measure sample-to-sample variation in relation to the housekeeping genes. Data was then expressed as a ratio of target mRNA to housekeeping RNA.

**Localization of CCL1 protein by immunohistochemistry**

To determine the cellular source of CCL1 during allergic inflammation, lung sections were prepared from paraffin-fixed sections obtained from OVA-sensitized mice sacrificed 24 h after the final aerosol challenge. These were compared with mice sensitized with alum/PBS. Lungs from the different experimental groups of mice were fixed in 10% neutral buffered formalin, paraffin embedded, and sectioned. An avidin/biotin staining protocol was used as described previously (23). Sections (4 μm) were rehydrated and microwaved twice for 5 min in 0.01 M sodium citrate (pH 6) before staining. All incubations were conducted under humidified conditions and slides were washed twice between steps for 5 min each in 0.1 M PBS. Endogenous peroxidase was blocked by incubation for 20 min in PBS containing 0.3% hydrogen peroxide. Sections were overlaid with 20% normal donkey serum in PBS for 15 min and then incubated overnight at 4°C with polyclonal goat anti-mouse CCL1 or purified goat Ig. Nonspecific staining due to endogenous avidin or biotin was blocked by incubation with avidin solution followed by biotin solution (Vector Laboratories, Burlingame, CA) both for 20 min. Bound Ab was visualized by incubation with biotinylated rabbit anti-goat Ig and then streptavidin peroxidase complex.
(DAKO, Carpinteria, CA), both for 1 h. Finally slides were flooded with peroxidase substrate solution (Vector Laboratories) for 10 min before counterstaining with hematoxylin. Control sections were included where the primary Ab, the biotinylated Ab, or the streptavidin complex were selectively omitted.

**Data analysis**

Data are expressed as mean ± SEM for bar graphs and medians for scatter plots. Statistical significance was accepted when \( p < 0.05 \) using the Mann-Whitney \( U \) test.

**Results**

**CCL1 is up-regulated in the lung during allergic airways disease**

A variety of chemokines are up-regulated in the lung following allergen challenge of sensitized mice; however, levels of CCL1 have not been described previously. We have used RNase protection assays to determine the levels of CCL1 mRNA in lungs isolated from mice at various time points following allergen challenge (Fig. 1A). Although only low levels of CCL1 were expressed in the lung in nonchallenged mice, there was a significant increase in expression by 3 h postchallenge. These levels peaked at 6 h and had started to decline by 24 h postchallenge. To determine which cells within the lung were responsible for the CCL1 expression, we performed immunohistochemical staining on sections from lungs isolated 24 h after allergen challenge as well as lungs from nonchallenged mice (Fig. 1B). Control sections incubated with goat Ig showed no positive staining (data not shown). Low levels of TCA-3 expression were observed in lungs from nonchallenged mice, with alveolar macrophages being the main source of CCL1 expression. After allergen challenge, CCL1 was expressed by macrophages within the inflammatory infiltrates as well as in alveolar macrophages.

**Eosinophil recruitment to the airway lumen and lung tissue is reduced after blockage of CCL1**

Because CCL1 is up-regulated in the lung after allergen challenge and is able to recruit CCR8-positive cells, we set out to establish the role that CCL1 plays in mediating inflammatory cell recruitment to the lung following allergen challenge. We used neutralizing Abs to block CCL1 function, administering Abs before each airway challenge with Ag. We determined the extent of cell recruitment in the airway lumen by examining bronchiolar lavage and in airway tissue by performing differential cell counts on isolated lung leukocytes. Total numbers of cells recovered from the lavage were significantly decreased in those mice receiving anti-CCL1 Ab compared with mice receiving control Ig (Fig. 2, i and iv). Differential cell counts of cytospins of isolated lavage and lung leukocytes revealed that the decrease in total cells was due to reduced numbers of eosinophils in both airway lumen (Fig. 2, ii) and airway tissue (v). There were no significant reductions in the other leukocyte populations (data not shown).

**Th2 recruitment is unaffected by the administration of anti-CCL1 Abs**

Because CCR8 is expressed on Th2 cells, we determined the effect of blockage of CCL1 on the recruitment of Th2 cells to the airway lumen and the lung. To determine the numbers of Th2 cells, leukocytes were isolated from the BAL and the left lobe of the lung and were stained with Abs for CD3, CD4, and the Th2-specific surface marker T1/ST2. There was no significant difference in the
number of CD3⁺ or CD3⁺/CD4⁺ cells after treatment with anti-CCL1 in either the lavage or the tissue (data not shown). The proportion of CD3⁺/CD4⁺ T1/ST2⁺ cells was determined, and the total number of each population was calculated from the total cell counts of lavage and lung samples. Serial pulmonary allergen challenge of sensitized mice increases the percentage and absolute number of T1/ST2-positive lymphocytes in the airway lumen and the lung (Fig. 2, iii and vi). However, treatment with anti-CCL1 did not significantly reduce either the percentage or absolute number of these T1/ST2-positive cells in either lung compartment.

AHR is unaffected by anti-CCL1 treatment

AHR is a cardinal feature of the pulmonary allergic response and is associated with the development of Th2 responses. To determine whether blockade of CCL1 resulted in a change in the development of airway dysfunction, AHR was measured by whole-body plethysmography 24 h following the final, serial OVA challenge (Fig. 3). Although allergen challenge induced an increase in enhanced pause (Penh) in sensitized, challenged mice, neutralization of CCL1 had no effect on levels of AHR at any concentration of methacholine studied.

Th2 mediators are not reduced after anti-CCL1 treatment

The pulmonary allergic response is characterized by high levels of Th2 cytokines in the bronchiolar lavage. In particular, high levels of IL-4, IL-5, and IL-13, and low levels of IFN-γ are associated with a robust Th2-directed response to allergen in the lung. Levels of these cytokines were measured in lavage supernatants and lung homogenates after anti-CCL1 or control Ig treatment. Although levels of the Th2 cytokines IL-4, IL-5, and IL-13 were up-regulated after serial allergen challenge in sensitized mice, levels in both the lavage and the lung remained unchanged after neutralization of CCL1 (Fig. 4 and data not shown). Similarly, levels of IFN-γ remained unaltered by treatment with anti-CCL1 Abs (data not shown).

Elevated levels of IgE are associated with the development of allergic pulmonary pathophysiology. We measured total IgE and OVA-specific IgE in sera from anti-CCL1 Ab- or Ig-treated mice following allergen challenge. Both groups of mice showed significantly enhanced production of total and Ag-specific IgE following allergen challenge (Fig. 4). However, there was no significant difference in levels of either IgE after treatment with anti-CCL1.

CCL1 is not involved in Th2 recruitment in a T cell transfer model of AAD

The data above show that anti-CCL1 has a significant effect in reducing eosinophilia in a sensitization model of AAD, suggesting
that the CCL1/CCR8 axis contributes to eosinophil recruitment. However, for the majority of other parameters that are characteristic of this disease, there was no change. In particular, there was an insignificant effect on the numbers of Th2 cells recruited. To look at Th2 cell recruitment in more detail, we determined the effect of CCL1 neutralization in a model of airway inflammation directly mediated by the adoptive transfer of allergen-specific Th2 cells. This model is characterized by eosinophilic inflammation, increased Th2 cytokines, and AHR (18). Figure 5 shows that treatment of mice with neutralizing Abs to CCL1 did not significantly decrease total leukocyte recruitment to either the lavage or the lungs in this model. Similarly, eosinophil numbers were only slightly reduced within BAL and the lung infiltrates.

In this model, it is possible to determine the number of transgenic OVA-specific Th2 cells that have been recruited to the airway compartments by staining infiltrates with the clonotypic Ab KJ126 (24). FACS staining of leukocytes isolated from the airway lumen or lung revealed that total numbers of lymphocytes were unaffected by the administration of anti-CCL1. Moreover, dual staining with Abs specific for CD4 and KJ126 determined that numbers of allergen-specific Th2 cells within the lungs or airways were similar in both groups of mice (Fig. 5, middle panel). Staining with the Th2 marker T1/ST2 also confirmed that there was no decrease in the Th2 population in either the lavage or lung compartments.

Treatment of mice with anti-CCL1 reduced levels of IL-4 in the lung, although other cytokines remained unchanged (Fig. 5, bottom panel). Moreover, there was no effect on the development of hyperreactivity after CCL1 Ab treatment in this model (data not shown).

**CCL1 induces pulmonary recruitment of eosinophils in allergen-sensitized mice**

Recent studies have determined that allergen-sensitized eosinophils possess an altered chemokine receptor repertoire to those from naive mice or IL-5 transgenic mice (25). Eosinophils from allergen-sensitized mice were found to express CCR8 (25), whereas those isolated from IL-5 transgenic mice do not (19). To determine whether CCL1 induces direct recruitment of eosinophils or Th2 cells into the airway, we instilled CCL1 directly into the lungs of allergen-challenged and -sensitized mice. Interestingly, the only cells to be affected in the airways or lung was the eosinophil population (Fig. 6). Mice that had been sensitized and challenged with OVA had significantly more eosinophils in their lungs and airways than did mice given PBS (Fig. 6A and data not shown). This increase in eosinophilia after instillation of CCL1 was not seen in mice sensitized with alum/PBS, but required a sensitization and challenge with OVA. In contrast, Th2 cell numbers in either the lung or the airways were unaffected by CCL1 instillation (Fig. 6B).

Moreover, the increase in eosinophil recruitment had no effect on the development of AHR, with similar levels of Penh being observed in mice given CCL1 and those given PBS (Fig. 6C).

**Discussion**

Chemokines provide directional signals for the recruitment of multiple populations of leukocytes to inflamed tissues. The temporal and spatial expression of chemokines and their receptors ensures that particular leukocyte subtypes traffic from the circulation into the tissue. Recruitment of eosinophils, lymphocytes, and monocytes to the lung is one of the hallmark characteristics of the pulmonary allergic response. Multiple chemokines have been shown to play a role in the recruitment of these cells to the allergic lung (26, 27). In the present study, we have determined the role of the CCR8 ligand CCL1 in the development of airway inflammation in mice. Neutralization of CCL1 in vivo was shown to reduce recruitment of eosinophils to the airways. In contrast, there was no effect on the migration of Th2 cells to the airways in any of the systems studied. Moreover, direct instillation of rCCL1 into the airways of allergen-sensitized/challenged mice enhanced the recruitment of eosinophils but not Th2 cells. These data imply that the CCR8-CCL1 axis is involved in the recruitment of eosinophils.

![FIGURE 4. Effect of CCL1/TCA-3 neutralization of Th2 mediator production. Cytokine levels were measured in lung homogenate (top panel) by ELISA. Serum total IgE and OVA-specific IgE were also analyzed by ELISA (bottom panel). Samples were collected from mice sacrificed 24 h after the final OVA challenge. For cytokine graphs, bars represent mean and SEM, and for IgE graphs, each dot represents an individual mouse with median values indicated for the group (n = 7–12/group).](image-url)
rather than Th2 cells, to the airways after allergen challenge in vivo.

The role of CCL1 in mediating allergic inflammation has not been investigated previously. Previous studies have shown that CCR8/CCL1 interactions mediate recruitment of Th2 cells rather than Th1 cells in vitro (10, 11) and that CCR8-positive T cells are present in the lung following allergen challenge of atopic asthmatics (14). In this study, we show for the first time that allergen challenge of sensitized mice increases the expression of both CCL1 RNA and protein in the lung. Analysis determined that CCL1 expression was up-regulated in the lung as early as 3 h following serial allergen challenge in sensitized mice. In comparison, very low expression was observed in lung homogenates by ELISA. Alveolar macrophages were observed to be the main cellular source of CCL1, and after allergen challenge, macrophages within the inflammatory infiltrate also positively stained for CCL1. In contrast, expression of CCL1 was not detected in biopsies from atopic asthmatics following allergen challenge, even though increased numbers of CCR8-positive T cells were documented (14). This led the authors to speculate that CCL17 acts as an in vivo ligand for CCR8 or that there is another as-yet-unidentified ligand for CCR8 in the airway mucosa. The interaction of CCR8 with CCL17 remains controversial, but evidence suggests that, at physiological concentrations, CCL1 remains the only ligand for CCR8 (28, 29).

Leukocyte infiltration of the airways is a prominent feature of allergic airway inflammation in both asthmatic patients and in animal models. Previous studies have determined that temporal and spatial expression of chemokines is important for the coordinated recruitment of inflammatory cells (30). In vivo blockage of CCL1 was seen to reduce the recruitment of inflammatory cells to both the airway lumen and the airway interstitium. This decrease was seen to be due to reduced numbers of eosinophils within cellular infiltrates to both lung compartments (Fig. 2). In contrast, recruitment of Th2 cells was unaffected. Th2 cells are thought to be important for the regulation of eosinophil recruitment, but our data indicate that eosinophil migration is directly affected by the neutralization of CCL1. This is similar to data obtained with the CCR8 knockout mouse, where a lack of CCR8 results in decreased eosinophilia in a number of different pulmonary inflammatory models (19). Moreover, a recent study found that, under certain conditions, CCR8 is also expressed on eosinophils (25). Although eosinophils from naive or IL-5 transgenic mice did not migrate to CCR8 ligands, eosinophils from mice sensitized with schistosoma...
egg Ags migrated to CCL1 in chemotaxis assays in vitro. To determine whether CCL1 directly mediates eosinophil recruitment to the lung after allergen challenge, we instilled rCCL1 directly into the lungs of Ag-sensitized and -challenged mice. Recruitment of eosinophils but no other leukocyte was increased in mice given intratracheal CCL1. In contrast, there was no effect in sham-challenged, allergen-sensitized mice, implying that sensitization and challenge with allergen is necessary for eosinophils to migrate in response to CCL1. Taken together, our data indicate that the decrease in eosinophil migration observed after anti-CCL1 Ab treatment occurred as a direct consequence of the CCR8-CCL1 axis being interrupted. Thus, CCR8-bearing eosinophils are not able to respond to CCL1 produced locally by alveolar macrophages. These data show that the CCR8/CCL1 axis is an important component of the complex combination of mediators responsible for mediation of eosinophil recruitment in vivo during AAD.

The reduction in eosinophils, although significant, was modest when compared with the effect of blocking other chemokines such as CCL11, CCL22, or CCL17, which are also thought to be involved in the development of Th2-mediated airway eosinophilia (15–17). It is likely that CCL1 plays an additional role to that of CCL11, CCL22, or CCL17 in the recruitment of eosinophils following allergen challenge. This seeming redundancy in the chemokine network may reflect the partial abrogation of eosinophilia observed after allergen challenge of sensitized mice genetically deficient in CCL1 (31). These data reinforce the idea that chemokines operate in a balanced, tightly regulated fashion but that deficiency in one molecule may be compensated for by the presence of other molecules with overlapping actions.

T cells are critical mediators of the allergic response, and in particular, the Th2 subset is thought to be vital for the development of airway eosinophilia and hyperreactivity. Indeed, blockade of Th2 function or neutralization of key Th2 cytokines abrogates eosinophil recruitment and AHR (2–5). However, the means by which this Ag-specific, Th2 population migrates to the lung has been the subject of much debate. The fact that chemokine receptor expression appears to be tightly regulated on effector T cells puts forward a possible mechanism for selective tissue recruitment of effector subsets in vivo. The attraction of Th2 cells by selected chemokines may represent a mechanism that drives allergen-induced airway inflammation with production of key Th2 cytokines such as IL-4, IL-5, and IL-13, which are essential for the differentiation and activation of eosinophils and development of airway inflammation. Previous studies have determined that CCL1 binding to CCR3, and CCL22 or CCL17 binding to CCR4 selectively attract Th2 cells in vitro and in vivo (8, 9, 18). CCR8 is also selectively expressed on Th2 cells (10, 11), but the role of this chemokine receptor in mediating Th2 recruitment in vivo has not been studied previously. Recent studies with mice genetically deficient in CCR8 show indirectly that CCR8 is important for development of Th2-mediated pathology in vivo (19). Although this study determined that the absence of CCR8 affected eosinophil recruitment in several different models of airway inflammation, the role of CCR8 in Th2 recruitment was not directly addressed. To determine whether CCL1 is important for the specific recruitment of Th2 cells to the allergic lung, we investigated Th2 cell recruitment in several different models. FACS staining of leukocytes isolated from the allergic lung was performed using an Ab specific for the Th2-specific surface marker T1/ST2. This marker is specific and selective for Th2 cells (2, 32, 33) and enables an estimation of the proportion of Th2 cells to be made. We determined that the proportion of CD4+ T1/ST2+ was not significantly affected by the neutralization of CCL1 in an allergen/sensitization model. Similarly, Th2 recruitment was unaltered after CCL1 treatment of mice following adoptive transfer of allergen-specific Th2 cells. Moreover, direct instillation of CCL1 after allergen challenge failed to increase the recruitment of Th2 cells in either lung compartment, suggesting that CCL1 is not involved in recruitment of Th2 cells in vivo. These findings are in contrast to previous studies showing that CCR3 and CCR4 mediate recruitment of Th2 cells to the lung (18). The data presented from this study indicate that either CCL1 is not critical for the specific recruitment of Th2 cells in vivo or that, in the absence of CCL1, the CCL11/CCR3 pathway or the CCL17/CCL22/CCR4 pathway can compensate. Further studies of how these chemokine pathways interact are ongoing.

The lack of effect of anti-TCA on Th2 cell recruitment is reflected in the fact that pathophysiology was not altered after CCL1 treatment. There was no change in development of bronchial hyperreactivity in any of the models studied, reinforcing the view that Th2 cells are critical in mediating changes in lung function (2, 18,
34, 35). Moreover, we found little change in cytokine secretion after neutralization of CCL1. The only decrease was in IL-4 in the adoptive transfer model, and this may be due to the modest reduction in eosinophils, because eosinophils are known to secrete IL-4 (36). This is in contrast to the studies with CCR8 knockout mice where Th2 cytokines were reduced in the lung in several models of allergen challenge (19). These differences probably reflect the nature of the different antigenic stimuli used to promote eosinophilia in vivo.

In summary, we have shown for the first time that CCL1 is involved in the recruitment of eosinophils in several models of allergic inflammation, but that this chemokine is not important for the recruitment of Th2 cells to the lung. These data highlight the complexity and pleiotropy of the chemokine system in mediating leukocyte recruitment. Moreover, they reinforce the idea that, although chemokines function in a tightly controlled manner, with particular chemokines operating at key stages of the immune response, deficiency in one chemokine/receptor axis may be compensated for. This has implications for our ability to design effective strategies for therapeutic intervention in vivo.

Acknowledgments

We thank Drs. Sara Rankin and Douglas Robinson for helpful discussion, and Lorraine Lawrence for excellent assistance with histology.

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