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Perspective

Chemokine Signaling Regulates Apoptosis as well as Immune Cell Traffic in Host Defense

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ABSTRACT

In the struggle for optimal host defense against infection with viruses, two major events are critical: death of the infected host cell and proper immune cell activation at the site of infection. Here we summarize our recent work indicating that chemokines exhibit a distinct capacity to regulate both of these events. We put particular emphasis on a recently completed study indicating that chemokine CCL5 may prevent cell death and thereby preserve innate immune cell function in the setting of viral infection. In addition, we introduce new work to support the more traditional role of CCL5 in mediating adaptive immune cell traffic and activation in this same setting.

INTRODUCTION

Programmed cell death is well-studied as a key regulatory event in developmental biology and cancer pathogenesis, but the same process may also be critical in defense against viral infection. In particular, viruses require host cell survival for initial replication. In some cases, the viruses also require the subsequent apoptotic death of the host cell to achieve the release of viral progeny and spread to neighboring cells. Thus, apoptosis may be protective in the early stages of infection by depriving the virus of its home but may instead favor viral spread in the later stages of infection. In the struggle between intracellular pathogen and host, it is therefore likely that the virus and the host aim to favorably influence this balance between life and death of the infected cell. From the standpoint of the host, this issue is often focused on the capacity of the adaptive immune system to target and kill the infected cells. In particular, the development, activation, and delivery of virus-specific cytotoxic T cells appears as a well-studied mechanism for clearance of viral infection. By contrast, the earlier events that govern the cellular decision for survival versus apoptotic death of the infected host cell still need to be fully defined. This aspect of host defense and its role in innate immunity forms much of the basis for the current studies. In the course of addressing this issue, we came to recognize a role for virus-inducible chemokines and consequent chemokine receptor signaling to regulate cell death during the antiviral response.

REGULATION OF APOPTOSIS

To address the issue of virus-induced cell death, we focused on the case of 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 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 apoptosis might significantly influence the outcome from viral infection.

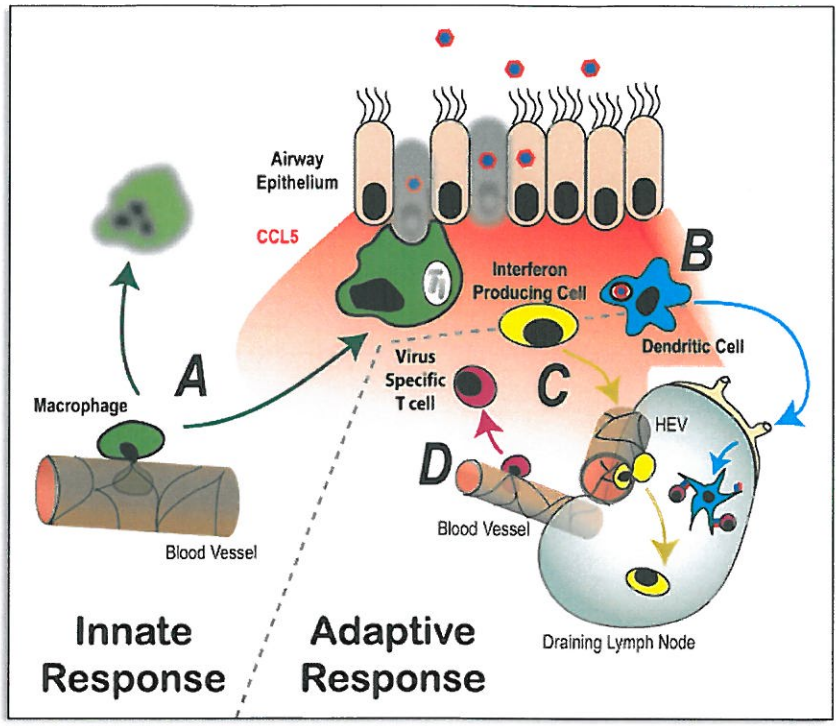

Figure 1. Innate and adaptive effects of the CCL5-CCR5 axis during a paramyxoviral infection. (A) In the innate response, CCL5 binds CCR5 on macrophages attracted to the inflamed lung and allows these macrophages to engulf and remove cellular corpses (right arrow). In the absence of CCL5-CCR5 signaling, the macrophages are still recruited to the organ but die by apoptosis (left arrow). (B) Homeostatic retention of lung dendritic cells depends upon the presence of CCL5 in the uninflamed lung. During viral infection, the dendritic cells mature and migrate to the draining lymph node initiating the adaptive immune response, a process that may depend in part on CCL5-CCR5 signaling. (C) Interferon-producing cells migrate to the draining lymph nodes via the blood vessels. CCR5 signaling is necessary for transendothelial migration across the high endothelial venule into the lymph node. (D) Effector T cells are recruited into the lung by CCL5 (and likely other chemokines) as part of the adaptive immune response to the 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 aspect of the epithelial immune response consisted of the production and release of the chemokine CCL5 (formerly designated RANTES).[1] This 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 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.[2] For initial experiments, we used mouse parainfluenza virus type I or 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 paramxyoviral infection.[3,4] 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, one of the receptors capable of mediating the CCL5 signal. 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.[5] 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.[4,6-8] 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 is no longer a variable. Under these conditions, endogenous

CCL5 or exogenous restoration of physiologic levels of CCL5 was 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, i.e., RSV and influenza virus.

Additional work aimed to define how CCL5 and CCR5 protected macrophages against virus-induced apoptosis. Since viral entry and replication was unchanged by CCL5 deficiency, we concentrated on how CCL5 might influence intracellular death pathways. Initial experiments indicated that CCL5 caused phosphorylation of downstream signaling proteins ERK1/2 and AKT in mouse and human macrophages. CCL5 concentrations in the range detected during viral infection (0.1–10 nM) activated ERK1/2 and AKT via a mechanism that depended on CCR5. Treatment with CCL5 concentrations above the physiologic range (100 nM) caused further activation of ERK1/2 that did not depend on CCR5. This finding is consistent with reports of CCL5 multimer formation at higher concentrations that bind cell surface GAGs and activate SRC kinase-dependent signaling.[9] Together, our observations indicate that high levels of CCL5 (capable of multimer formation) signal through HCK independently of the CCR5 G-protein coupled receptor, but at physiologic levels, CCL5 (acting as a monomer) signals through CCR5 and independently of any SRC family kinases. In this case, CCR5 activation initiates dual signals to G_{ai}/MEK/ERK or G_{ai}/PI3K/AKT. Consequently, losing either of these two pathways should lead to loss of protection from virus-induced apoptosis. This possibility was confirmed when we observed that inhibition of either AKT or ERK pathways (under conditions not affecting baseline apoptosis) caused substantial increases in virus-induced apoptosis in wild-type but not *Ccl5*$^{-/-}$macrophages.

In a final set of experiments, we aimed to more firmly link our observations in vivo with those in vitro. Similar to our observations in cultured macrophages, we found that *Ccl5*$^{-/-}$ and wild-type mice manifest similar activation of ERK1/2 and AKT at baseline but *Ccl5*$^{-/-}$ mice showed blunted activation after viral infection. These findings indicated that the same defect in intracellular death signaling was found in vivo as was identified in vitro in the setting of CCL5 deficiency. In addition, we tested whether macrophage depletion was sufficient to reproduce the pathology predicted by loss of macrophage anti-apoptotic signaling in the *Ccl5*$^{-/-}$ phenotype. We accomplished selective macrophage depletion of wild-type mice during SeV infection using clodronate liposomes. We found that the phenotype for macrophage-deficient mice follows closely the one predicted for defects in *Ccl5*$^{-/-}$ and *Ccr5*$^{-/-}$ mice, i.e., decreased clearance of virus and apoptotic, infected cells and concomitant decrease in survival from respiratory compromise. Moreover, wild-type and *Ccl5*$^{-/-}$ mice no longer exhibit differences in survival if both are macrophage depleted. The findings thereby establish a requirement for macrophage-dependent clearance of virus-infected cells that could be sufficient to explain the observed immune compromise in the setting of viral infection.

REGULATION OF IMMUNE CELL TRAFFIC

Our studies established a distinct role for CCL5-CCR5 signaling in 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).[6,10] However, we found little evidence of a change in the effector arm of the immune response, at least by 12 days after inoculation, in *Ccl5*$^{-/-}$ mice. 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.[11] 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*.[12] Whether this response is also dependent upon CCL5 signaling is not yet known. Nonetheless, if plasmacytoid dendritic cells condition the subsequent antigen-specific T cell response, this traffic 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 found that CCL5-CCR5 interaction may regulate the recruitment of differentiating and maturing dendritic cells from the lung parenchyma to the draining lymph nodes. Thus, *Ccl5*$^{-/-}$ and *Ccr5*$^{-/-}$ mice appear to have fewer dendritic cells migrating to the draining lymph nodes compared to wild-type control mice at 3–5 days after inoculation with SeV. As noted above, we did not find much difference in the adaptive immune response by 12 days after inoculation. However, 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. Nonetheless, our findings to date 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.

SUMMARY

Host defense against intracellular pathogens is traditionally defined by innate and adaptive immune responses aimed at death of infected cells. Chemokines and macrophages influence both arms of the immune response,[13-15] so it is not surprising that, in other models of infection, loss of chemokine expression results in decreased immune cell recruitment.[6,7] Based on this anti-inflammatory action, chemokine antagonism may be beneficial in the therapy of inflammatory diseases.[16] CCR5 signaling has also been targeted for specific blockade based on CCR5 capacity to serve as a viral coreceptor.[17,18] In the present experiments, however, we show that CCL5 has a distinct role in host defense based on activation of G-protein-dependent signaling pathways that are essential to inhibit apoptosis of virus-infected macrophages. In some circumstances, apoptosis of infected cells is helpful for host defense,[19] but in the present case, increased

apoptosis is harmful to the host since macrophages must resist cell death to efficiently clear virus-infected, apoptotic cells from the tissue. These studies define a new role for chemokine action during the innate immune response but do not yet fully exclude an additional role in the development of the adaptive immune response to viral infection. Recent work suggests that chemokines might influence both the afferent and efferent arms of this adaptive immune response. Defining this aspect of chemokine biology is already underway and will provide for a more complete framework for understanding the antiviral response and thereby improving it.

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