Expression and function of chemokines during viral infections: from molecular mechanisms to in vivo function

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Abstract: Recruitment and activation of leukocytes are important for elimination of microbes, including viruses, from infected areas. Chemokines constitute a group of bioactive peptides that regulate leukocyte migration and also contribute to activation of these cells. Chemokines are essential mediators of inflammation and important for control of viral infections. The profile of chemokine expression contributes to shaping the immune response during viral infection, whereas viral subversion of the chemokine system allows the virus to evade antiviral activities of the host. In this review, we discuss the role of chemokines in host-defense against virus infections, and we also look deeper into the virus-cell interactions that trigger chemokine expression as well as the cellular signaling cascades involved. *J. Leukoc. Biol.* 74: 331–343; 2003.

Key Words: *host-defense · leukocytes · signal transduction · NF-κB · IRF-3*

INTRODUCTION

Host-defense against viral infections

When facing a viral infection, most cell types in the body respond by secreting high levels of type 1 interferons (IFN-α/β). This induction occurs within a few hours of the infection, and IFN-α/β has a number of antiviral functions [1]. First, IFN-α/β directly induces antiviral activities in the uninfected, neighboring cells. This prevents viral spread by increasing the resistance of uninfected cells toward the virus. Second, IFNs can activate natural killer (NK) cell-mediated cytotoxity toward virus-infected cells [2, 3]. Third, there is accumulating evidence that IFN-α/β contributes to driving the adaptive-immune response in the T helper cell type 1 (Th1) direction via stimulation of IFN-γ expression [4, 5].

The cellular antiviral response is initiated by activation of NK cells, which kill virus-infected cells directly and also activate other cells of the innate- and adaptive-immune system through production of cytokines, notably IFN-γ [6]. One of the IFN-γ-activated leukocytes is the macrophage, which is sensitive to IFN-γ and other proinflammatory cytokines, including tumor necrosis factor α (TNF-α) [7]. Macrophages participate in the antiviral response through production of free radicals and proinflammatory cytokines [8] as well as by working as an antigen-presenting cell (APC). It is also possible that macrophages contribute to antiviral defense via phagocytosis of extracellular virus and infected apoptotic cells [7]. Dendritic cells (DCs) also participate in the antiviral response through several mechanisms [9]. First, DCs are probably the main APC, thus playing a pivotal role in development and maturation of the specific immune response. Second, this cell type participates in coordinating the inflammatory response, through production of cytokines and chemokines (see below). In particular, cells of the DC lineage have recently been shown to represent the main source of IFN-α/β during viral infections [10, 11].

Another prominent response to viral infection is the expansion and activation of CD4+ and CD8+ T cells, which have central roles in antiviral immunity and work through various mechanisms to inhibit replication and clear infection [12]. First, CD8+ cells have a direct effector role through cytotoxic T lymphocyte-mediated lysis. Second, this cell type produces IFN-γ and TNF-α, cytokines found to possess a prominent portion of the antiviral function of CD8+ cells during some viral infections [13]. Third, CD8+ cells play a central role in maintenance of the antiviral response by production of chemokines, which modulate the immune response and attract the appropriate leukocyte subsets to the site of infection [2, 14]. The role of CD4+ T cells in antiviral immunity is highly dependent on production of cytokines, notably IFN-γ [15, 16], and the cytolytic activity exerted by a subset of CD4+ T cells [17].

Activation, coordination, and regulation of the above-described antiviral response are mediated by complex mechanisms, where cytokines play important roles. Within the large group of cytokines, the subgroup of chemotactic cytokines, chemokines, is now also recognized to participate in defense against viral infections. Moreover, chemokines can in some instances be important mediators of the immunopathology associated with many viral infections. In this review, we focus our attention on chemokines and viral infections.

CHEMOKINES

Chemokines constitute a family of small, secretory proteins that are expressed constitutively or in an inducible manner.

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Received November 25, 2002; accepted March 20, 2003; doi: 10.1189/jlb.1102577.

Their main functions are to attract leukocytes to sites of infection and inflammation and to contribute to the homeostatic circulation of leukocytes through tissue [18]. Chemokines also have the capacity to directly activate leukocytes, e.g., to release granula contents and produce cytokines [19].

Today, ~50 chemokines and 20 chemokine receptors have been identified [19]. Chemokines are 80–130 amino acid residues in size and have a homologous structure consisting of antiparallel β-strands with connecting loops held together by disulfide bonds between cysteine residues. The disulfide bonds keep together two domains in the molecule, which are both important for receptor binding and activation [20]. The amino-terminal cysteine residues also form the basis for the systematic classification of chemokines, based on their numbers and mutual placement. The two major groups are CXC chemokines and CC chemokines, both groups having four cysteine residues. In CXC chemokines, the two first cysteine residues are separated by one amino acid, whereas in CC chemokines, the two cysteine residues lie adjacent. Besides the CC and CXC chemokines, there are two minor groups, CX_3C and C chemokines, with one and two members, respectively. The CX_3C chemokine CX3CL1/neurotactin (murine) or fractalkin (human), which is a membrane-bound glycoprotein, has three residues between the two amino-terminal cysteines, and the C chemokines have only one amino-terminal cysteine residue [18].

Chemokines can also be classified based on their pattern of expression. First, homeostatic chemokines are expressed constitutively and participate in recirculation of leukocytes between tissue and lymphatics and in the traffic of leukocytes within compartments of lymph nodes and thymus [21]. Second, inflammatory chemokines are induced by infection and other proinflammatory stimuli and function as mediators of inflammation by attracting leukocytes to the site of infection.

Recently, the nomenclature of chemokines has been changed to a systematic system. The CC chemokines have been renamed CC chemokine ligand (CCL) 1, 2, 3, etc., and the CXC chemokines, CXCL1, 2, etc. [22]. In this paper, we use the new nomenclature but also provide the traditional name when introducing a new chemokine, as most workers in this field, including ourselves, will need some time to get used to the new nomenclature.

The chemokine receptors are seven-transmembrane-spanning, G-protein-coupled receptors that have a number of conserved motifs. They are classified based on the class of chemokines they bind, e.g., CC chemokine receptors (CCRs) bind CCLs, and CXC chemokine receptors (CXCRs) bind CXCLs. Within the group of inflammatory chemokines, there is a great degree of redundancy; i.e., most receptors bind several chemokines, and most chemokines use more than one receptor. In the group of homing chemokines, conversely, most receptors have only one ligand, although there are exceptions (e.g., CCR7). Chemokine receptors are expressed primarily on leukocytes but also on other cells, e.g., endothelial cells. The specific expression of chemokine receptors is dependent on cell type as well as the state of differentiation and activation of the cell [23].

Besides these receptors, chemokines can bind to nonsignaling molecules. First, in the tissue, chemokines can bind to heparan sulfate proteoglycans in the extracellular matrix or on the surface of cells. As the chemokines retain their chemotactic activity during this binding, they establish a concentration gradient contributing to the attraction of leukocytes [18]. Second, chemokines can bind to the Duffy antigen receptor for chemokines (DARC), expressed on erythrocytes and endothelial cells. DARC can bind CC as well as CXC chemokines and may function as a sink, clearing chemokines from the circulation [18]. A third chemokine-binding protein is D6, which binds CC chemokines but not C, CXC, or CX_3C chemokines [24]. The function of this receptor, which is expressed on many cell types within and without the hemopoietic system, remains to be understood in detail.

In this review, we discuss what is currently known about chemokines in antiviral defense and review the literature on molecular mechanisms regulating chemokine expression during virus infections. As this review focuses on chemokines in antiviral defense, other aspects of chemokine biology, although important, are not described here. These include the ability of chemokine receptors to function as coreceptors for human immunodeficiency virus (HIV), which is excellently reviewed elsewhere [25, 26].

EXPRESSION OF CHEMOKINES DURING VIRAL INFECTIONS

To understand the mechanisms governing recruitment of leukocytes to infected areas, knowledge about the chemokine expression profile during viral infections is important. Although most of this work has been done in vitro, a substantial body of information about chemokine expression in vivo is now available. **Table 1** summarizes the data on chemokine expression during viral infection in humans as well as in experimental animals. From the available data, it appears that expression of CC chemokines dominates over the expression of CXC chemokines during viral infections. Among the CC chemokines, CCL3/macrophage inflammatory protein (MIP)-1α and CCL5/regulated upon activation, normal T cell-expressed and secreted (RANTES) seem to be almost invariably associated with viral infections, with some interesting exceptions. For instance, during an intranasal influenza virus infection in mice, only CCL2/monocyte chemotactic protein-1 and CXCL10/IFN-γ-inducible protein of 10 kDa (IP-10) were detected in the lungs at various time-points post-infection, whereas other chemokines including CCL3 and CCL5 were not expressed [36]. By comparison, RSV-infected mice display high levels of expression of numerous chemokines in the lungs, including CCL3 and CCL5 [38]. This observation in mice correlates very well with the results of a comparative study in pediatric patients infected with RSV and influenza virus [45]. Although serum levels of CCL5 were very high in RSV-infected children, this was not the case in influenza virus-infected patients. This difference in chemokine response to these two viruses may be explained by the ability of influenza virus to inhibit various activities of the immune response, in particular the production and activity of IFNs [46].

Although CC chemokine expression seems to dominate, CXC chemokines are also expressed. Among the CXC chemokines, expression of CXCL10 is found in many viral infections

TABLE 1. Expression of Chemokines during Virus Infections in vivo

Virus	Virus family/genome	Species (strain)	Site of expression	Virus-induced chemokine expression	Refs.
HSV-1[a]	Herpesvirus/DNA	Mouse (B)	Cornea	CCL2, CCL3, CCL4, CXCL1, CXCL2/3, XCL1	[27–29]
		Mouse (ICR)	TG (latency)	CCL5	[30]
		Man	CSF	CCL2, CCL3, CCL5, CXCL8	[31]
HSV-2	Herpesvirus/DNA	Mouse (B)	Liver	CCL5, CCL8	Note[b]
			Spleen	CCL5	Note[b]
			Brain	CCL3, CCL5	Note[b]
			Peritoneum	CCL3, CCL5, CCL7, CCL8, CCL9	Note[b]
MCMV	Herpesvirus/DNA	Mouse (C)	Liver	CCL3, CXCL9	[32, 33]
MHV-68	Herpesvirus/DNA	Mouse (B)	Lung	CCL1, CCL3, CCL5, CXCL2/3, CXCL10	[34]
HCV	Flavivirus/RNA	Man	Liver	CCL5, CXCL9	[35]
Influenza virus	Orthomyxovirus/RNA	Mouse (Cx129)	Lung	CCL2, CXCL10	[36]
			Lymph	CCL5	[36]
PVM	Paramyxovirus/RNA	Mouse (C)	Lung	CCL2, CCL3, CCL5, CCL7, CCL11	[37]
RSV	Paramyxovirus/RNA	Mouse (B)	Lung	CCL1, CCL2, CCL3, CCL4, CCL5, CXCL2/3, CXCL10, XCL1	[38]
		Man	UST	CCL3, CCL5	[39]
MHV	Coronavirus/RNA	Mouse (C, C*)	Brain	CCL2, CCL3, CCL4, CCL5, CXCL1, CXCL9, CXCL10	[40, 41]
TMEV	Picornavirus/RNA	Mouse (SJL)	Brain	CCL2, CCL4, CCL5, CCL6, CXCL10	[42]
pMuLV	Retrovirus/RNA	Mouse (R)	Brain	CCL2, CCL3, CCL4, CCL5, CXCL10	[43]
VSV	Rhabdovirus/RNA	Mouse (C)	Brain	CCL2, CCL3, CCL4, CCL5, CXCL2/3, CXCL10	[44]
LCMV	Arenavirus/RNA	Mouse (C)	Brain	CCL2, CCL3, CCL4, CCL5, CXCL2/3, CXCL10, XCL1	[44]

[a] HSV, Herpes simplex virus; B, BALB/c; TG, trigemina ganglia; CSF, cerebrospinal fluid; MCMV, murine cytomegalovirus; C, C57BL/6; MHV-68, murine γ-Herpes virus-68; HCV, hepatitis C virus; PVM, pneumonia virus of mice; RSV, respiratory syncytial virus; UST, upper respiratory tract; MHV, mouse hepatitis virus; C*, C6129F2/J; TMEV, Theiler's murine encephalomyelitis virus; pMuLV, polytropic murine leukemia viruses; R, Rocky Mountain white mice; VSV, vesicular stomatitis virus; LCMV, lymphocytic choriomeningitis virus. [b] Sørensen, L. N., Paludan, S. R. (2003), submitted.

[36, 38, 40, 43]. CXCL10 together with CXCL9/monokine induced by IFN-γ (MIG) and CXCL11/IFN-inducible T cell α chemoattractant constitute the non-ELR CXC chemokines, which all use CXCR3 as receptor [18]. Given the strong activity of this receptor as mediator of T cell recruitment, expression of the non-ELR CXC chemokines may be central for mounting an efficient antiviral response. This idea is supported by the finding of a statistically significant association between intrahepatic expression of CXCL9 and the inflammatory activity of chronic hepatitis C [35]. However, as will be discussed below, CXCL9- and CXCL10-dependent functions can also lead to excessive immune activation and accompanying immunopathology [47].

Another interesting observation from the information shown in Table 1 is the expression of CCL5 in areas of latent herpesvirus infections. For instance, CCL5 expression was detectable in HSV-1-infected TG long after the virus had been cleared [30], and a different study showed that MHV-68 infection strongly induced expression of many chemokines in the lungs [34]. Moreover, although the virus was rapidly cleared from the lungs, CCL5 protein was present up to 1 month after the infection. These findings show that latently infected cells do stimulate the immune system, despite lack of detectable viral replication, and also explains why CD8+ T cells are recruited to TGs latently infected with HSV-1 [48].

Altogether, viral infections are associated with expression of chemokines, and CC chemokines dominate over the other classes. In the section below, we review the literature regarding the role of chemokines in defense and disease during viral infections.

ROLE OF CHEMOKINES IN HOST-DEFENSE AGAINST VIRAL INFECTIONS

Viruses themselves provide the best indication that chemokines are important for antiviral defense. The existence of more than 30 virally encoded chemokine and chemokine receptor mimics [49], at least one of which has been demonstrated to contribute to inhibition of the antiviral host response [50], strongly suggests that chemokines are important for restriction of virus infections.

Although much remains to be learned about the role of chemokines during viral infections in man, some information is available. In one study, it was found that expression of CCL5 and CXCL9 correlated statistically with the inflammatory activity in patients with chronic HCV infection [35]. This observation is interesting in light of a recent publication by the group of Chisari (Ref. 47), where the authors demonstrated that neutralization of CXCL9 and CXCL10 in the murine model for HBV infection did not prevent antiviral defense but diminished liver pathology [27]. Therefore, certain chemokines, although potentially contributing to antiviral activity, may also fuel the host response responsible for the pathology of chronic viral hepatitis.

Chemokines have also been studied in viral meningitis and encephalitis in humans. Among chemokines detected in patients with viral meningitis, notably CCL2 and CXCL10 were abundantly expressed [51]. CCL2 and CXCL10 were identified in the CSF of 97% and 79%, respectively, of the patients and in concentrations sufficient to mediate chemotaxis of mononuclear cells. In a different study, the expression of chemokines

in patients with HSV-1 encephalitis was evaluated and compared with the clinical status of the patients [31]. Although several chemokines were detected in the CSF, CCL2 was particularly interesting, as the authors found a strong, reciprocal correlation between the clinical status of the infection and the levels of CCL2. Together, these findings place CCL2 at a cardinal position with respect to defense against virus infections in the central nervous system. As will be discussed below, this idea is supported by studies performed in mice lacking CCR2, the receptor for CCL2 [41].

Much of our knowledge about the role of specific chemokines and receptors during various viral infections has been gathered through studies in mice. Experimentally, this has been done using specific gene-deleted mice strains or through blocking specific members of the chemokine system with antibodies. As shown in **Table 2**, there is now evidence that several chemokines and receptors contribute to antiviral defense and immunopathology. Selected examples are described in more detail below.

Mice deficient in the chemokine receptor CCR1 show increased viral titers after lower respiratory tract infection with PVM, which was found to correlate with enhanced mortality [52, 53]. Conversely, lack of CCR1 does not affect the ability to clear RSV infections, despite high production of the CCR1 ligand CCL3 [53]. Also, mice deficient in CCL3 exhibit delayed viral clearance when infected with influenza virus, PVM, or MCMV [32, 52, 56]. In an impressive series of studies, Biron and colleagues [32, 33, 61] described the leukocyte-cytokine/chemokine interactions responsible for the antiviral activity of CCL3 in the liver. They identified IFN-α/β as the main inducer of CCL3 expression, which in turn, triggered recruitment of NK cells. This cell type subsequently produced IFN-γ, which was then responsible for induction of expression of CXCL9, a chemokine mediating chemotaxis toward T cells [32, 33, 61]. Collectively, the studies discussed above indicate that CCR1 and its ligands are necessary to raise a sufficient immune response against many viruses. In other viral infections, however, CCL3 has been found not to be important for viral clearance. For instance, lack of CCL3 does not affect viral titers after intraperitoneal Coxsackie B3 virus infection, intranasal RSV infection, and ocular HSV-1 infection [38, 56, 57]. However, in these cases, CCL3 contributes to the pathology of the infections, as CCL3-deficient mice did not develop myocarditis or keratitis after Coxsackie B3 virus and HSV-1 infections, respectively [56, 57], and the CCL3-deficient mice also displayed lower pulmonary inflammation after RSV infection as compared with wild-type mice [38].

In another series of studies, respiratory tract infection with influenza virus in CCR2 −/− mice caused a decreased inflammatory response, increased viral titers, but reduced mortality when compared with wild-type mice [36]. A similar outcome was seen in mice deficient in CCR2 and CCL3. CCR5 −/− mice, however, showed increased infiltration of macrophages, increased pathological changes in the tissue, enhanced mortality, but unchanged viral titers compared with control mice when facing the same infection [36]. A role for CCR5 in immunopathology has also been found during intracerebral infection with MHV. Glass et al. [55] observed that deletion of CCR5 did not affect viral titers and mortality but did decrease postinfectious demyelination when compared with control mice.

Besides attraction of leukocytes to the site of infection, studies have indicated that chemokines also have a role in the development of a Th1 versus Th2 immune response [62]. In mice immunized with DNA vaccines encoding HSV-2 glycoprotein D, coimmunization with CCL5 or CXCL8/IL-8 enhanced an antigen-specific Th1 CD4+ T cell-dependent immune response, which correlated with decreased morbidity and mortality during the infection [63]. Moreover, mice lacking CCR2 display an impaired Th1 cytokine profile in the brain after MHV infection despite higher viral titers [41]. In agreement with this, a study in humans showed that in patients infected with HIV, expression of CCL3, CCL4, and CCL5 correlated with a Th1 type of immune response [64].

Together, these results indicate that the chemokine system is necessary for control of some viral infections and also seems to contribute to shaping the immune response, which in some cases, may lead to an overwhelming inflammatory response with the poitential to cause tissue damage.

MOLECULAR MECHANISMS GOVERNING VIRUS-INDUCED CHEMOKINE EXPRESSION

In the section above, we have described what is currently known about the functions of chemokines during virus infections in vivo. As these studies have revealed that chemokines are involved in host-defense and immunopathology, there is increasing interest in the molecular mechanisms that govern chemokine expression in virus-infected cells. From Table 1 and the discussion in the text, it is apparent that among the chemokines produced during viral infection, notably, CCL3, CCL5, and CXCL10 are abundantly expressed. Among these, CCL5 is the most studied with respect to molecular mechanisms governing virus-induced chemokine expression. Therefore, the discussion below focuses mainly on CCL5, but we will provide information on other chemokines when available. First, we review the basic principles in virus-cell interactions and virus-activated cellular signal transduction, and subsequently, we discuss the literature on molecular mechanisms involved in regulation of chemokine expression during virus infection.

VIRUS-CELL INTERACTIONS IN VIRUS-INFECTED CELLS

Virus infection can lead to activation of cellular signal transduction through a number of mechanisms. These include interactions of viral surface proteins with cellular receptors, accumulation of viral double-stranded RNA (dsRNA) produced during the viral life cycle, overload of the cellular protein synthesis machinery, mitochondrial oxidative stress, and virus-encoded signaling molecules [65]. Below these mechanisms are described in brief.

First, activation of cellular signal transduction by viral surface proteins occurs through engagement of cellular receptors, which undergo oligomerization or conformational changes following ligand binding and thus, initiate signaling events [66].

TABLE 2. Role of Chemokines and Chemokine Receptors in the Host Response to Virus Infections

Knockout/Ab treatment	Outcome of infection	Refs.
CCR1–/–	PVM[a]—lower respiratory tract infection Leukocyte infiltration ↓[b] CCL3 expression ↑ Virus titers ↑ Mortality ↑	[52]
CCR1–/–	RSV—lower respiratory tract infection Leukocyte infiltration ↔ Virus titers ↔	[53]
CCR2–/–	Influenza virus—respiratory tract infection Macrophage and T cell infiltration ↓ Delayed pulmonary tissue damage Virus titers ↑ Mortality ↓	[36]
CCR2–/–	MHV—intracerebral infection $CD4^+$, $CD8^+$, and macrophage infiltration ↓ IFN-γ and CCL5 expression ↓ Virus titers ↑ Mortality ↑	[41]
CCR5–/–	LCMV infection Systemic: generation of $CD4^+$ and $CD8^+$ ↑ Virus titers ↔ Intracerebral: accelerated mortality Mononuclear cell infiltration ↔	[54]
CCR5–/–	MHV—intracerebral infection Delayed $CD4^+$ and $CD8^+$ infiltration Macrophage infiltration ↓ Virus titers ↔ (↑ early in infection) Mortality ↔ Demyelination ↓	[55]
CCR5–/–	Influenza virus—respiratory tract infection Macrophage infiltration ↑ Accelerated pulmonary tissue damage Virus titers ↔ Mortality ↑	[36]
CCR1, CCR5 (Met-RANTES)	HSV-2—intraperitoneal infection Virus titers (liver, brain) ↑ Proinflammatory cytokines ↑ NK cell recruitment to peritoneum ↓	Note[c]
CCL3–/–	Influenza virus—respiratory tract infection Mononuclear infiltration and tissue damage ↓ Virus titers ↑/delayed clearance	[56]
CCL3–/–	PVM—lower respiratory tract infection Leukocyte infiltration ↓ Virus titers ↑	[52]
CCL3–/–	RSV—lower respiratory tract infection Mononuclear cell infiltration ↓ Pulmonary inflammation ↓ CCL2, CCL5, CXCL2, and XCL1 ↓ Virus titers ↔ (day 5)	[38]
CCL3–/–	Cocksackievirus B3 virus—intraperitoneal infection Myocardial pathology ↓ Virus titers ↔	[56]
CCL3–/–	MCMV—intraperitoneal infection NK cell infiltration in liver ↓ IFN-γ and CXCL9 induction in liver ↓ Virus titers ↑/delayed clearance Mortality ↑	[32]
CCL3–/–	HSV-1—corneal infection $CD4^+$ and neutrophil infiltration ↓ IFN-γ, IL-2, CCL2, CXCL2 expression ↓ Virus titers ↔ Corneal opacity ↓	[57]
CCL5 (RANTES; Ab)	MHV-68—respiratory tract infection Lymph node cell chemotaxis ↓ (ex vivo)	[58]
CXCL2 (Ab)	HSV-1—corneal infection Neutrophil infiltration ↓ Corneal opacity ↓	[27]
CXCL9 (MIG; Ab)	MHV–intracerebral infection $CD4^+$ and $CD8^+$ infiltration ↓ IFN-γ and IFN-β expression ↓ IL-10 production ↑ Delayed virus clearance	[40]
CXCL9 (Ab) Treatment from day 12	MHV—intracerebral infection $CD4^+$ and macrophage infiltration ↔ CCL5 production ↔ Infiltration and demyelination ↔	[59]
CXCL9, CXCL10 (Ab)	HBV—transgenic mice with hepatic HBV replication Virus titers ↔ Infiltration of lymphomononuclear cells ↓ Liver disease ↓	[47]
CXCL10–/–	MHV—intracerebral infection $CD4^+$, $CD8^+$, and macrophage infiltration ↓ IFN-γ, CXCL9, and CXCL11 expression ↓ Virus titers ↑ (day 12)/delayed clearance Demyelination ↓	[60]
CXCL10 (Ab) Treatment from day 12	MHV—intracerebral infection $CD4^+$ and macrophage infiltration ↓ CCL5 production and IFN-γ expression ↓ Demyelination ↓ and initiation of remyelination	[59]
CXCL10 (Ab)	MHV-68—respiratory tract infection Lymph node cell chemotaxis ↓ (ex vivo)	[58]

[a] Ab, Antibody; IL, interleukin. [b] Symbols used in the text: ↓, Parameter decreased in knockout or antibody-treated mice relative to control mice; ↑, parameter increased in knockout or antibody-treated mice relative to control mice; ↔, parameter unchanged in knockout or antibody-treated mice relative to control mice. [c] Sørensen, L. N., Paludan, S. R. (2003), submitted.

Second, viral dsRNA is produced during viral replication and is sensed by the cell through at least two mechanisms: the dsRNA-activated protein kinase (PKR) and Toll-like receptor (TLR) 3 [67, 68]. Third, overload of viral proteins in the endoplasmic reticulum as well as virus-induced mitochondrial oxidative stress triggers release of calcium from the organelles, which activate kinase cascades, ultimately leading to expression of proinflammatory genes (ref. [69]; and T. H. Mogensen et al., *J. Immunol.* in press). Finally, some viral proteins have the capacity to specifically activate certain signal-transduction pathways in infected cells [70]. Although it may not seem beneficial for the virus to deliberately activate signal transduction that promotes antiviral defense, the virus may profit from this by stimulating chemokine expression and hence promote recruitment of new cells to infect, which may subsequently relocate and hence support viral spread in the body [71]. Additionally, some viruses harbor sequences in their gene promoters that are recognized by transcription factors, specifically activated by viral proteins [72]. In the literature, each of the above mechanisms has been reported to activate signal-transduction pathways necessary for cytokine and chemokine production [73].

To illustrate the above principles, the Epstein-Barr virus (EBV) glycoprotein gp350 induces expression of CXCL8 and CCL3 in human neutrophils [74]. This viral glycoprotein uses the cellular surface protein CD21 as receptor and triggers intracellular signaling [75]. Although neutrophils are not the main target for EBV, this finding does demonstrate that gp350 is capable of inducing chemokine expression. Another EBV protein, latent membrane protein 1, which is produced in certain stages of EBV latency and is required for transformation of infected cells, is also able to induce expression of certain chemokines [76].

Altogether, viruses infect cells and affect the intracellular milieu in a profound manner. The sections below describe the cellular signal-transduction pathways activated by virus infections and how these contribute to chemokine expression.

VIRUS-ACTIVATED SIGNAL TRANSDUCTION

Virus-activated signal transduction promotes expression of many proinflammatory proteins, including chemokines. In this section, we describe these pathways, which are depicted in **Figure 1**.

NF-κB is an important cellular, proinflammatory transcription factor, present in the cytoplasm in a latent form associated with IκB [77]. Following the appropriate stimulus, IκB is serine-phosphorylated by the IκB kinase, ubiquitinated, and eventually degraded by the 26S proteasome. Released from IκB, the nuclear localization signal of NF-κB is exposed, and the transcription factor migrates to the nucleus and activates transcription. Viruses are able to activate NF-κB through many different mechanisms. (For a more detailed description, see ref. [73].) As will be described below in detail, activation of NF-κB is indispensable for expression of certain inflammatory chemokines during viral infections.

The IRF family of transcription factors is involved in many aspects of the immune response [78]. For instance, IRF-1-deficient mice fail to develop NK cells and are hyporesponsive to IFN-γ. With respect to virus-induced signal transduction, IRF-3 and IRF-7 are activated in a virus-specific manner and play crucial roles in clearance of virus infections [79]. Like NF-κB, the two IRFs require post-translational modification to become activated. Following infection, IRF-3 and IRF-7 are serine/threonine-phosphosphorylated by a kinase, VAK, allowing the proteins to adapt a conformation compatible with homo- or heterodimerization. The dimers migrate to the nucleus and activate transcription. There is evidence that IRF-3 and IRF-7 play important roles in virus-induced expression of the cytokine IFN-α/β [79] as well as CCL5 [80] (see below). Recently, IRF-5 has also been linked to virus-induced production of chemokines [81].

A third group of transcription factors activated by many viruses includes AP-1 and ATF2/Jun. Activation of these transcription factors is dependent on up-stream kinases of the mitogen-activated protein (MAP) kinase family, e.g., p38 and JNK [82]. The transcription factors are active as dimmers, and Jun family members are part of the ATF2/Jun complex and AP-1. The involvement of AP-1 and ATF2/Jun in virus-induced cytokine and chemokine induction is well documented. For example, HSV-2-induced expression of IL-6 and TNF-α production relies on ATF2/Jun [83, 84], and HIV-1 up-regulates production of CCL2 via AP-1 [85].

Another transcription factor activated in response to virus infection is NF-AT, which is constitutively present in the cytoplasm of some cell types, notably T cells, in a latent, phosphorylated form. Increasing levels of cytoplasmic calcium activate the calmodulin-dependent phosphatase calcineurin, which activates NF-AT by dephosphorylation [86].

In addition to the above-described examples, virus infection has been reported to affect other cellular transcription factor activities. These include C/EBPs, selective promoter factor 1 (Sp-1), and CRE-binding protein [87, 88].

In summary, many signal-transduction pathways are activated in virus-infected cells, and our knowledge about the underlying molecular mechanisms for these activities is increasing. Below, we focus the discussion on CCL5 and to a lesser extent, CCL3, CXCL8, and CXCL10 and go through the current knowledge on the mechanisms of chemokine expression in virus-infected cells.

REGULATION OF CHEMOKINE PRODUCTION AT THE TRANSCRIPTIONAL LEVEL

Many chemokines are induced in response to virus infections (Table 1), and a number of studies have addressed the underlying mechanisms in detail; it appears that common as well as virus-specific activities are involved (**Table 3**). Expression of most chemokines is regulated primarily at the level of transcription, and their gene promoter regions contain recognition sites for many virus-activated transcription factors. Figure 1 depicts the promoters of CCL3, CCL5, CXCL8, and CXCL10, all of which have been studied in the context of viral infections. As it appears from Figure 1, these promoters contain common as well as promoter-specific regulatory elements, which explains why many chemokines display partly overlapping, yet

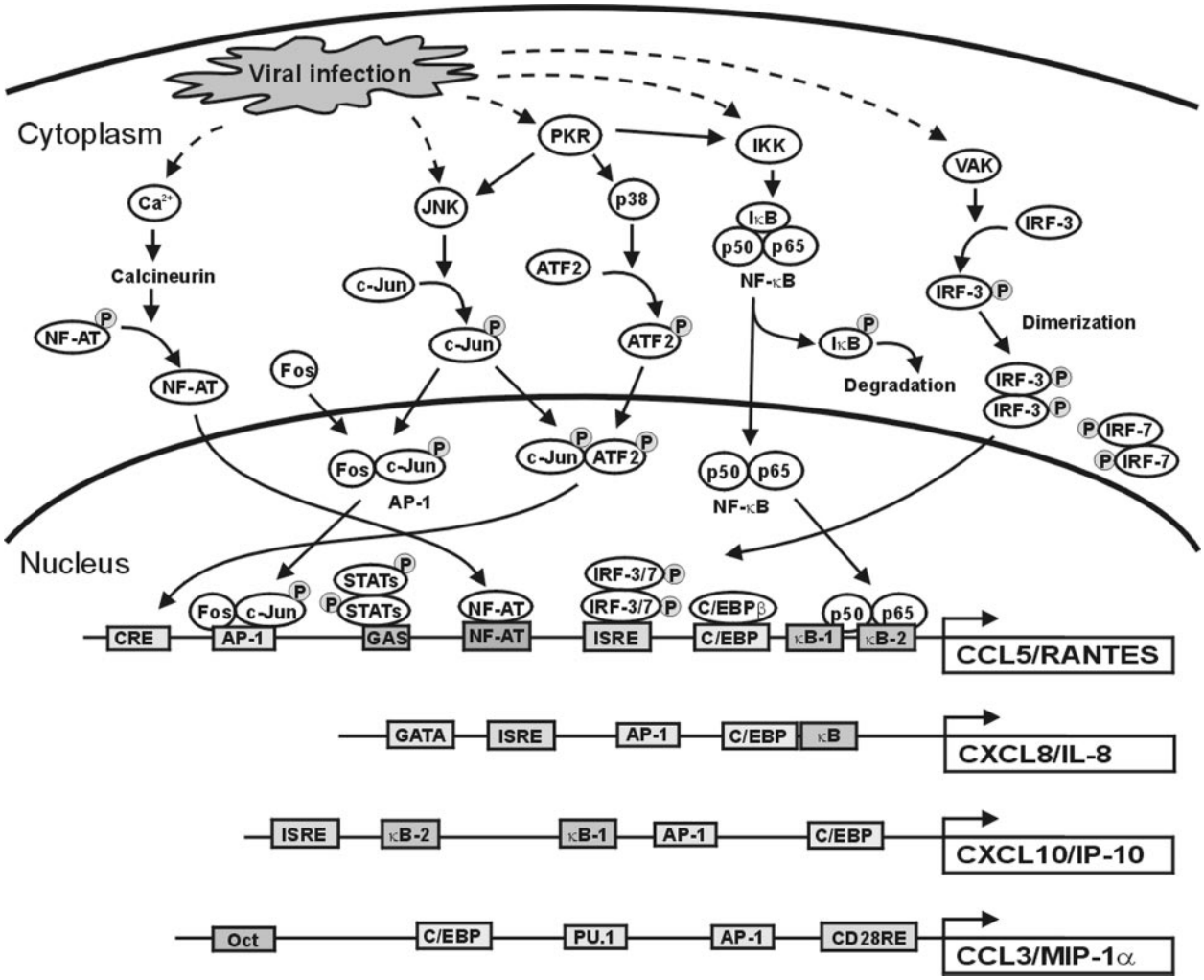

Fig. 1. Virus-activated signal transduction. Cells respond to virus infection by activating a number of signal-transduction cascades, which lead to nuclear translocation of a specific set of transcription factors. Ultimately, the activated transcription factors stimulate expression of chemokines and other proinflammatory mediators. For a more detailed description of virus-activated signal transduction, see the text. This figure depicts the gene promoter regions of CCL5/RANTES, CCL3/MIP-1α, CXCL8/IL-8, and CXCL10/IP-10, which are discussed in more detail in the text. NF-AT, Nuclear factor of activated T cells; JNK, Jun N-terminal kinase; ATF2, activating transcription factor 2; AP-1, activator protein-1; STAT, signal transducer and activator of transcription; IKK, inhibitor of κB (IκB) kinase; VAK, virus-activated kinase; IRF, IFN regulatory factor; CRE, cyclic adenosine monophosphate response element; GAS, IFN-γ activation site; ISRE, IFN-stimulated response element; C/EBP, CCAAT enhancer-binding protein; CD28RE, CD28 response element.

distinct, patterns of expression. As will be described below, the cooperation between transcription factors binding these promoter elements is of utmost importance in the regulation of chemokine expression.

For CCL5, most of the characterized promoter elements have been shown to contribute to expression of this CC chemokine during virus infection, with NF-κB and IRFs being ascribed particularly important roles. This phenomenon was recently explained mechanistically by the finding that NF-κB and IRF-3/7 synergistically promote CCL5 expression in Sendai virus-infected fibroblastic or myeloid cells [96]. Subsequently, this observation has been demonstrated also to apply to HSV-induced CCL5 expression (J. Melchjorsen and S. R. Paludan, *J. Gen. Virol.* in press). The ability of NF-κB to act synergistically with IRFs in stimulation of promoter activation has long been appreciated [103]. The above-described, cooperative action of NF-κB and IRF factors bears some resemblance to the mechanisms involved in virus-induced expression of CXCL10. Although differences between the gene promoters of CCL5 and CXCL10 are noticeable, significant similarities are also apparent (Fig. 1). Most notably, both promoters contain a functional ISRE and two NF-κB sites. In fact, as for CCL5, these elements play important roles in regulation of CXCL10 expression during virus infection as well as in response to cytokines [104–108]. Analysis of the CXCL10 promoter showed that infection with measles virus, NDV, and Sendai virus as well as treatment with dsRNA lead to activation of the wild-type promoter and that this activation is dependent on the region of the promoter encompassing the ISRE and the distal κB site [106, 107]. As these findings were done before the identification of IRF-3 and IRF-7 as the main virus-activated IRFs, it was not demonstrated which IRF family member is responsible for the ISRE-dependent CXCL10 transcription. However, more recent work has indicated that IRF-3 and IRF-7 are involved, given the correlation between hantavirus-induced activation of these two transcription factors and expression of CXCL10 [105]. In the

TABLE 3. Regulation of RANTES/CCL5 Expression During Viral Infection

		NF-κB pathway		MAP kinase system				IRF family members					
Virus	Cell type	NF-κB	Specific kinases	p38	ERK	JNK	AP-1	1	2	3	5	7	Refs.
HSV	Many different	+[a]	+[b]	(+)						+	(+)		[81][c]
HHV-8[d]	Epithelial cells	+		(+)									[89]
Adenovirus	Epithelial cells	+											[90]
HPV-16	Keratinocytes	(+)	(+)				(+)						[91]
RSV	Epithelial cells	+		+	+	(+)				(+)	(+)	(+)	[92–94]
Measles virus	Epithelial cells									+			[95]
Sendai virus	Epithelial cells	+								+		+	[80, 96]
	Macrophages	+						+					[97]
NDV	Epithelial cells									(+)	(+)	(+)	[81, 98]
Influenza virus	Macrophages	+											[97]
	Epithelial cells			+		+							[99]
Dengue virus	Epithelial cells	(+)											[100]
Hantavirus	Epithelial cells	(+)						(+)		(+)		(+)	[101]
VSV	Fibroblasts										(+)		[81]
Reovirus	Fibroblasts	(+)					(+)	(+)	(+)				[102]

[a] Symbols used in the table: +, A direct connection between the specific protein and induction of CCL5 expression has been established; (+), indirect evidence for involvement in CCL5 expression has been published. [b] Mogensen, T. H., Melchjorsen, J., Höllsberg, P., Paludan, S. R. (2003) Activation of NF-κB in virus-infected macrophages is dependent on mitochondrial oxidative stress and calcium release: down-stream involvement of the kinases TAK1, MEKK1, and IKKβ, *J. Immunol.*, in press. [c] Data from this laboratory conclusively demonstrate a role for NF-κB and IRF-3 in HSV-induced CCL5 expression in macrophages and fibroblasts (Melchjorsen, J., Paludan, S. R. (2003) Induction of RANTES/CCL5 expression by herpes simplex virus is regulated by NF-κB and IRF-3, *J. Gen. Virol.*, in press). [d] ERK, extracellular-regulated kinase; HHV, human herpesvirus; HPV, human papillomavirus; NDV, Newcastle disease virus.

same study, cooperation between IRFs and NF-κB was again reported, and this cooperation was found to be enhanced in magnitude and duration by cotreatment with IFN-γ.

NF-κB-dependent pathways also regulate CCL3 [109], whereas IRFs do not seem to participate, an observation explained by the lack of an ISRE element in the CCL3 promoter. The involvement of NF-κB, which is activated through a mechanism independent of virus replication, most likely proceeds through the CD28RE. This element is a variant NF-κB site, which requires adjacent promoter elements, such as NF-κB, Sp-1, or AP-1, for proper function [110]. The CD28RE of the CCL3 promoter lies in close proximity to an AP-1 site (Fig. 1).

In addition to IRFs and NF-κB, C/EBPs and MAP kinase-dependent pathways have been demonstrated to stimulate chemokine expression during viral infections [85, 92, 99]. For instance HIV-1 up-regulates production of CCL2 via AP-1 [85], and influenza A virus infection of human bronchial epithelial cells leads to expression of CCL5 through a mechanism dependent on p38 and JNK [99]. Given the well-described substrate specificities of these two MAP kinases, this indicates that ATF2/Jun stimulates expression of CCL5 in influenza-virus-infected cells. ATFs and Jun have previously been identified to promote expression of CCL5 in response to RSV infection as well as bacterial lipopolysaccharide (LPS) [92, 111]. However, it should be noted that p38 also regulates expression of CCL5 at the post-transcriptional level in RSV-infected airway epithelial cells [93]. Therefore, the role of p38 in expression of CCL5 remains to be fully characterized.

CXCL8, which potently elicits chemotaxis of neutrophils, is expressed during many virus infections in vitro through an AP-1-dependent mechanism [112–117]. In most reported cases, this occurs through cooperation with NF-κB, and examination of the CXCL8 promoter (Fig. 1) reveals two closely located regulatory elements for these transcription factors. AP-1 can also act together with other transcription factors to induce CXCL8 expression. One study suggested that induction of CXCL8 expression proceeds through an AP-1-dependent mechanism during RSV infection, and AP-1 cooperates with ISRE-binding proteins rather than NF-κB [115].

Altogether, a review of the literature demonstrates that the mechanisms through which viruses stimulate expression of chemokines display common as well as virus-type-specific features. In addition, cooperative action of transcription factors plays central roles in the regulation of chemokine expression. For instance, NF-κB can stimulate expression of CCL5 through synergistic action with IRFs and also promote expression of CXCL8 via cooperation with AP-1.

VIRUS-CELL INTERACTIONS TRIGGERING CHEMOKINE EXPRESSION

As to the virus-cell interactions responsible for activation of signal transduction, important advances have been made recently. Hiscott and colleagues [95] have studied measles virus and IRF factors and found that the nucleocapsid (N) protein is able to activate IRF-3. The authors showed that overexpression of the N protein led to activation of the IRF-3 kinase, VAK, and subsequent phosphorylation of IRF-3. Moreover, the N protein associated with IRF-3 as well as VAK, thus indicating that IRF-3 or an IRF-3-containing protein complex is able to recognize the N protein and transduce this signal to VAK, which in turn, activates IRF-3. The ability of capsid proteins to activate IRF-3 appears not to be specific to papamyxoviruses, as the capsid protein of the flavivirus West Nile virus also induces expression of CCL5 [118], thus strongly indicating

activation of IRF-3. Moreover, HSV-1 has been shown to activate IRF-3 in different human cell lines through a mechanism dependent on viral entry but independent of de novo transcription of viral or cellular genes [119], again pointing toward a mechanism involving viral capsid proteins.

Although full insight into the mechanism of IRF-3 activation still remains to be gained, the available data suggest that several mechanisms exist. For instance, IRF-3 was initially shown to be activated in response to dsRNA [120]. Moreover, receptor-mediated activation of IRF-3 has also been demonstrated by the finding that LPS activates IRF-3 through engagement of TLR4 [121]. Even highly related viruses may use different mechanisms to activate IRF-3. Krug and associates [122] have compared the ability of influenza A and B viruses with respect to activation of IRF-3 and found that the ability of influenza A virus to activate IRF-3 is sensitive to UV treatment of the virus, whereas that of influenza B virus is not. Thus, activation of IRFs, which can occur through several different mechanisms in virus-infected cells, stimulates expression of CCL5.

Activation of NF-κB is very well studied, and a wealth of virus-induced mechanisms has been demonstrated (ref. [73] for a review). For instance, dsRNA activates NF-κB through at least two mechanisms. First, the RNA accumulates intracellularly and activates PKR, which in turn, activates the signal transduction leading to degradation of IκB and activation of NF-κB [67]. Second, dsRNA, in intracellular compartments or extracellularly after release from lysed cells, binds to TLR3 and thus activates NF-κB [68]. Studies from this laboratory have shown that HSV infection of macrophages triggers expression of CCL5 and that this relies on PKR, NF-κB, and viral-infected cell protein 0 (ref. [123]; and J. Melchjorsen and S. R. Paludan, *J. Gen. Virol.*, in press). Subsequent studies have identified the mechanism of NF-κB activation by HSV to include mitochondrial oxidative stress and subsequent calcium release from this organelle (Mogensen et al., submitted). The laboratory of Dr. Muruve [90, 104] has studied the virus-cell interactions responsible for NF-κB-dependent expression of CCL5 and CXCL10 during adenovirus infection. They found that activation of NF-κB was mediated by the viral capsid and was dependent on viral entry. Together, these studies show that many different mechanisms contribute to activation of NF-κB during viral infections, an essential factor in virus-induced chemokine expression.

The MAP kinase pathways, which contribute to expression of chemokines in response to some virus infections, have also been studied with respect to the viral mechanism of activation [93, 99]. The picornavirus encephalomyocarditis virus and dsRNA have been demonstrated to activate p38 and JNK through PKR-dependent pathways [124]. Others [125, 126] have subsequently confirmed the involvement of PKR in activation of JNK, whereas some controversy remains with respect to the involvement of PKR in activation of p38 by dsRNA. Virus can also activate p38 through dsRNA-independent mechanisms. For instance, adenovirus has been reported to activate the kinase within 20 min of infection [127], and Muruve and colleagues showed that activation of p38 and ERK by adenovirus was inhibited by bafilomycin A1 or ammonium chloride, suggesting that endosomal escape triggered activation of the MAP kinase pathways. More research is needed to get a better overview of the mechanisms governing activation of MAP kinase pathways during virus infections.

Analysis of the CCL5 promoter reveals at least two additional transcription factor-binding sites potentially involved in regulation of chemokine expression during viral infection, although no direct evidence has been provided yet. First, the GAS element, known to be highly active in response to IFN-γ [128], may be activated by homodimers of STAT1 formed in response to IFN-α/β, which has been shown to stimulate expression of several chemokines [104]. Second, NF-AT is activated during infections with human T lymphotropic virus type 1 and Sendai virus and probably many more [129, 130]. The presence of a NF-AT-binding site in the CCL5 promoter therefore opens up the possibility that this transcription factor might also play a role in virus-induced CCL5 expression. At this point, it is appropriate to mention that in addition to direct virus-induced chemokine expression, cytokines expressed during the course of infection contribute substantially to stimulation of chemokine production, notably at later stages of infection after the initial virus-host interaction. Therefore, promoter elements not directly targeted by virus-activated transcription factors may still contribute to chemokine production during infection. One study by Matikainen et al. [97] showed that influenza A virus infection of human monocytes triggered expression of a number of chemokines, some of which (CCL2, CCL7, and CXCL10) were dependent on intermediary IFN-α/β expression. Biron and colleagues [61] also found a role for IFN-α/β in virus-induced chemokine expression in the MCMV model, where the authors demonstrated that expression of CCL3 in the liver was induced by type I IFN.

CONCLUDING REMARKS

When chemokines were first discovered, it was found that this class of cytokines is a central regulator of leukocyte migration. More recently, chemokines have been ascribed multiple functions, most notably leukocyte activation. Given these properties, it is of little surprise that chemokines contribute significantly to the ability of the organism to mount a proinflammatory response and fight infections. The latter is underscored by the fact that many viruses encode proteins that antagonize chemokine function.

In this paper, we have reviewed what is currently in the literature about the molecular mechanisms through which different viruses activate chemokine production and also what is known about chemokines in viral infections in vivo. Investigations of the molecular events that occur during the clinch between the virus and the host cell have revealed that a wealth of mechanisms is involved. These include interactions between viral glycoproteins and cellular surface receptors; accumulation of viral dsRNA, which is sensed by the cell by various mechanisms; cellular stress through protein overload of the endoplasmatic reticulum or production of reactive oxygen intermediated from the mitochondria; and intracellular accumulation of viral proteins that interact with cellular signal transduction.

As to the role of chemokines during viral infections in vivo, a clear picture still remains to emerge, given the limited number of chemokine knockout mice analyzed in the context of viral infections. The available data seem to support the conclusion that Th1-promoting chemokine receptors and their ligands support viral clearance and in some cases, immunopathology. However, the great degree of redundancy in the chemokine system makes it difficult to predict the role of one specific chemokine or chemokine receptor during infection and hence, emphasizes the importance of thorough experimentation. With the present focus on chemokine receptor antagonists as potential treatment against HIV infections and chronic inflammatory diseases, it is important to fully understand the role of these receptors in defense against infections. Moreover, in-depth understanding of the chemokine system may in turn allow development of vaccines and immunostimulatory drugs as treatment against virus infections in immunocompromised hosts prone to opportunistic infections.

ACKNOWLEDGMENTS

This work was supported by grants from The Carlsberg Foundation, Kathrine og Vigo Skovgaards Fond, and LEO Pharma Research Foundation. J. M. and L. N. S. were supported by fellowships from the Faculty of Health Sciences, University of Aarhus, and the Danish Health Science Research Foundation (Grant 22-01-0524), respectively, and contributed equally to the manuscript.

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