The role of chemokines in linking innate and adaptive immunity

Andrew D Luster

It is becoming clear that chemokine function is necessary to translate an innate-immune response into an acquired response. Dendritic cells activated by innate stimuli and loaded with foreign antigen travel to regional lymph nodes to activate the acquired-immune system. Subsequently, the activated acquired-immune cells move into tissue, where the innate immune system sets-off the danger signal. The chemokine system has emerged as an essential regulator of this dendritic cell and lymphocyte trafficking, which is necessary to turn an innate immune response into an adaptive response.

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Abbreviations

CCR	CC-chemokine receptor
CMV	cytomegalovirus
CXCR	CXC-chemokine receptor
LPS	lipopolysaccharide
PRR	pattern-recognition receptor
STAT6	signal transducer and activator of transcription 6
TLR	Toll-like receptor

Introduction

The host response to foreign challenge requires the coordinated action of both the innate and acquired arms of the immune system. The adaptive response is mediated by T and B cells that have undergone germline gene rearrangement, and is characterized by exquisite specificity and long-lasting memory. Innate immunity was formerly thought to be nonspecific, but it has become clear that the innate immune system can recognize pathogens from self.

Once thought of as separate, parallel systems, the innate and adaptive immune systems are now known to be two interdependent parts of a single integrated immune system. The innate immune response not only provides the first line of defense against microorganisms, but also provides the biological context — the 'danger signal' — that instructs the adaptive immune system to mount a response [1]. The adaptive immune response calls on the innate immune system to provide the professional phagocytes (e.g. macrophages and neutrophils) and specialized granulocytes (e.g. eosinophils and basophils) necessary to engulf small pathogens and contain larger parasites.

The primary adaptive immune response takes place in the draining lymph nodes and not in the tissue itself. Antigen is picked up by dendritic cells in the tissue and carried into regional lymph nodes, where the dendritic cells activate naive T and B cells. Activated T and B cells then leave the lymph node and find their way back to the site of inflammation. Although the trafficking patterns of dendritic cells and lymphocytes have been appreciated for some time, the molecules that control their movement *in vivo* have only recently been identified as members of the chemokine family (Figure 1). The chemokine system has emerged as the crucial regulator of the dendritic-cell and lymphocyte trafficking that is needed, first, to bring together antigen-loaded dendritic cells and naive T and B cells to generate an adaptive immune response and, second, to deliver this adaptive effector response to sites of inflammation and infection.

In this review I focus on how innate immune stimuli, through activation of Toll-like receptors (TLRs), induce the expression of a subset of chemokines from resident tissue macrophages and alter chemokine-receptor expression on dendritic cells to link activation of innate immune cells effectively with the generation and delivery of an adaptive immune response. In addition, I discuss the concept that chemokines are used by the adaptive immune system to amplify antigen-specific lymphocyte responses with the destructive power of the innate immune system.

The Toll connection

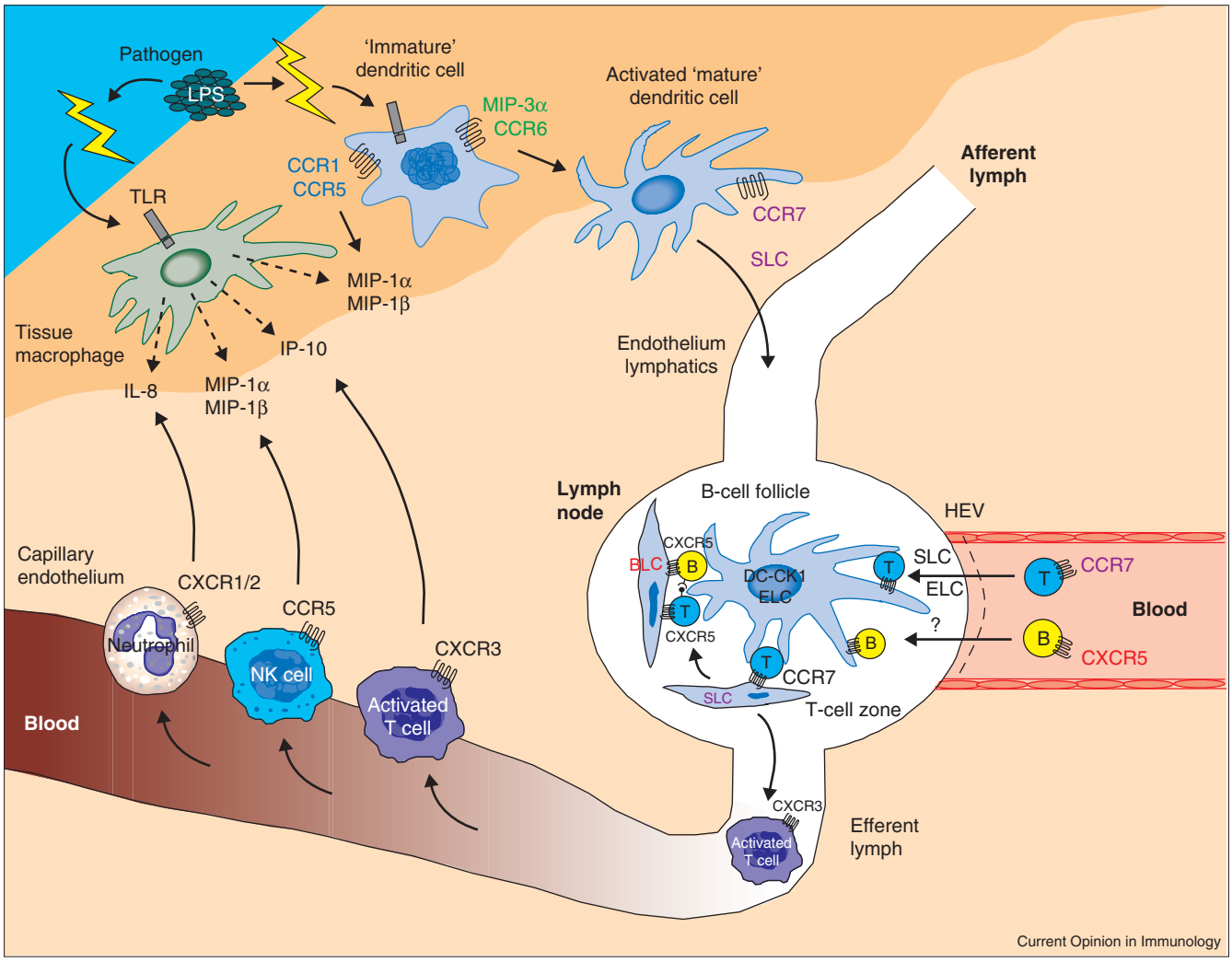
The innate immune system provides the biological context for the adaptive immune response. Recognition of pathogens is mediated by a set of germline-encoded receptors that are referred to as pattern-recognition receptors (PRRs). These receptors recognize conserved molecular patterns (i.e. pathogen-associated molecular patterns), which are shared by large groups of microorganisms. TLRs function as PRRs and are essential for translating the recognition of microbial components to activation of the immune system [2].

Activation of TLRs leads to the release of several inflammatory mediators, including chemokines, from resident tissue macrophages and dendritic cells, and modulates the expression of chemokine receptors on dendritic cells. These TLR-mediated events are essential for both the recruitment of immature dendritic cells to sites of pathogen entry and their ultimate journey back to lymph nodes to activate naive T cells. In addition, chemokines released by resident tissue cells after TLR activation guide these activated T cells into the site of pathogen entry and/or replication. In this way, chemokines link innate immune cell activation in the tissue to the recruitment of antigen-specific T cells generated in the lymph nodes into tissues.

TLR activation of resident tissue macrophages and dendritic cells

Resident tissue macrophages and dendritic cells play a critical role in initiating the innate immune response in

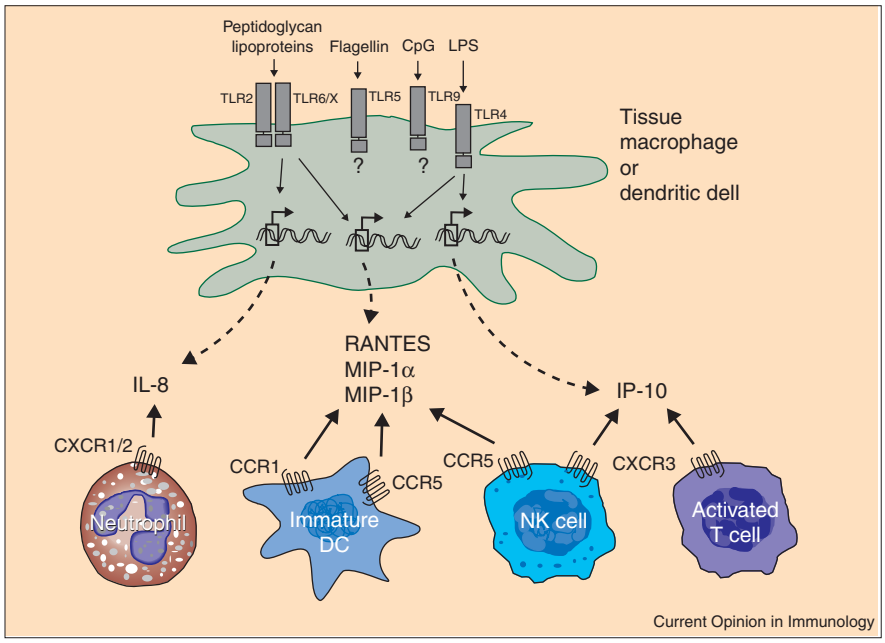
Figure 1



Chemokines orchestrate the trafficking of dendritic cells, T cells and B cells needed to generate an immune response. By activating TLRs, LPS on the surface of bacterial pathogens stimulates the local release of chemokines such as MIP-1α, MIP-1β, MIP-3α and IP-10. Immature dendritic cells are attracted to this site through activation of chemokine receptors, such as CCR1, CCR5 and CCR6, that they constitutively express. Immature dendritic cells are efficient at picking-up antigen, but must mature and differentiate into cells that can activate naive T cells. The local milieu into which the immature dendritic cell is attracted contains pathogen-associated molecular patterns (e.g. LPS) that are recognized by PRRs such as CD14 and TLRs, which induce the differentiation and maturation of dendritic cells into potent antigen-presenting cells. During this process, the dendritic cell downregulates expression of CCR1, CCR5 and CCR6 and upregulates expression of CCR7, causing its migration into the afferent lymphatic system. The CCR7 ligand SLC, which is expressed on the endothelium of the afferent lymphatic system, plays an important role in directing the migration of the antigen-loaded mature dendritic cells. Chemokines are also involved in bringing naive T cells and B cells from blood across high endothelial venules (HEVs) into the lymph nodes and into contact with the activated dendritic cell. The molecular details remain to be elucidated, but it is likely that chemokines such as DC-CK1 and ELC are important in juxta-positioning these cells in the lymph node. SLC and ELC are produced from stromal cells in the T-cell zone and BLC is expressed from stromal cells in the B-cell follicle, helping to guide cells to T- and B-cell areas, respectively. Some activated T cells downregulate expression of CCR7 but upregulate CXCR5 and so become directed toward the follicle to deliver help to B cells, whereas other activated T cells upregulate CXCR3 and are attracted into inflamed tissue. T cells that are activated in regional lymph nodes, after encountering antigen-loaded dendritic cells, subsequently return to sites of inflammation by sensing chemokine gradients established at these local sites. Chemokines such as IP-10, which is induced by LPS and IFN-γ, and a ligand for CXCR3, which is highly expressed on activated T cells, are believed to be important in this process in immune responses mediated by Th1 cells.

the tissue. These professional phagocytes express many PRRs, including CD14, scavenger receptors and TLRs. These PRRs have the ability to recognize foreign antigens as pathogens and set in motion the innate immune response by activating one or more of the TLRs. TLR activation ultimately leads to the activation of NF-κB — a key transcription factor that mediates early host defenses. NF-κB induces a genetic program that is essential for host defense, including the induction of a subset of chemokines.

Figure 2



TLR activation induces the release of chemokines from resident macrophages and dendritic cells (DCs). Pathogens selectively activate distinct TLRs expressed on resident tissue macrophages and dendritic cells. For example, the TLR2–TLR6 or TLR2–TLRX heterodimers are activated by different microbial products such as peptidoglycan from Gram-positive organisms, and bacterial lipoproteins; TLR4 is activated by LPS from Gram-negative bacteria; TLR5 is activated by flagellin; and TLR9 is activated by bacterial DNA, which contains unmethylated CpG dinucleotides. Activation of different TLRs induces the expression of different sets of chemokines. To date, it has been shown that activation of TLR2 induces the expression of IL-8, RANTES, MIP-1α and MIP-1β, whereas activation of TLR4 induces the expression of IP-10, RANTES, MIP-1α and MIP-1β. In turn, these chemokines recruit distinct subsets of leukocytes. Thus, discrimination of the pathogen by TLRs, and the subsequent production of a specific subset of chemokines, may be the first point at which the immune system tailors its response to specific pathogens.

Chemokines downstream of TLR activation in macrophages and dendritic cells include IL-8 (also known as CXCL8), MIP-1α (CCL3), MIP-1β (CCL4), RANTES (CCL5) and IP-10 (CXCL10) [3•]. MIP-1α, MIP-1β and RANTES were found to be induced by agonists of both TLR2 and TLR4 (Figure 2). Interestingly, IP-10 was preferentially induced by TLR4 agonists (e.g. *Escherichia coli* lipopolysaccharide [LPS]), whereas IL-8 was preferentially induced by TLR2-specific agonists (e.g. *Staphylococcus aureus* peptidoglycan and yeast zymosan). These studies suggest that pathogens can determine the nature of the immune response through differential activation of TLRs and the subsequent patterns of chemokine expression.

The early production of chemokines is essential in shaping the immune response that follows in the tissue. For example, the production of IL-8 will induce the recruitment of neutrophils; MIP-1α and MIP-1β will induce the influx of NK cells, macrophages and immature dendritic cells; and IP-10 will guide activated T cells back into tissues. The discrimination of the pathogen by TLRs and the subsequent production of a specific subset of chemokines may be the first point at which the immune system tailors its response to specific pathogens.

The NK-cell connection

The early pathogen-induced release of MIP-1α and MIP-1β has been shown to be vital for the initial influx of NK cells into the liver in a murine model of cytomegalovirus (CMV) [4,5••]. These NK cells are an important source of IFN-γ, which induces the expression of the chemokines IP-10 and Mig (CXCL9). In turn, IP-10 and Mig lead to the recruitment of activated T cells into the liver. Neutralization of Mig in this model impaired the ability of the host to control CMV replication and led to increased mortality [5••].

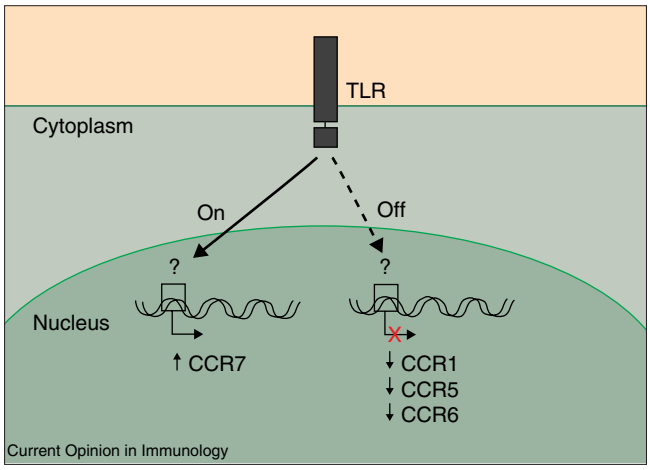
Similar observations have been made studying the role of chemokines in the host response to the intracellular protozoal parasite *Toxoplasma gondii*. Infection by this parasite induces an early and rapid burst in the production of MIP-1α and MIP-1β from dendritic cells in infected tissue [6••]. Recently, my co-worker and I have found that MIP-1α and MIP-1β recruit IFN-γ-secreting NK cells into infected tissue in a manner dependent on CC-chemokine receptor (CCR)5, establishing CCR5 as a critical NK-cell homing receptor (AD Luster, I Khan, unpublished data).

IFN-γ secreted from NK cells induces the production of chemokines including IP-10 from resident tissue cells; these chemokines attract antigen-specific $CD4^+$ and $CD8^+$ lymphocytes into infected tissue to establish organ-specific immunity [7••]. Thus, analogous to antiviral defenses described for CMV [5••], the host response to *T. gondii* requires a 'chemokine to cytokine to chemokine' cascade. This probably represents a general principle that links the innate and acquired immune response to intracellular pathogens and is essential for protective immunity. Chemokines — operating at the level of recruiting NK cells into tissues and then converting the innate NK-cell response into a T-cell response through the function of IFN-γ-inducible chemokines — thus constitute an important link between the innate and adaptive immune response.

The IP-10 connection

As mentioned above, NK-cell production of IFN-γ is important in inducing the expression of the IFN-γ-inducible

Figure 3



TLR activation modulates chemokine-receptor expression on dendritic cells. The maturation of dendritic cells that is induced by TLR activation downregulates the expression of CCR1, CCR5 and CCR6, and upregulates the expression of CCR7. Because TLR stimulation occurs when a dendritic cell is likely to have internalized pathogen-associated antigens, this switch in chemokine-receptor expression ensures that dendritic cells loaded with pathogen-associated antigens leave the tissue and are attracted into the lymphoid system. This modulation of chemokine-receptor expression and subsequent pattern of dendritic-cell migration are crucial for the induction of an adaptive immune response.

chemokines IP-10, Mig and I-TAC (CXCL11). These chemokines bind to and activate CXC-chemokine receptor (CXCR)3, which is expressed on activated $CD4^+$ and $CD8^+$ T cells and NK cells, and is important for the recruitment of these cells into tissues.

IP-10 is also directly induced by bacterial products and viruses, and therefore may play an early role in recruiting the first T cells and NK cells into tissue. In fact, in an allogeneic cardiac transplant model IP-10, but not Mig or I-TAC, was induced in endothelial cells immediately after surgical manipulation of the graft [8••]. This early expression of IP-10 induced by endogenous 'innate' danger signals, such as hypoxia or trauma, was found to be essential for the influx of $CXCR3^+$ NK cells and T cells. Without IP-10 or CXCR3 function, the grafts showed markedly increased survival. Interestingly, many endogenous danger-signaling molecules, such as heat shock proteins, have been shown to activate TLRs [9].

Modulation of chemokine-receptor expression

The early pathogen-induced release of MIP-1α and MIP-1β is also involved in attracting immature dendritic cells into the immediate vicinity of the pathogen. Immature dendritic cells act as sentinels in the tissue and pick up foreign antigen very efficiently, but are not yet potent activators of naive T cells [10]. Dendritic cells have been shown to express TLR-2, -3, -4, -5, -6 and -9 and respond to various pathogen-associated molecular patterns, such as LPS, bacterial lipoproteins, peptidoglycan and CpG dinucleotides [11,12]. The activation of TLRs on dendritic cells has a key role in linking innate and adaptive responses. TLR stimulation induces dendritic-cell maturation, which is characterized by the production of proinflammatory cytokines, upregulation of co-stimulatory molecules and altered expression of chemokine receptors [13]. TLR stimulation occurs when a dendritic cell is likely to have internalized pathogen-associated antigens.

Immature dendritic cells express several chemokine receptors, including CCR1, CCR5 and CCR6 [14]. These receptors help to keep the immature dendritic cell in the tissue. After activation, however, dendritic cells down-modulate the expression of these chemokine receptors and hence their responsiveness to MIP-1α, MIP-1β and MIP-3α (CCL20) [15,16]. At the same time, they upregulate the expression of CCR7, allowing them to respond to SLC (CCL21) and ELC (CCL19) [15,16]. This switch in chemokine-receptor expression and chemokine responsiveness results in the dendritic cells leaving the tissue, and being drawn into the lymphatics and ultimately into the T-cell-rich regions of lymph nodes (Figure 3). This dendritic-cell migration pattern is vital for the induction of an adaptive immune response.

Localization of immature dendritic cells

Cells of the dendritic-cell lineage are now known to be the primary cells responsible for activating naive T and B cells. Dendritic cells, which are formed in the bone marrow, must move into tissues to pick up and respond to foreign antigen. This process is probably under the control of chemokines, but a single chemokine–chemokine-receptor pair has not yet emerged as a dominant player in this process. CCR6 was thought to have a role in this process, but the impairment of dendritic cell function in CCR6-deficient mice has been reported to be limited to the gastrointestinal tract [17•,18].

In a recent study, treating rats with an amino-terminally modified form of RANTES that functions as the selective CCR1 and CCR5 antagonist (Met-RANTES) reduced baseline numbers of tracheal intraepithelial dendritic cells by about 50% [19•]. In addition, pretreating the animals with Met-RANTES before they inhaled an aerosol containing heat-killed bacteria abolished the rapid influx of dendritic cells into the epithelium that occurred in untreated controls. These findings implicate CCR1and CCR5 and their ligands in the recruitment of immature dendritic-cell precursors into the resting airway tissue and during acute bacteria-induced inflammation. But Met-RANTES did not inhibit the influx of dendritic cells into the airway epithelium after airway infection with mucosal Sendai virus or after aerosol challenge in mice that were sensitized with antigen. Thus, many chemokine–chemokine-receptor pairs may be involved in dendritic-cell recruitment into the tissue depending on the nature of the eliciting stimulus. Alternatively, different dendritic-cell subsets may express different chemokine receptors and respond to different chemokines.

T-cell and B-cell trafficking within lymphoid organs

Although the molecular details remain to be elucidated in full, the expression of chemokines by lymph-node stroma and dendritic cells coordinates the juxtaposition of antigen-loaded dendritic cells with re-circulating T and B cells. Chemokines that may be involved in this process include DC-CK1 (CCL18), MDC (CCL22), MIP-3α, SLC and ELC.

SLC and ELC are expressed by T-zone lymph-node stroma and dendritic cells, and recruit naive T cells [20,21]. DC-CK1 is expressed by activated mature dendritic cells and also recruits naive T cells [22]. In contrast, MDC is induced in Langerhans cells migrating from contact-sensitized skin during maturation into lymph-node dendritic cells, and chemoattracts antigen-specific T cells but not naive T cells [23]. BLC is expressed from stromal cells in the B-cell follicle and brings together CXCR5+ T-helper cells and B cells [24–27].

Effector-T-cell trafficking to sites of infection and inflammation

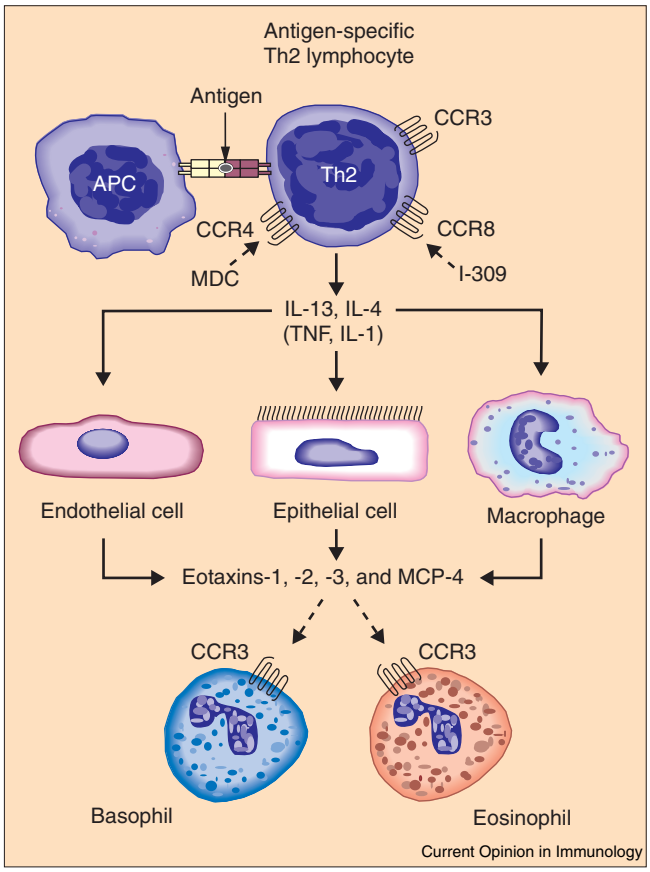
Thus, some activated T cells downregulate CCR7, upregulate CXCR5, become directed toward the follicle to instruct B cells in class switching and have been called 'follicular T cells' [24,27]. In a Th2-polarizing environment, other T cells downregulate CCR7, upregulate CCR3, CCR4 and CCR8, and are attracted into sites of Th2 inflammation. In contrast, in a Th1-polarizing environment, certain T cells upregulate CXCR3 and CCR5, and are attracted into sites of Th1 inflammation [28].

T cells that are activated in regional lymph nodes after encounter with activated antigen-loaded dendritic cells are recruited to sites of inflammation by sensing chemokine gradients established at these local sites. For Th2 cells, chemokines induced by Th2 cytokines and regulated by the transcription factor STAT6 (signal transducer and activator of transcription 6), such as eotaxins-1, -2 and -3 (CCL11, CCL24 and CCL26, respectively), MDC, TARC (CCL17) and I-309 (CCL1) appear to control the trafficking of Th2 cells into sites of allergic inflammation [29••]. For Th1 cells, chemokines induced by IFN-γ, including IP-10, Mig and I-TAC, recruit Th1 cells into tissues.

Chemokines link innate cells to the activation of adaptive immune cells in the tissue

Once in the tissue, Th2 cells secrete IL-4 and IL-13, and amplify Th2-cell recruitment by inducing the release of STAT6-inducible chemokines, which are active on Th2 cells. Interestingly, many of these same chemokines are also active on eosinophils, basophils and mast cells. In this way, Th2 cells control the trafficking of eosinophils, basophils and perhaps mast cells into sites of allergic inflammation (Figure 4). Thus, activation of adaptive immune cells in the tissue leads to the production of chemokines, which recruit innate immune cells into the tissue. In the case of Th2 inflammation, activation of Th2 cells in tissue leads to the influx of eosinophils, basophils and mast cells.

Figure 4



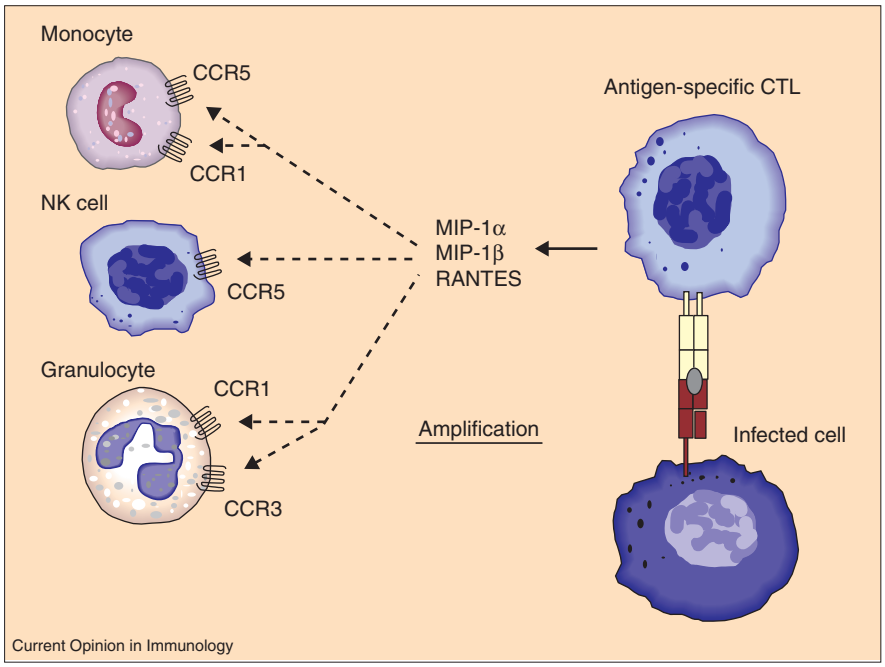
Chemokines link the activation of Th2 lymphocytes and tissue eosinophilia. Antigen-activated CD4+ Th2 cells express IL-4 and IL-13, which synergize with proinflammatory cytokines such as IL-1 and TNF, and stimulate from epithelial cells, endothelial cells and tissue macrophages the production of eosinophil chemoattractants, including eotaxin-1, eotaxin-2, eotaxin-3 and MCP-4. In turn, these chemokines attract activated eosinophils and basophils into the tissue, which results in the hallmarks of allergic diseases. APC, antigen-presenting cell.

Chemokines also link activation of the Th1 adaptive cellular immunity in the tissue to the innate immune response. Chemokines are released from cytotoxic CD8+ T lymphocytes after antigen-specific activation. My co-workers and I have found that cytotoxic lymphocytes specific for HIV-1, HBV (hepatitis B virus) or HCV vectorially release large amounts of MIP-1α, MIP-1β and RANTES from their cytotoxic granules [30]. The release of chemokines directly onto the infected cell recruits other inflammatory cells, including professional phagocytes, to the site of viral replication, amplifying the response to include innate immune effector cells (Figure 5).

Conclusions

Chemokines are essential for the trafficking of immune effector cells to sites of infection, and it is becoming increasingly clear that their function is necessary to translate an innate immune response into an acquired

Figure 5



Chemokines released from cytotoxic T lymphocytes (CTLs) localize and amplify the immune response by recruiting leukocytes to the site of viral replication. Antigen expressed on infected cells induces activation of $CD8^+$ CTLs and results in the release of MIP-1α, MIP-1β and RANTES directly onto the target cell. The release of these chemokines at the site of infection also serves as a beacon to call in additional leukocytes, such as monocytes/macrophages, granulocytes and NK cells, resulting in the amplification of the local immune response.

response. Innate immune stimuli — through activation of TLRs — set in motion a genetic program that induces the expression of a subset of chemokines from resident tissue macrophages and dendritic cells, and modulates the expression of chemokine receptors on dendritic cells.

These changes in chemokine/chemokine-receptor expression orchestrate the movement of antigen-loaded dendritic cells from the tissue into lymphoid tissue to activate naive T and B cells to initiate the adaptive immune response. Chemokines downstream of TLR activation also help guide the newly activated T cells back into the tissue where the innate immune system first sensed the foreign challenge.

In addition, during secondary immune responses chemokines induced by antigen-specific lymphocyte responses recruit innate immune cells into sites of inflammation, serving to amplify the adaptive response with innate immune effector cells. Thus, chemokines and their receptors serve a critical function in coordinating the interdependent innate and adaptive immune responses.

Future studies will undoubtedly continue to unravel the complexity of the chemokine system. It is likely that these studies will continue to add to our knowledge that the chemokine system finely regulates leukocyte trafficking and that this trafficking is critical to immune-cell and immune-system function.

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