Chemokine Complexity The Case for CCL5

William of Ockham (of Ockham's Razor fame) and Albert Einstein both urged us to keep it simple, but biology, perhaps in comparison to philosophy or physics, is often a more complicated matrix. An example of the complex nature of nature is the case of chemokine action in mediating inflammatory disease. Thus, chemokines were associated initially with the development of inflammatory and allergic disease primarily through their role as *chemoattractant cytokines*. In turn, increased lung levels of the chemokine CCL5 in patients with asthma and in mouse models of asthma (1, 2) were presumed to cause further recruitment of inflammatory cells, especially eosinophils, into airway tissue and consequent worsening of airway disease. However, this view of CCL5, and chemokines in general, turns out to be quite naïve, as underscored by an additional article in the current issue of the *AJRCMB* (3).

In this new article, Koya and colleagues (pp 147–154) show that CCL5 selectively dampens the long-term effect of allergen challenge in mice (3). In this model, prolonged allergen exposure (out to 48 d after initial sensitization) led to airway hyperreactivity and mucous cell metaplasia, and administration of CCL5 decreased these traits, whereas treatment with anti-CCL5 antibody significantly increased them. Administration of CCL5 also caused an increase while anti-CCL5 antibody caused a decrease in BAL fluid levels of IFN- γ as well as IFN- γ -producing CD4 T cells. Moreover, anti-IFN- γ antibody treatment caused an increase in airway hyperreactivity. Together, the results suggest that CCL5 somehow stimulates the generation of IFN- γ -producing Th1 cells that protect against a prolonged allergic response. Whether concomitant changes in lung IL-12 levels are linked to this mechanism remains uncertain. Similarly, why this action takes place after longer- but not shorter-term allergen exposure still needs to be defined, but presumably relies on the delayed development of an activated Th1 cell population.

Non-T Cell Explanations: CCL5 and the Macrophage

Consistent with the present report by Koya and colleagues, the history of CCL5 is closely linked to its actions in regulating T cell performance (3). Indeed, the initial naming of CCL5 (i.e., Regulated And Normal T cell Expressed and Secreted or RANTES) was based on T cell behavior. Is it possible, however, that CCL5 has a broader activity on other immune cell types that better explains its effects on the allergic response? In support of this prospect, we now recognize that several (non-T) cell types express CCL5-responsive receptors, including macrophages and dendritic cells (via CCR1 and CCR5) as well as eosinophils (via CCR3). In fact, inspection of the present data after allergen challenge indicates that the only significant change in lung immune cell levels takes place in macrophages, and in linkage with functional data, this change in macrophage levels also occurred

selectively in the setting of the long-term allergen challenge. But, whether these concomitant changes in lung macrophage levels were driving the change in airway behavior was not pursued. This omission is not unusual. The whole subject of CCL5 signaling to macrophages was barely even considered until recently.

Our research group can take some credit for this redirection to the macrophage based on studies of CCL5 function during common respiratory viral infections. For these types of infections, the site of initial viral replication is often the airway epithelium. This pattern is especially typical of the most common cause of serious respiratory infection in childhood (i.e., respiratory syncytial virus [RSV]) as well as other common paramyxoviruses. For these viruses (and others), the initial immune cell response is relatively nonspecific and is conducted by cells of the innate immune system. Natural killer cells and neutrophils are rapidly attracted to the infected tissue and activated at the site of viral replication. However, this response is not likely enough to fully clear the infection. Thus, while this innate immune response is developing, there is a simultaneous development of an adaptive immune response. This response is initiated by maturation and migration of dendritic cells to the draining lymph nodes. Once in the nodal tissue, the dendritic cells instruct rare virus-specific CD4 and CD8 T cells to proliferate and activate. Ultimately, the CD8 T cells will migrate back to the lung and directly kill the infected host cells. The virus-specific CD4 T cells will in turn direct B cells to make neutralizing antibody that will eventually lead to resolution of the viral infection.

In addition to these well-characterized events, the period of time following the early recruitment of neutrophils and the subsequent appearance of the adaptive immune response is dominated by the recruitment of macrophages into the airway tissue. Macrophages represent the primary phagocytic cells of the immune system and are therefore critical for the removal of cellular corpses and debris. In addition to these housekeeping duties, macrophages are also capable of presenting antigen to lymphocytes; however, the relative importance of this aspect of their biology in a viral infection is unknown. Macrophages, like airway epithelial cells, may also be productively infected by paramyxoviruses and thereby secrete inflammatory cytokines that help to further activate lymphocytes to clear the virus. The precise contribution of macrophages to antiviral defense was incompletely defined. Nonetheless, it seemed reasonable that macrophage as well as epithelial cell behavior might significantly influence the outcome from viral infection.

Our initial approach to these issues aimed at defining the pattern of gene expression in response to RSV infection in primary cultures of human airway epithelial cells. The results revealed that a prominent (perhaps the most prominent) aspect of the epithelial immune response consisted of the production and release of CCL5 (1, 4). This high level of induction was also found for other common respiratory viruses, including rhinovirus and influenza virus. In the case of RSV, viral induction of CCL5 gene expression depended on both transcriptional and post-transcriptional events. The synergy inherent in this combined biochemical mechanism may be responsible for the pronounced induction of CCL5 compared to all other immune-response genes. In any case, the prominence of the induction of CCL5

This research is supported by grants from the National Institutes of Health (NIAID and NHLBI), Martin Schaeffer Fund, and Alan A. and Edith L. Wolff Charitable Trust.

Am J Respir Cell Mol Biol Vol 35, pp 143–146, 2006
Internet address: www.atsjournals.org

gene expression suggested a special role for this chemokine in antiviral defense.

To test the role of CCL5 in the antiviral response, we developed a *Ccl5*^{-/-} mouse and examined its response to respiratory viral pathogens (5). For initial experiments, we used mouse parainfluenza virus type I (Sendai virus; SeV) to model respiratory infection, since we found that mice are relatively resistant to infection with RSV. The experimental conditions for SeV infection allow for high-level viral replication and a pattern of illness in wild-type mice that is similar to human paramyxoviral infection (6, 7). In later experiments, we also used a mouse-adapted strain of influenza virus. For both viruses, we found that CCL5 was required for host survival. The same phenotype developed in mice that were deficient in CCR5. This finding fit with coordinated induction of CCR5 in concert with CCL5 expression during viral infection. Other receptors for CCL5 (i.e., CCR1 and CCR3) did not exhibit similar induction under these conditions. Thus, CCL5–CCR5 interaction appeared necessary for the antiviral defense system and specially tailored for activation during viral infection.

Since CCL5 is a potent chemotaxin, we initially reasoned that the viral susceptibility of *Ccl5*^{-/-} and *Ccr5*^{-/-} mice was due to decreased recruitment and/or activation of immune cells (especially macrophages and effector T cells) at the site of infection (8). However, lymphocyte infiltration into the airways was no different in *Ccl5*^{-/-} and *Ccr5*^{-/-} mice compared to wild-type control mice. Moreover, T cell activation levels also appeared unchanged as assessed by flow cytometry of immune cells isolated from spleen, lung, and BAL fluid of *Ccl5*^{-/-} and control mice. In contrast, loss of CCL5–CCR5 interaction was associated with an increased level of macrophages in the airway tissue. Serial tissue sections indicated that this macrophage population was persistently infected with virus and was undergoing apoptosis at increased levels in *Ccl5*^{-/-} and *Ccr5*^{-/-} mice compared to wild-type control mice.

We recognized that the observed phenotype for *Ccl5* deficiency was distinct from those found in other experimental models for chemokine blockade. In those models, chemokine deficiency is associated with a decrease (not an increase) in immune cells at the site of infection (9–11). In fact, our results might also have been compatible with defective macrophage traffic due to loss of chemotactic signal, resulting in higher levels of macrophage infection and death rates. However, the distinct phenotype in *Ccl5*^{-/-} mice was better explained when we found that the CCL5–CCR5 interaction was necessary to prevent virus-induced apoptosis in isolated macrophages, where chemotaxis was no longer a variable. Under these conditions, endogenous CCL5 or exogenous restoration of physiologic levels of CCL5 were each protective against virus-induced apoptosis and so fully reversed the *Ccl5*^{-/-} defect. In addition, the absence or blockade of CCR5 caused increased virus-inducible apoptosis at levels equivalent to those observed in *Ccl5*^{-/-} macrophages. Thus, in *Ccl5*^{-/-} and *Ccr5*^{-/-} mice, accumulation of apoptotic macrophages in tissue could be explained by premature cell death before reaching the airspace. In addition, similar to mouse macrophages, we found that CCR5 blockade caused increased apoptosis in human macrophages infected with SeV as well as viruses that are commonly pathogenic in humans (e.g., RSV and influenza virus). In each case, physiologic levels of CCL5 activate CCR5 and initiate dual signals to G_{αi}/MEK/ERK or G_{αi}/PI3K/AKT. Consequently, losing either of these two pathways leads to loss of protection from virus-induced apoptosis. Similarly, macrophage depletion was sufficient to reproduce the pathology predicted by loss of macrophage anti-apoptotic signaling in the *Ccl5*^{-/-} phenotype. The findings thereby establish a requirement for macrophage-dependent clearance of virus-infected cells that could be suffi-

cient to explain the observed immune compromise in the setting of viral infection.

The Case for the Dendritic Cell

These studies established a distinct role for CCL5–CCR5 signaling in the innate immune response to viral infection, but we still questioned whether chemokines in general, and CCL5 in particular, might somehow influence the adaptive immune response to viral infection. As noted above, previous work made it likely that chemokine influence is directed at traffic and activation of immune cells. For example, *Ccl3*^{-/-} mice exhibit decreased inflammation and delayed clearance of virus during infection with influenza virus or pneumonia virus of mice (PVM) (9, 12). However, we found little evidence of a change in the effector arm of the immune response, at least by 12 d after viral inoculation, in *Ccl5*^{-/-} mice. By this time, *Ccl5* deficiency had no influence on the levels of virus-specific T cells recruited to the lung. We therefore questioned whether earlier events that are important for the initiation of the adaptive immune response might also be influenced by chemokine action.

In support of this possibility, others have reported that CCR5 (as well as CCR1) appears to regulate the homeostatic recruitment of lung dendritic cells. For example, treatment with a CCL5 antagonist (met-CCL5) decreases the number of dendritic cells in the rat lung (13). In addition, others recently showed that interferon-producing cells (also known as plasmacytoid dendritic cells) enter the lymph node via high endothelial venules using a CCR5-dependent mechanism during infection with *Mycobacterium tuberculosis* (14). Whether this response is also dependent upon CCL5 signaling is not yet known. Nonetheless, if plasmacytoid dendritic cells can condition the subsequent antigen-specific T cell response, this trafficking defect represents an additional way in which CCL5–CCR5 interaction may influence the adaptive immune response.

Perhaps similar to the findings with tuberculosis models, we have recently found that CCL5–CCR5 interaction may regulate the recruitment of differentiating and maturing dendritic cells from the lung to the draining lymph nodes. Thus, both *Ccl5*^{-/-} and *Ccr5*^{-/-} mice appear to have over 3-fold fewer dendritic cells migrating to the draining lymph nodes compared to wild-type control mice at early times (1 d) after inoculation with virus (15). This defect is accompanied by a partial failure to up-regulate CCR7 expression that may at least partially explain the decrease in DC traffic to the lymph node. As noted above, we did not find much difference in the effector arm of the adaptive immune response by 12 d after inoculation, but it is still possible that earlier events might be influenced by CCL5. Whether a significant degree of delay in the development of the adaptive immune response contributes to immune compromise during viral infection still needs to be defined. For example, a significant delay in the development of the adaptive response could contribute to immune compromise during a secondary viral infection. The findings also suggest that the afferent arm of the adaptive immune response may be influenced more strongly by CCL5–CCR5 signals, whereas previous work has focused on the effector arm of the response.

A Broader Role for CCL5 in Allergy

We earlier raised the possibility that CCL5 influences the allergic response through non-T cell mechanisms. Based what we have learned about CCL5, is it possible that CCL5 influences the chronic allergic response via actions on macrophages and dendritic cells? For example, could prolonged allergen exposure lead to CCL5-dependent recruitment and survival of macrophages? The

observations by Koya and coworkers suggest that this is the case (3). Could these macrophages then contribute to down-regulating the allergic response? This issue was not examined in the present work, but certainly macrophages could be a major source of IFN- γ in this setting. If so, IFN- γ could skew T cells towards Th1-style behavior and thereby decrease the allergic response. By contrast, CCL5 inhibition might decrease macrophage recruitment and/or survival and so allow up-regulation of the allergic Th2 response. Studies of macrophage depletion by either genetic or chemical methods would serve to define these possibilities. Since macrophage activation is detectable in patients with asthma (6), the same issue may be translatable into humans with airway disease.

It is also possible that CCL5 influences the allergic response via actions on dendritic cells. For example, is the compromise in dendritic cell traffic found in *Ccl5*^{-/-} and *Ccr5*^{-/-} mice after viral infection also found after allergen challenge? As noted above, CCR5 is necessary for plasmacytoid dendritic cell traffic to draining lymph nodes after exposure to a microbial stimulus (14). Furthermore, plasmacytoid dendritic cells are a major source of type I IFNs. Thus, regulating the migration of this cell population to the draining lymph node may also influence the development of Th1 cells.

Finally, the role of CCR5 signaling may also extend to other immune cell types. For example, naïve CD8 T cells appear to use CCR5 to move to sites of CD4 T cell–dendritic cell interaction in

the draining lymph nodes (16). Presumably, this interaction allows for the generation of memory CD8 T cells. In addition, CCR5 signals can facilitate activation-induced apoptosis of NKT cells as well as skew NKT cell production from IL-4 towards IFN- γ (17). Since both CD8 T cells and NKT cells may influence the development of the allergic response and asthma (18, 19), it is possible that CCL5 and/or CCR5 actions on these cell populations may also influence the response to allergen challenge. This complexity of CCL5 action during immune and/or allergen challenge is summarized in Figure 1. Answers to these new questions about CCL5 function during allergen challenge will likely provide a more complete and perhaps simpler scheme for the multiple actions of CCL5–CCR5 signaling in airway immunity and inflammatory disease.

Conflict of Interest Statement: Neither author has a financial relationship with a commercial entity that has an interest in the subject of this paper.

MITCHELL H. GRAYSON

MICHAEL J. HOLTZMAN

Departments of Medicine and Cell Biology
Washington University School of Medicine
St. Louis, Missouri

References

1. Taguchi M, Sampath D, Koga T, Castro M, Look DC, Nakajima S, Holtzman MJ. Patterns of RANTES secretion and intercellular adhesion molecule-1 expression mediate transepithelial T cell traffic based on analyses in vitro and in vivo. *J Exp Med* 1998;187:1927–1940.
2. Castro M, Block SR, Jenkerson MV, De S, Martino DL, Hamilos RB, Cochran XE, Zhang L, Wang H, Bradley JP, et al. Asthma exacerbation after glucocorticoid withdrawal reflects T cell recruitment to the airway. *Am J Respir Crit Care Med* 2004;169:842–849.
3. Koya T, Takeda K, Kodama T, Miyahara N, Matsubara S, Balhorn A, Joetham A, Dakhama A, Gelfand EW. RANTES (CCL5) regulates airway responsiveness after repeated allergen challenge. *Am J Respir Cell Mol Biol* 2006;35:147–154.
4. Koga T, Sardina E, Tidwell RM, Pelletier MR, Look DC, Holtzman MJ. Virus-inducible expression of a host chemokine gene relies on replication-linked mRNA stabilization. *Proc Natl Acad Sci USA* 1999; 96:5680–5685.
5. Tyner JW, Uchida O, Kajiwar N, Kim EY, Patel AC, O'Sullivan MP, Walter MJ, Schwendener RA, Cook DN, Danoff TM, et al. CCL5–CCR5 interaction provides antiapoptotic signals for macrophage survival during viral infection. *Nat Med* 2005;11:1180–1187.
6. Walter MJ, Kajiwar N, Karanja P, Castro M, Holtzman MJ. IL-12 p40 production by barrier epithelial cells during airway inflammation. *J Exp Med* 2001;193:339–352.
7. Walter MJ, Morton JD, Kajiwar N, Agapov E, Holtzman MJ. Viral induction of a chronic asthma phenotype and genetic segregation from the acute response. *J Clin Invest* 2002;110:165–175.
8. Schall TJ, Bacon K, Toy KJ, Goeddel DV. Selective attraction of monocytes and T lymphocytes of the memory phenotype by cytokine RANTES. *Nature* 1990;347:669–671.
9. Cook DN, Beck MA, Coffman TM, Kirby SL, Sheridan JF, Pragnell IB, Smithies O. Requirement for MIP-1 α for an inflammatory response to viral infection. *Science* 1995;269:1583–1585.
10. Lu B, Rutledge BJ, Gu L, Fiorillo J, Lukacs NW, Kunkel SL, North R, Gerard C, Rollins BJ. Abnormalities in monocyte recruitment and cytokine expression in monocyte chemoattractant protein 1-deficient mice. *J Exp Med* 1998;187:601–608.
11. Lukacs NW, Strieter RM, Warmington K, Lincoln P, Chensue SW, Kunkel SL. Differential recruitment of leukocyte populations and alteration of airway hyperreactivity by C-C family chemokines in allergic airway inflammation. *J Immunol* 1997;158:4398–4404.
12. Domachowske JB, Bonville CA, Gao J-L, Murphy PM, Easton AJ, Rosenberg HF. The chemokine macrophage-inflammatory protein-1 α and its receptor CCR1 control pulmonary inflammation and antiviral host defense in paramyxovirus infection. *J Immunol* 2000;165:2677–2682.
13. Stumbles PA, Strickland DH, Pimm CL, Proksch SF, Marsch AM, McWilliam AS, Bosco A, Tobagus I, Thomas JA, Napoli S, et al. Regulation of dendritic cell recruitment into resting and inflamed

Figure 1. Scheme for CCL5 actions during allergen exposure. During acute allergen challenge, Th2 cells are recruited to the lung and produce cytokines (such as IL-4 and IL-13) that cause mucous cell metaplasia and airway hyperreactivity. During chronic allergen challenge, CCL5 production and/or CCR5 activation allows for: (1) NKT cell switching towards IFN- γ and away from IL-4 production; (2) recruitment and survival of macrophages that produce IFN- γ and foster Th1 cell recruitment to the airway; (3) recruitment of naïve CD8 T cells that may influence CD4 T cell–dendritic cell interactions in the draining lymph nodes; (4) migration of conventional dendritic cells and IFN-producing cells to lymph nodes where IFN- α drives allergen-specific CD4 T cells to develop towards Th1 cells that are also recruited to the airway. In turn, IFN- γ from all sources serves to down-regulate the Th2 response and the consequent asthmatic phenotype.

- airway epithelium: use of alternative chemokine receptors as a function of inducing stimulus. *J Immunol* 2001;167:228–234.
14. Diacovo TG, Blasius AL, Mak TW, Cella M, Colonna M. Adhesive mechanisms governing interferon-producing cell recruitment into lymph nodes. *J Exp Med* 2005;202:687–696.
 15. Grayson MH, Rohlfing MM, Holtzman MJ. Lung dendritic cells require CCL5-CCR5 interaction to migrate to lymph nodes during paramyxoviral infection. *Proc Am Thorac Soc* 2006;3:A827.
 16. Castellino F, Huang AY, Altan-Bonnet G, Stoll S, Scheinecker C, Germain RN. Chemokines enhance immunity by guiding naive CD8⁺ T cells to sites of CD4⁺ T cell-dendritic cell interaction. *Nature* 2006; 440:890–895.
 17. Ajuebor MN, Aspinall AI, Zhou F, Le T, Yang Y, Urbanski SJ, Sidobre S, Kronenberg M, Hogaboam CM, Swain MG. Lack of chemokine receptor CCR5 promotes murine fulminant liver failure by preventing the apoptosis of activated CD1d-restricted NKT cells. *J Immunol* 2005;174:8027–8037.
 18. O'Sullivan S, Cormican L, Faul JL, Ichinohe S, Johnston SL, Burke CM, Poulter LW. Activated, cytotoxic CD8⁺ T lymphocytes contribute to the pathology of asthma death. *Am J Respir Crit Care Med* 2001; 164:560–564.
 19. Akbari O, Faul JL, Hoyte EG, Berry J, Wahlstrom J, Kronenberg M, Dekruyff RH, Umetsu DH. CD4⁺ invariant T-cell-receptor⁺ natural killer T cells in bronchial asthma. *N Engl J Med* 2006;354:1117–1129.

DOI: 10.1165/rcmb.f318