Tipping the balance in favor of protective immunity during influenza virus infection

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Calibrating immune responses to levels that control infection while minimizing damage to host tissues is the primary challenge facing the immune system. Disease manifestations associated with most infections result from host inflammatory responses that, in some cases, are of sufficient magnitude to result in death. Influenza virus infections occasionally fall into this category. The outcome of human influenza virus infection is heavily influenced by the virulence of the viral strain and the host's immune status. In this issue of PNAS, Aldridge et al. (1) investigate inflammatory responses induced by influenza virus and discover that recruitment of TNF and inducible NOS (iNOS)-producing dendritic cells (TipDCs) correlates with viral strain virulence. Recruitment of TipDCs is substantially greater in mice infected with highly pathogenic influenza virus, and recruitment depends on chemotactic cytokine receptor 2 (CCR2), a chemokine receptor that responds to monocyte chemotactic protein (MCP)-1, MCP-3, and MCP-5. Aldridge et al. demonstrate that CCR2-mediated recruitment of TipDCs enhances viral clearance at later stages of infection by enhancing virus-specific T cell responses.

CCR2-mediated recruitment of inflammatory monocytes is essential for defense against a range of microbial pathogens (2). Early studies demonstrated that CCR2-deficient mice are extremely susceptible to infection with the intracellular bacterial pathogen *Listeria monocytogenes* (3), and subsequent studies have also implicated CCR2-mediated recruitment of inflammatory cells in defense against *Cryptococcus neoformans* (4), *Toxoplasma gondii* (5), and *Mycobacterium tuberculosis* (Mtb) (6), which, like influenza virus, is principally, but not exclusively, a respiratory tract pathogen.

CCR2-deficient mice are more susceptible to Mtb infection (6) and have diminished T cell responses in lung-draining lymph nodes. These results suggested that CCR2-recruited monocytes might promote T cell priming. This notion is supported by a subsequent study (7), which used bone marrow chimeras to show that monocyte recruitment to the lungs of tuberculosis-infected mice requires their expression of CCR2 but that T cells traffic to the lung regardless of whether they express CCR2. The role of CCR2-mediated monocyte recruitment during Mtb infection varies, however, depending on the size of the initial inoculum used to infect mice. CCR2-deficient mice infected with a low inoculum of aerosolized Mtb survive normally and control infection (8), whereas infection with a high inoculum of Mtb is poorly controlled in CCR2-deficient mice.

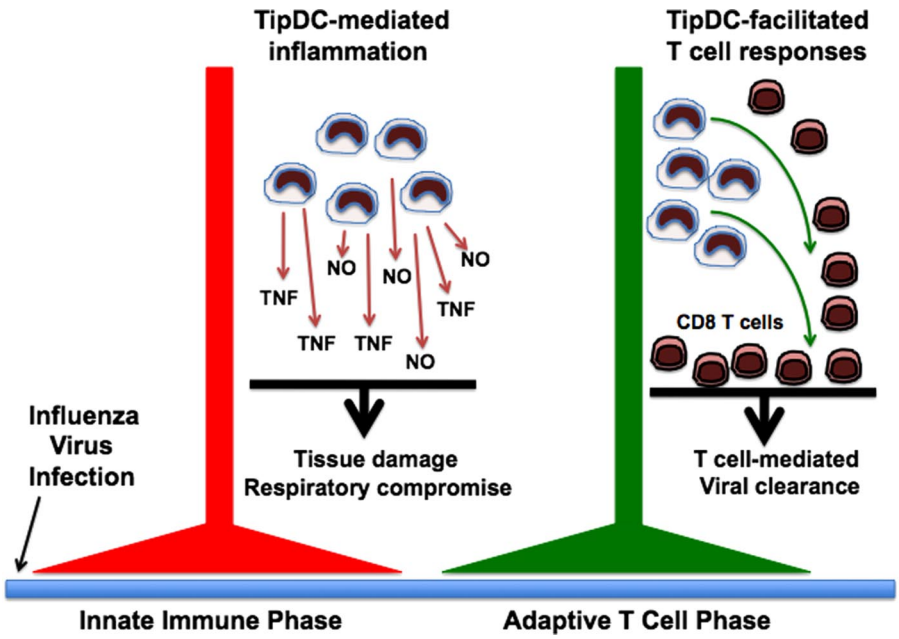

Fig. 1. Monocyte recruitment and TipDC differentiation make distinct contributions to early and late immune responses to influenza virus infection. TNF and NO production during early infection (depicted in red) can lead to increased pulmonary inflammation and increased morbidity, especially after infection with highly-virulent viral strains. However, TipDCs facilitate T cell responses during later stages of infection, promoting viral clearance (depicted in green).

Although the magnitude of initial Mtb infection determines whether CCR2-mediated monocyte recruitment contributes to protection, after influenza virus infection the benefits of CCR2-mediated monocyte recruitment may depend on the virulence of the viral strain. Much has been learned about influenza virus virulence and virally-induced inflammatory responses from studies of the recently reconstructed highly-virulent influenza virus strain that caused the calamitous 1918 influenza epidemic. This viral strain, in mouse models, grows more rapidly and induces much greater neutrophil and macrophage recruitment than less virulent viruses (9). An analysis of the inflammatory response induced in infected mice by the 1918 influenza virus after pulmonary inoculation demonstrated, compared with other influenza viruses, the most rapid and dramatic induction of inflammatory cytokines and activation of inflammatory cascades (10). Additional studies also showed that the 1918 influenza virus induces markedly greater recruitment of macrophages to lungs of infected mice (11).

CCR2-mediated recruitment of monocytes during influenza virus infection contributes to early innate immune responses and adaptive T cell responses.

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Histologic analysis demonstrated that monocyte/macrophage recruitment is diminished in influenza-infected, CCR2-deficient mice (12). Furthermore, the frequency of activated T cells was also diminished in infected CCR2-deficient mice. Influenza virus infection induces production of MCP-1, the major chemokine ligand for CCR2 (13), and in vitro infection of alveolar epithelial cell monolayers with influenza virus induces monocyte transepithelial migration that is CCR2-dependent (14). CCR2-deficient mice survive influenza virus infections (15) but have increased neutrophil recruitment to the lungs. CCR2-recruited monocytes make iNOS and TNF and promote recruitment of activated T populations to the lung (16). It has been demonstrated that iNOS and TNF production worsen influenza virus infections (17, 18), but it remains unclear whether expression of these proteins by TipDCs is deleterious.

CCR2-recruited monocytes make two major contributions to the immune response against influenza virus infection (see Fig. 1). The initial contribution is to enhance the innate inflammatory response, in part by producing iNOS and TNF, which, after bacterial infection may be beneficial, but after influenza virus infection appears to be deleterious. Thus, depending on the viral strain, robust recruitment of monocytes and their differentiation into TipDCs may be either neutral or deleterious. The second contribution, as demonstrated by Aldridge et al, (1), is to enhance influenza virus-specific T cell responses. Although previous studies have correlated CCR2-mediated monocyte recruitment with the size of activated T cell populations, Alldridge et al. have made several important additional steps by demonstrating that TipDCs isolated from infected mice present antigens and that, upon adoptive transfer, they enhance pulmonary influenza virus-specific T cell responses. Their results indicate that TipDCs do not prime CD8 T cells in draining lymph nodes, but that TipDCs increase the frequency of virus-specific T cells in the lung. It is possible that TipDCs promote proliferation of influenza virus-specific T cells in the lung. Alternatively, TipDCs may enhance survival of influenza virus-specific T cells and in this way increase their frequency. It remains unclear how and where within the lung T cells and TipDCs interact. Because TipDC-mediated stimulation of influenza virus-specific CD8 T cells is antigen specific, it seems likely that T cells and TipDCs are physically contacting each other. Alternatively, TipDCs may transfer antigens to other DCs. Bronchial-associated lymphoid aggregates have, albeit in rather restricted circumstances (19), been demonstrated after influenza virus infection and may provide a site for TipDCs and virus-specific CD8 T cells to interact.

TipDCs may enhance survival of influenza virus-specific T cells.

Attempts to diminish inflammatory responses to improve survival after influenza virus infection have met with mixed success. Tumpey et al. (20) depleted neutrophils or alveolar macrophages and demonstrated that depletion at later stages of infection has little impact, whereas depletion before infection converted a sublethal influenza virus infection into a lethal infection. However, in some cases CCR2 deficiency enhances survival, presumably because early TNF- and iNOS-mediated inflammatory responses are attenuated. Aldridge et al. (1) have successfully walked a tightrope and diminished inflammatory responses with pioglitazone, which decreases chemokine production, while maintaining protective T cell responses. Although this approach is exciting, given the spectrum of inflammatory responses induced by different influenza strains and the diversity within human populations exposed to these viral strains, pharmacologically calibrating inflammatory responses to optimize antiviral responses will require a great deal of further investigation. That said, the findings by Aldridge et al. point us in an exciting and potentially important direction.

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