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Chemokines in Respiratory Viral Infections: Focus on Their Diagnostic and Therapeutic Potential

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ABSTRACT: Chemokines are small chemoattractant cytokines involved in cell trafficking and activation. Despite the general nonspecific nature of chemokine activity in certain instances, specific chemokine expression patterns have been associated with specific disease states. In the field of respiratory viral infection, evidence suggests that response to viral invasion is regulated by a distinct chemokine expression profile involving more CC chemokines than CXC chemokines. Moreover, among the CC chemokines, CCL3 and CCL5 appear to be most commonly implicated in viral respiratory disease. Most data available in this field have been derived from *in vitro* studies, as well as studies conducted in animal models with limited evidence obtained in settings of actual human disease. In the present review, we focus on the diagnostic, prognostic, and therapeutic potential of virus-induced chemokine activity as reflected by studies conducted in actual disease states, either in animal models or humans. We further discuss whether these data advocate chemokines as a realistic clinical tool for the management of viral infection.

KEY WORDS: respiratory tract, viral infection, chemokines, therapeutic target

ABBREVIATIONS: IFN, interferon; kDa, kilodaltons; NK, natural killer; TNF, tumor necrosis factor; DARC, Duffy antigen receptor for chemokines; RSV, respiratory syncytial virus; LPS, lipopolysaccharide; FMLP, formyl-methionylleucyl-phenylalanine; IL, interleukin; BAL, bronchoalveolar lavage; HMPV, Human metapneumovirus; SARS, severe acute respiratory syndrome; ELR, glutamic acid-leucine-arginine; Th, T helper; ELISA, Enzyme-linked immunosorbent assay; RT PCR, Reverse transcription polymerase chain reaction; qPCR, quantitative real-time polymerase chain reaction; RPA, RNase protection assays; ELISPOT, enzyme-linked immunosorbent spot.

I. INTRODUCTION

A. Host Defense Against Viral Infections

The human body responds to any type of biological offence by activating two distinct defense mechanisms. The first line of defense, known as innate immunity, comprises a number of nonspecific effectors that act early in the course of the infection to limit the spread of the infecting agent. In innate immunity, rapid response necessitates the use of general motifs for the purpose of non–self recognition.[1–3] Thus, during microbial invasion, certain substances that are common products of microbial pathogens such as endotoxin, flagellin, peptidoglycans, single- or double-stranded RNA, and unmethylated DNA containing motifs that are uncommon in the human genome, activate cellular toll-like receptors on macrophages, dendritic cells, and other cells to initiate the innate immune response. Therefore, the innate response is not specific; nevertheless it is rapid because it

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does not require cellular proliferation.[1] In contrast, activation of adaptive immunity requires highly specific recognition that necessitates a clonal distribution of the involved receptors and therefore a delay in activation due to the time required for clonal expansion and differentiation.[1–3]

In the context of viral infection, most cell types in the body respond to invasion by secretion of type 1 interferons (IFN α/β). Interferons directly induce antiviral activities in uninfected, neighboring cells, preventing viral spread. Interferons are also capable of activating natural killer cell-mediated cytotoxicity toward virus-infected cells and contributing to the rotation of the adaptive-immune response towards a T helper cell type 1 direction via the stimulation of IFN-γ expression.

The cellular antiviral response is initiated by the activation of NK cells, which eliminate infected cells directly and also activate other cells of the innate- and adaptive-immune system through the production of cytokines, including IFN-γ. IFN-γ activates macrophages that participate in the antiviral response through the production of free radicals and pro-inflammatory cytokines as well as by functioning as antigen-presenting cells. Macrophages further contribute to antiviral defence via phagocytosis of extracellular virus and apoptotic cells.[3–5] Dendritic cells are the main antigen-presenting cells and play a pivotal role in the orchestration of the specific immune response. Furthermore, they participate in the coordination of the inflammatory response, through the production of cytokines and chemokines. Dendritic cells are the main source of type 1 IFNs during viral infections.[3–5]

Another prominent response to viral infection is the expansion and activation of CD4- and CD8-positive T cells, which play key roles in antiviral immunity. CD8-positive cells have a direct effector role through cytotoxic T lymphocyte-mediated lysis. These cells further produce cytokines, such as IFN-γ and TNF-α, and chemokines, which modulate the immune response and attract the appropriate leukocyte subsets to infected areas. The role of CD4-positve T cells in antiviral immunity is highly dependent on the production of cytokines, particularly IFN-γ, and on the cytolytic activity exerted by a subset of CD4-positve T cells.[3–5]

Activation, coordination, and regulation of the above-described antiviral response are mediated by complex mechanisms, in which cytokines play important roles. Within the large group of cytokines, the subgroup of chemotactic cytokines, also known as chemokines, is also currently known to be involved in the defense against viral infections. Moreover, chemokines, in some instances, are considered to be important mediators of the immunopathology associated with viral infection.[6,7]

B. Chemokines

Chemokines are small, secreted chemoattractant proteins (approximately 8–17 kDa) that serve as regulatory molecules in leukocyte chemotaxis and activation.[8] Chemokines share substantial homology and most importantly, a conserved tetracysteine motif. They are classified into four subfamilies based on the number and structural arrangement of the conserved cysteine residues within their amino-terminal polypeptide sequence. CXC chemokines possess a single amino acid separating the two amino-terminal cysteine residues of the protein, whereas CC chemokines lack this type of amino acid sequence. CX3CL1 is the only member of the CX3C subfamily and has three amino acids separating the two amino-terminal cysteine residues. Finally, XCL1 and XCL2 are the only currently known members of the C subfamily that lack two of the four conserved cysteines in the final protein.[8] The complicated nomenclature used for the identification of each member created confusion in the scientific literature. A revised nomenclature was introduced in 1999 based on the protein structure of chemokines. The latter nomenclature is used throughout this manuscript.[8]

Chemokines promote cell activation and chemotaxis by interacting with specific seven-transmembrane G-protein coupled cell-surface receptors on target cells. Chemokines promote chemotaxis through the development of a chemotactic gradient that mobilises the inflammatory cell toward an area of increased chemokine concentration. *In vivo,* the chemotactic gradient may be generated by the binding of chemokines to basement membrane proteins. This gradient aids in transferring cells toward the site of inflammation and retaining them once they have reached the inflamed area. Chemokines interact with their receptors on the cell surface leading to the generation of an intracellular signal via the G-protein complex, and subsequently to cell chemotaxis towards a chemokine gradient.[9–12] Cell movement and migration are driven by the dynamic remodelling of the actin cytoskeleton. In chemotaxis, chemokine gradients strongly bias actin assembly to the leading edge of the cell and, thus, the direction of cell movement. Decoy receptors, also known as interceptors (internalizing receptors), bind ligands with high affinity but do not trigger signal transduction. Three decoy receptors have been identified: D6, DARC, and CCX-CKR.[12] Chemokine receptor CCX-CKR is a scavenger of CCR7 ligand chemokines, while D6 is thought to act as a chemokine scavenger for proinflammatory CC chemokines. DARC is considered a decoy receptor for both CC and CXC motif chemokines. It is speculated that decoy receptors act as a clearing system balancing local concentrations of chemokine ligands; nevertheless, their exact biological role remains to be elucidated. Chemokines also interact with glycosaminoglycans as a part of the presentation process of chemokines on endothelial layers and for leukocyte migration *in vivo*.[11,12]

Inflammatory cells represent both the main source and the main target of chemokines. However, most human cellular populations have been reported to be capable of producing or responding to certain chemokine ligands. This applies to respiratory epithelial cells, particularly after exposure to pathogens. Upper and lower respiratory epithelial cells exposed to RSV have been shown to alter their chemokine expression profile. The existence of distinct genetic responses to different types of airway-derived epithelial cells has been also reported.[13,14]

An unusual characteristic of most chemokine receptors is their high affinity for multiple ligands and vice versa. Chemokines can also dimerize while their receptors also form dimers and/or higher-order oligomers at the cell membrane. Moreover, functional studies indicate substantial synergy between chemokines. Synergic action augments target cell chemotaxis and activation.[8]

Regarding the mechanisms involved in the regulation of chemokine gene expression, special interest should be focused on post-transcriptional regulation. A wide spectrum of stimuli has been found, in different cell types, to trigger changes in the mRNA turnover of a number of chemokines, as follows: proinflammatory and immunomodulatory cytokines, such as TNF-α, IL-1, IL-4, IFN-γ and IL-10; stress-related signals, such as hypoxia; infectious agents, such as viruses or bacterial-derived products such as lipopolysaccharide (LPS) or formyl-methionylleucyl-phenylalanine (FMLP) and other stimuli, such as nitric oxide and activated protein C.[15–18] Of note is that T-cell-derived products selectively expressed in polarized inflammatory responses, such as IFN-γ and IL-4, may utilize post-transcriptional pathways to exert opposite effects on the chemokine gene expression. For example, in human monocytes, IFN-γ has been found to upregulate the expression of CXCL8 by increasing in mRNA stability. On the other hand, in the same cell type, IL-4 downregulates CXCL8 expression by decreasing the half-life of its mRNA.[19–20]

The levels of chemokines in body fluids/tissues or culture supernatants can be measured by a variety of assays as shown in Table 1.

TABLE 1. Assays Used for the Measurement of Chemokine Levelsin Body Fluids/Tissues or Culture Supernatants

Measurement of chemokines in culture supernatants and serum
ELISA/Colorimetric methods/bioassays for soluble receptor levels
Chemokine production of different populations of immune cells
Flow cytometric analysis
Differential gene expression technologies for transcripts for chemokines/chemokine receptors, chemokine gene profiling
mRNA-based assays (RT-PCR, qPCR, Northern-blot, RPA, gene array analysis)
Single cell assays for chemokine secretion
ELISPOT

ELISA, enzyme-linked immunosorbent assay; RT PCR, reverse transcription polymerase chain reaction; qPCR, quantitative real-time polymerase chain reaction; RPA, RNase protection assays; ELISPOT, enzyme-linked immunosorbent spot.

C. Chemokines in Virally Induced Respiratory Tract Infection

Viruses are the most common cause of respiratory tract disease and represent a major public health problem in all age groups. The implication of chemokine pathways in the course of viral respiratory tract infection has been substantiated through evidence derived from *in vitro* studies, studies in animal models, and detection of soluble chemokines in actual human disease. These data suggest that response to viral invasion is regulated by a distinct chemokine expression profile. However, several deviations from this profile indicate a virus-specific pattern, at least for some types of infection.[21,22] For instance, serum levels of CCL5 are reportedly high in RSV-infected children but not in influenza virus-infected patients.[23] As a general rule, it appears that the expression of CC chemokines dominates over that of CXC chemokines, while among the CC chemokines, CCL3 and CCL5 appear to be almost invariably associated with viral infections. Among these, CCL5 is the most extensively studied with respect to molecular mechanisms governing virus-induced chemokine expression. Concerning the less expressed CXC chemokines, CXCL9 and CXCL10 are those most commonly associated with viral infection.[21,22]

Another noteworthy observation is that chemokine pathways are activated in the course of viral infection, although not necessarily in favor of the host. Viruses are capable of exploiting chemokine pathways through chemokine mimicry to facilitate viral propagation.[24] Tripp et al. demonstrated that the interaction of viral G glycoprotein with CX3CR1 plays a crucial role in the pathogenesis of RSV infection.[25] In a nonglycosylated, central conserved region, G glycoprotein contains a CX3C chemokine attachment motif at amino acid positions 182–186. Due to this structural similarity, G glycoprotein has the ability to interact with the CX3C chemokine receptor. This interaction appears to have at least two important roles in the pathogenesis of RSV infection. First, G glycoprotein, through binding to CX3CR1, facilitates infection. Secondly, this interaction appears capable of modifying the host's immune response.[24] More than 30 virally encoded chemokines and chemokine receptor mimics have been recognized to date. Therefore, certain chemokines, although potentially contributing to antiviral activity, may also fuel the host response responsible for the pathology of acute or chronic viral disease.[24] Thus, in this review, we present data on chemokine involvement in viral-induced respiratory tract infection. Our search was limited to data derived from animal models of human disease and data derived from actual human disease focusing on the potential role of chemokines as prognostic and diagnostic markers or therapeutic targets.

II. CHEMOKINES IN VIRALLY INDUCED RESPIRATORY INFECTION: DATA FROM ANIMAL MODELS

Among members of the Paramyxoviridae family, RSV is characterized as the most important pathogen causing serious lower respiratory tract disease in infants, young children, as well as the elderly and immune-compromised individuals, worldwide.[26] Despite its high prevalence, the pathogenesis of RSV infection is not yet fully understood. Studies in animal models have highlighted the biology of RSV infection and have provided important information concerning the indistinct balance between host immunity and disease pathogenesis. Several of these studies have demonstrated enhanced chemokine activity modulating cell recruitment and infiltration to the inflammation site, while evidence suggests that the pattern of upregulated chemokines affects the balance between virus clearance and exacerbation of the disease, leading to more severe RSV infection.[27] The BALB/c mouse is the most preferable animal model employed due to its close similarity to humans in respect to the pathogenesis of RSV-induced lower respiratory disease.[28] In 2001, Haeberle et al. provided the first direct evidence that RSV infection may induce lung inflammation via the early production of inflammatory chemokines. These authors demonstrated that the intranasal infection of BALB/c mice with RSV-A results in an inducible expression of lung chemokines including CXCL2, CXCL10, CCL5, CCL11, CCL3, CCL4, CCL2, and XCL1.[29] Miller et al. further demonstrated that viral replication is necessary for optimal chemokine production, while Culley et al. directly correlated the chemokine expression pattern by prior sensitisation to individual RSV proteins.[30,31] Among the induced chemokines in the course of RSV infection, CCL5 and CCL2 have been associated with severe RSV bronchiolitis and post-infection airway hyper-responsiveness.[32,33] A correlation of CCL5 production in RSV-infected mice with the subsequent development of allergic airway inflammation has also been reported by several research groups.[34–37] On the other hand, Tekkanat et al. reported that RSV-infected animals treated with the anti-CCL5 antibody demonstrated a significant decrease in airway hyperreactivity and an increase in IL-12 production. These authors also demonstrated that CCL5 production was regulated by IL-13, a cytokine that was correlated with RSV-induced hyperreactivity in the utilised murine model.[32] Culley et al. similarly used a murine model to further investigate the timing of CCL5 production in association with the pathology of the viral disease. The authors concluded that the role of CCL5 both in the recruitment of inflammatory cells and in controlling virus infection is time dependent and this may complicate the use of chemokine blockers as potential therapeutic agents in viral lung diseases.[38]

CCL3 is a cell-specific chemokine that attracts eosinophils to the site of infection. It is an important mediator of virus-induced inflammation *in vivo*. CCL3 has also been implicated in airway hyperreactivity. Haeberle et al. were the first to investigate the role of CCL3 as an airway inflammatory mediator in mice with gene deletions of CCL3. These mice had significantly less lung inflammation and milder clinical manifestations than the wild-type strain.[29] Matsuse et al. studied the effect of recurrent RSV infections in allergen-sensitized mice. They demonstrated that a secondary RSV infection increases the expression of CCL3 in the lung tissues of allergen-sensitized mice and persistently enhances airway responsiveness.[39]

Another chemokine that represents a selective eosinophilic chemotactic mediator is CCL11. Matthews et al. used a mouse model to investigate the role of CCL11 in the pathogenesis of eosinophilic RSV-induced bronchiolitis. They concluded that treatment with anti-CCL11 greatly reduces lung eosinophilia and disease severity.[40]

Tripp et al. studied mice infected with either wild-type RSV or an RSV mutant lacking G or SH genes, comparing the chemokine response

on each occasion. They reported that the G and/or SH protein expression was associated with reduced CCL2, CCL3, and CCL4 mRNA production from bronchoalveolar lavage (BAL) cells.[41] The CCR1 and CCR5 receptors of these chemokines are mostly expressed on Th1 cells. The authors concluded that the G and/or SH protein expression may weaken Th1 immune responses mediated by CCL2, CCL3, and CCL4, suggesting that these chemokines are important in RSV immunity or disease pathogenesis. The same group demonstrated that the RSV glycoprotein G has structural similarities with the CX3CL1 chemokine and that it is capable of interacting with its receptor, CX3CR1. These authors also demonstrated that the G glycoprotein competes with CXCL1 for binding to CX3CR1 and inhibits CXCL1-mediated leukocyte chemotaxis, facilitating viral infection.[25] Additional studies in murine models have shown that the immune response to primary RSV infection is characterized by a mixed Th1/Th2-type cell response. CX3CL1 is a potent mediator for Th1 and NK cell responses, because these cell types express high levels of CX3CR1.[42] Tripp et al. further hypothesised that modification of the CX3CL1-mediated immune responses by RSV G glycoprotein may alter the Th1-type cell and NK cell responses and affect the pattern of chemokine expression. To test their hypothesis, the investigators assessed (BAL) leukocytes from BALB/c mice infected with an RSV mutant lacking G and SH genes. They reported that these mice express increased Th1-type cytokines and increased CC and CXC chemokine mRNAs, and that they have increased numbers of pulmonary NK cells compared to wild-type-infected mice.[43] Moreover, Tripp et al. reported that the absence of the G glycoprotein or G glycoprotein CX3C motif during FI-RSV vaccination or RSV challenge of FI-RSV-vaccinated mice, or treatment with anti-substance P or anti-CX3CR1 antibodies, reduces or eliminates enhanced pulmonary disease, modifies T-cell receptor Vβ usage, and alters CC and CXC chemokine expression. The authors suggest that the G glycoprotein, and in particular the G glycoprotein CX3C motif, is an essential mediator in the enhanced inflammatory response to FI-RSV vaccination, possibly through the induction of substance P.[44]

Human metapneumovirus (HMPV) is a newly identified member of the Pneumovirinae subfamily of Paramyxoviridae that can cause severe respiratory disease, particularly in infants and young children and in the elderly with concomitant conditions.[45] Currently, many factors involved in the development of disease, such as HMPV-related immunopathogenesis and possible viral persistence, remain to be determined.[46,47] Several studies conducted using animal models of HMPV infection demonstrated that the production of inflammatory chemokines play a crucial role in the immunopathogenesis of HMPV disease. Huck et al. investigated immune induction in BALB/c mice infected either with HMPV or RSV. They reported higher CCL2 levels in the BAL of HMPV-infected mice compared to RSV-infected ones.[48] Guerrero-Plata et al. also compared chemokine production in BALB/c mice infected either with HMPV or RSV. These investigators demonstrated different chemokine patterns in the airways of each group of virus-infected mice, with HMPV being a stronger inducer of the CXCL1 murine analogue production than RSV.[49] In a recently published study, Herd et al. investigated virus-directed cellular immunity induced by HMPV infection in a murine model and found an increased pulmonary expression of CCL3, CCL4, CXCL9, CXCL10, and CX3CL1 chemokines.[50]

Parainfluenza and influenza viruses share many clinical and immunologic features. Infection with these viruses has been associated with the development of a wide range of clinical diseases ranging from mild upper respiratory tract illness to severe bronchiolitis and pneumonia. Infection by parainfluenza or influenza virus has been also associated with the likelihood of developing asthma later in life.[51,52] Evidence derived from animal models implies an association between chemokine activity and the

clinical severity of the influenza virus infection. To clarify the development of the cell-mediated immune response to influenza A virus, Wareing et al. examined the chemokine expression pattern in lung tissue from A/PR/8/34-infected C57BL/6 mice. They reported the upregulation of CCL2, CCL3, CCL4, CCL20, CCL5, CXCL2, and CXCL10 mRNA expression between post-infection days 5 and 15. The authors concluded that the detected chemokines play a role in the regulation of leukocyte trafficking to the lung during influenza infection.[53]

Human rhinovirus is a member of the Picornaviridae family. It is responsible for the majority of virus-induced asthma exacerbations. The major group serotypes bind to intercellular adhesion molecule 1.[54] Studies in mouse models have been conducted to clarify the proinflammatory molecules implicated in airway inflammation and subsequent hyper-responsiveness.[55,56] Nagarkar et al. used a CXCR2-knockout mouse model infected with human rhinoviruses to determine the role of CXCR2, the receptor for ELR-positive CXC chemokines, in the course of rhinovirus-induced airway infection. The authors concluded that CXCR2 is required for neutrophilic airway inflammation and hyperresponsiveness following rhinovirus infection.[57] To further determine the immunologic mechanisms underlying rhinovirus-induced asthma exacerbations, Nagarkar et al. infected a murine model of allergic airway disease with human rhinoviruses. These authors reported that augmented airway eosinophilic inflammation and hyperresponsiveness is directed, in part, by the CCL1 chemokine.[58]

Human adenovirus underlies a wide range of upper and lower respiratory tract infections in children, while it is a common cause of more aggressive respiratory disease in immunocompromised patients. A potential long-term consequence of persistent adenovirus infection is an increased risk for the development of asthma and chronic obstructive pulmonary disease.[59–61] A variety of models have been used to study chemokine responses to adenovirus infection. *In vitro* systems have used infection of cell lines with adenovirus or transduction with adenoviral vectors. An *ex vivo* lung slice model has also been used to examine chemokine responses to adenoviral infection. The majority of these studies have confirmed the upregulation of CXCL8.[62] Relatively few studies have used animal models to examine chemokine responses in respiratory infection with human adenovirus, while *in vivo* studies of human adenovirus pathogenesis relative to chemokine production are not yet available. In the broader investigation of chemokine profile post–respiratory adenoviral infection, Weinberg et al. reported that the intranasal inoculation of adult C57BL/6 mice with mouse adenovirus type 1 resulted in the upregulation of a broad spectrum of chemokines. However, the authors observed a certain time profile, with CXCL10 and XCL1 reaching higher levels at day 7 post-infection, whereas levels of CCL3, CCL4 and CCL5 expression peaked at day 14. CCL2 and CCL1 expression was increased to similar levels at days 7 and 14.[63]

III. CHEMOKINES IN VIRUS-INDUCED RESPIRATORY INFECTION: DATA FROM HUMAN DISEASE

Chemokines have been identified in the blood, but they also in the upper and lower respiratory secretions in the course of viral respiratory infection in humans. Efforts have focused on the detection of a particular chemokine expression pattern that may be used for the differential diagnosis of viral and nonviral respiratory disease. There are also data indicating that the chemokine expression pattern can differ even among different viral pathogens in the course of respiratory disease (Table 2).[15,64–87] Respiratory syncytial virus has been the most extensively studied virus in infants and young children, while coronavirus related to severe acute respiratory syndrome (SARS) has been mostly studied in adult populations. Yet, data are limited, and most are derived from small, single center, case-

TABLE 2. Studies Assessing Diagnostic and Prognostic Potential of Chemokine Expression in Respiratory Viral Disease

Author	Year	Disease group (n)	Age group	Disease	Biological specimen	Virus	Predominant chemokine expression	Association
Arankalle et al.[64]	2010	41	Adult	LRTI	Lung aspirates/ plasma	H1N1	CCL3, CCL4	CCL3/CCL4 and severity
Takano et al.[65]	2011	34	Children	21 patients with pneumonia	Serum	H1N1	CXCL8 and CCL2 in pneumonia cases	CCL2 and severity
Sumino et al.[66]	2010	283	Adult	LRTI	BAL	Predominantly rhinovirus	CXCL10, eotaxin	
Kato et al.[67]	2010	267	Children	Virus-induced wheezing	Nasal/serum	Predominantly rhinovirus	CXCL10	
El Feghaly et al.[68]	2010	153	Infants-children	LRTI/URTI	Nasal washes	Parainfluenza virus 1-4	CXCL8, CCL3, CCL4, CXCL9 and CCL5 in cases	CXCL8 and severity
Quint et al.[69]	2010	126	Adults	COPD exacerbation	Serum	Rhinovirus		CXCL10 and rhinovirus load
Bermejo-Martin et al.[70]	2009	35	Adults	NvH1N1 infected patients	Serum	A/H1N1	CXCL10, CCL2, CCL4 in cases	CXCL8 and severity
Gill et al.[23]	2008	104	Children	LRTI	Nasal/serum	RSV vs. Infuenza A	CCL5, CCL2	CCL5 and Infuenza A CCL2 and RSV
Bermejo-Martin et al.[71]	2007	22	Children	LRTI	Nasopharyngeal aspirates/plasma	RSV	CXCL10, CXCL8, CCL3, CCL4	CXCL8 and severity
Murai et al.[72]	2007	70	Children	LRTI	Nasopharyngeal aspirates	RSV	CCL5	CCL5 and RSV
Chien et al.[73]	2006	14	Adults	SARS	Serum	SARS coronavirus	CXCL10	CXCL10 and SARS
Kim et al.[74]	2005	18	Children	RSV bronchiolitis	Bal	RSV	none	CXCL8 and RSV
Grissell et al.[75]	2005	59	Adult	Acute exacerbation of asthma	Sputum	Predominantly rhinovirus	CCL3, CCL5	CCL3/CCL5 and airway neutrophilia
McNamara et al.[76]	2005	47	Infants	RSV LRTI requiring mechanical ventilation	Bal	RSV	CXC over CC	CXC chemokines and RSV infection

Tang et al.[77]	2005	255	Adults	SARS	Serum	SARS coronavirus		CXCL10 and adverse outcome
Jiang et al.[78]	2005	23	Adults	SARS	Serum	SARS coronavirus	CXCL10	
Wong et al.[79]	2004	20	Adults	SARS	Plasma	SARS coronavirus	CXCL8, CCL2 and CXCL10	
Noah et al.[80]	2002	47	Infants	RSV bronchiolitis	Nasal Lavage	RSV	CCL5	Higher CCL5/CXCL8 ratio in RSV bronchiolitis
Chung et al.[81]	2002	30	Infants	RSV bronchiolitis	Nasal secretions	RSV	CCL5 in cases	CCL5 and RSV bronchiolitis CCL5 and recurrent wheezing
Tripp et al.[82]	2002	20	Infants	RSV LRTI requiring hospitalization	PBMCs	RSV	Predominant CC chemokine expression	CC chemokines and RSV LRTI
Sung et al.[83]	2001	21	Infants	Coryzal illness	Serum	RSV vs, Infuenza	CCL5 in cases	
Pacifico et al.[84]	2000	25	Infants	Wheezing/acute upper respiratory illness	Nasal washes	rhinovirus	CCL5/CXCL8 in cases	CCL5 and severity
Bont et al.[85]	1999	50	Infants	RSV LRTI	Plasma	RSV		CXCL8 and RSV LRTI requiring mechanical ventilation
Bonville et al.[86]	1999	100	Children	Suspected respratory viral infection	Nasal washes	predominantly RSV	CCL5/CCL3 in cases	
Harrison et al.[87]	1999	10	Infants	RSV LRTI requiring mechanical ventilation	Lower respiratory secretions	RSV	CCL5/CCL3/CXCL8 in cases	

controlled studies. Most studies on RSV confirmed the predominance of CC chemokines in respiratory secretions in the course of viral infection, while a predominance of the CXC family was documented in coronavirus infection.

In respect to RSV-induced disease, Harrison et al. investigated the chemokine expression pattern in the lower airway secretions of 10 intubated infants with RSV bronchiolitis and 10 control subjects. They reported increased levels of CCL3, CXCL8, and CCL5 in RSV patients, compared with the control subjects.[87] Bonville et al. also reported the detection of chemokines CCL3 and CCL5 in nasopharyngeal secretions of paediatric patients with upper respiratory tract infections, suggesting further investigation of their role in disease immunopathogenesis.[86] Tripp et al. demonstrated a mixed Th1/Th2 cellular immune response and predominant CC chemokine expression in children hospitalized due to severe RSV disease.[82]

In respect to the chemokine response in the course of coronavirus-induced SARS, Tang et al. studied plasma samples from 255 patients with SARS and proposed that the elevated plasma levels of CXCL10 during the first week of SARS symptoms were an independent predictor of adverse disease outcome.[77] Chien et al. demonstrated a predominance of CXCL10 expression in serum derived from patients infected with the SARS coronavirus, compared with patients diagnosed with community acquired pneumonia. In contrast, the authors reported CXCL8 and CXCL9 to be significantly elevated in CAP patients, but not in SARS patients, compared with the levels in healthy controls.[73]

Sumino et al. assessed 27 inflammatory mediators in patients presenting with serious acute respiratory illness.[66] The presence of a respiratory virus—predominantly rhinovirus—was associated with increased levels of CXCL10 and CCL11. Arankalle et al. investigated the role of host immune response in the differential outcome of a pandemic H1N1 influenza virus infection in Indian patients and correlated disease severity with increased plasma levels of CCL3 and CCL4.[64]

IV. DISCUSSION

A. Diagnostic and Prognostic Potential of Chemokines

Multiple functions have been attributed to chemokines, most notably leukocyte activation, accumulation and migration. Given these properties, it is to be expected that chemokines contribute significantly to the ability of the host to orchestrate an anti-inflammatory response and fight infections.

Our knowledge of the role of chemokines in the course of viral infections is evolving; however, a clear picture remains to emerge. The available data suggest that several chemokine receptors and their ligands support viral clearance and in some cases, underlie immunopathology. However, the complexity of the chemokine system makes it difficult to predict the role of one specific chemokine or chemokine receptor during infection. This complexity is reflected in the multiple sources of chemokines in the course of a disease; the fact that cellular populations can be both a source and a target of a specific chemokine ligand, the multiple levels involved in the regulation of chemokine activity, particularly regarding decoy receptors, and the ability of chemokines to form dimers and act in synergy with each other. Moreover, the mainstream theory proposes that chemokines act in "a collaborative network" in the course of a disease. The latter theory suggests that different chemokines might be upregulated at different time points depending on the level of immune activity and the type of cells that are to be recruited in the inflamed area. Most likely this theory applies to viral infection as well. In fact, in a murine model of RSV-induced lower respiratory tract infection, Culley et al. demonstrated a dynamic change in chemokine expression.[31,38] These authors reported a biphasic response for CCL11 and CCL5 consisting

of an early phase, probably associated with resident cells and a later phase, characterized by an influx of lymphocytes. The complexity of chemokine activity suggests that secreted levels of chemokines may differ depending on the phase of the disease and the type of immune activation. This characteristic may be a serious limitation of chemokine-based diagnostic tools. Moreover, taking into account the complexity of chemokine activity, the discovery of a single-chemokine-based marker with prognostic or diagnostic potential also seems a utopia. Indeed, most available studies seeking a single-molecule marker have failed to identify a clinically applicable marker of viral infection.

B. Therapeutic Potential

No data are currently available on the safety and efficacy of chemokine-based therapies in viral-induced respiratory disease derived from human studies.

According to animal-derived data, CCL5/CCR5 interaction may be a promising therapeutic target in the field of RSV-induced lower respiratory tract disease. The blockade of this pathway has already been tested in humans in other disease states. CCR5 monoclonal antibodies have been shown to be effective in avoiding T cell infection by CCR5-tropic HIV-1. Other chemokine receptor antagonists are currently under clinical evaluation as therapeutic targets in other nonviral inflammatory diseases; for example, the efficacy and safety of CCR1 antagonists have been evaluated for the treatment of rheumatoid arthritis.[88] A monoclonal antibody blocking the binding of CCL2 to CCR2 was also tested for the treatment of rheumatoid arthritis.[89] At least theoretically, the previous data suggest that antibodies able to block the interaction between chemokine ligands and receptors, such as the CCR5/CCL5 interaction in RSV disease, could decrease lung inflammation and improve outcomes.

Nevertheless, as in any immune-modulating therapeutic approach, several drawbacks need to be considered. First, tolerability and safety issues for a potential therapeutic agent that blocks a non-specific chemotactic pathway need to be addressed. Secondly, chemokines are an important mediator of several aspects of the immune response. Therefore, the complete blockade of a chemokine signalling pathway may not be desirable. Thus, techniques need to be applied to ensure the localized and reversible blockade of chemokine pathways.

C. Perspectives

The available data in the scientific literature do not appear to support the role of chemokines either as diagnostic markers or therapeutic targets. The chemokine network is extremely large and ambiguous and has been only partly investigated. Future studies should therefore assess chemokine response as a whole as opposed to investigating the response of each independent molecule. Until recently, technical limitations prevented researchers from assessing chemokines as a network and research focused on target-gene/protein analysis. However, genome-wide analysis has made the identification of certain chemokine responses feasible. The identification of a chemokine pattern versus a single molecule as a prognostic and diagnostic marker in viral diseases seems to be a more promising hypothesis. Moreover, a better understanding of the activation of the chemokine network might also reveal new potential therapeutic targets. However, this would require significantly powered prospective cohorts assessing hard clinical endpoints in the course of viral infection.

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