Structure–Function Relationships of Antimicrobial Chemokines

Mauricio Arias, Sebastian A.J. Zaat, and Hans J. Vogel

Abstract The chemokines are a group of small chemotactic cytokines that play an important role in the innate and adaptive immune system. Their main function is related to the recruitment of white blood cells to sites of infection. They bind to specific chemokine receptors, which subsequently triggers signaling pathways in the leukocytes. Recently the discovery of chemokines that possess a direct antimicrobial activity against a broad range of pathogenic bacteria has generated increased interest in the role of these proteins in the innate immune system. Prior studies regarding ligand and receptor binding have already established the structural elements important for chemokine interaction and activation of their receptors. In the same manner, it is important to study the structural features required for the antimicrobial activity of this group of chemokines in order to establish key elements related with this new activity. This review will focus on the structure–function relationships that appear to be related to the direct antimicrobial activity of the chemokines. A close similarity of the C-terminal domain of many chemokines to cationic α-helical antimicrobial peptides suggests that this C-terminal helical region is responsible for the chemokine antimicrobial activity. However, for several chemokines, the antimicrobial activity resides in other parts of the protein, indicating that each chemokine needs to be examined individually. We also discuss the role of dimerization and of linearization of chemokines in their antimicrobial activity.

M. Arias • H.J. Vogel (✉)
Department of Biological Sciences, Biochemistry Research Group, University of Calgary, 2500 University Dr. NW, Calgary, AB, Canada
e-mail: vogel@ucalgary.ca

S.A.J. Zaat
Department of Medical Microbiology, Centre for Infection and Immunity Amsterdam (CINIMA), Academic Medical Center, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands

P.S. Hiemstra and S.A.J. Zaat (eds.), *Antimicrobial Peptides and Innate Immunity*, Progress in Inflammation Research, DOI 10.1007/978-3-0348-0541-4_8,

Abbreviations

AMPs	Antimicrobial peptides
CA-MRSA	Community-associated methicillin-resistant *Staphylococcus aureus*
CTAP-3	Connective tissue-activating protein-3
DC	Dendritic cells
DOPE	1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine
DOPG	1,2-Dioleoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol)
ENA-78	Epithelial neutrophil-activating protein 78
GCP-2	Granulocyte chemotactic protein-2
GRO	Growth-regulated oncogene
hBD	Human beta-defensin
IL-8	Interleukin-8
IP-10	Interferon-inducible protein-10
I-TAC	Interferon-inducible T-cell alpha chemoattractant
LDL	Low-density lipoprotein
MCP-4	Monocyte chemoattractant protein-4
MEC	Mucosa-associated epithelial chemokine
MIG	Monokine induced IFN-gamma
MIP-3α	Human macrophage inflammatory protein-3α
NAP-2	Neutrophil-activating peptide-2
NMR	Nuclear magnetic resonance
PBP	Platelet basic protein
PF-4	Platelet factor-4
PG-1	Protegrin-1
PGLa	Peptidyl-glycylleucine-carboxyamide
PMP	Platelet microbicidal protein
RANTES	Regulated upon activation normal T-cell expressed and secreted
SDS	Sodium dodecyl sulfate
SIC	Streptococcal inhibitor of complement
SPA	Staphylococcal protein A
SpyCEP	*Streptococcus pyogenes* cell envelope proteinase

1 Chemokines

The chemokines are a family of small chemotactic cytokines, with a size ranging from 8 to 13 kDa (Allen et al. 2007). Their role in the innate and adaptive immunity has been studied since the first chemokines were discovered 28 years ago (Sauder et al. 1984; Yoshimura et al. 1987a). Their primary function concerns the activation and recruitment of specific leukocytes to the site of injury or infection through the creation of a chemokine concentration gradient. The localization and activation of

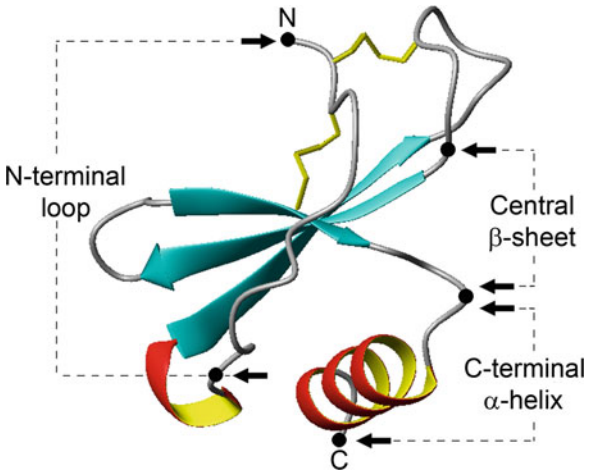

Fig. 1 Stereo ribbon diagram of CCL20 (PDB code 1M8A) showing the highly conserved three-dimensional structure of the chemokine family. The two disulfide bonds are represented in *yellow*, and the N and C termini are labeled. The structural elements normally analyzed for antimicrobial activity, such as the N-terminal loop, the central β-sheet, and the C-terminal α-helix, are indicated between the *arrows*. Figure prepared with MolMol software

the white blood cells is achieved by binding of the chemokines to their cognate chemokine receptors, which are all part of the G-protein-coupled transmembrane receptor family (Proudfoot 2002; Allen et al. 2007). In addition to their chemotactic activity, several chemokines and chemokine receptors are known to play a role in various diseases (Viola and Luster 2008; Proudfoot 2002; Allen et al. 2007). The chemokine family is usually classified into four groups which can be distinguished by the organization of the cysteine residues in the N-terminal region of the protein, i.e., C, CC, CXC, and CX_3C, where X represents any nonconserved amino acid residue (Murphy et al. 2000). The CXC group of chemokines can be further divided into two subgroups, where the presence or absence of the amino acid sequence ELR in the N-terminal region creates the ELR and non-ELR CXC chemokine subfamilies. All chemokines of the CC, CXC, and CX_3C classes form two disulfide bonds, while the C class only possesses one stabilizing disulfide linkage. To date, approximately 50 chemokines and 20 chemokine receptors have been reported (Allen et al. 2007). Although the sequence identity among all the members of the chemokine family is highly variable (20–90 %), their three-dimensional structures display a remarkable conserved topology. Most chemokines are formed by an N-terminal extended loop region, in which the first two cysteine residues are located, a bundle of three antiparallel β-strands, and a C-terminal α-helix (Allen et al. 2007) (Fig. 1). Several structural features are important for the signaling induced by the chemokines upon binding to their receptors. The N-terminal domain has emerged as the main recognition site in these proteins. Deletion of this region inhibits chemokine-induced signaling and in some cases also affects the interaction with the receptor. The N-loop located between the first cysteine residues and the first β-strand is important for the interaction with the receptor and is generally considered as the first docking site during the two-step binding process of the chemokines to their receptors. The loops connecting the β-strands also contribute to the interaction with the receptor. In some cases other residues in the chemokine structure have also been shown to be important for the interactions with the receptors (Allen et al. 2007; Clark-Lewis et al. 1995).

2 Antimicrobial Activity of Chemokines

A different part of the innate immune defense against bacterial infections is formed by a large group of antimicrobial peptides (AMPs) which often have a potent activity against bacteria, fungi, and other organisms, including some viruses. The AMPs are an important part of the host defense system of widely divergent organisms including bacteria, plants, insects, and vertebrates. Antimicrobial peptides act by directly perturbing bacterial membranes or by entering the bacterial cell and interfering with important processes, e.g., DNA transcription (Epand and Vogel 1999; Nguyen et al. 2011). However, recently some antimicrobial peptides have been shown to be involved in the regulation of the immune response as well, by binding to the same receptors as used by the chemokines (Yang et al. 2004). Conversely, it has also been demonstrated that numerous chemokines can have a direct antimicrobial activity against Gram-positive and Gram-negative bacteria, as well as an antifungal activity (see Table 1). Several studies have reported on the antimicrobial activity of individual or small groups of chemokines. Unfortunately, there is no consensus about the methodology and experimental conditions used to determine the antimicrobial activity of chemokines. The liquid-phase antimicrobial and microbicidal assay is a solution-phase test widely used in antimicrobial experiments. In this assay bacteria are incubated with different concentrations of chemokines or antimicrobial agents in liquid media. The assay is based on the microtiter broth dilution assays used to assess minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) of antibiotics (Amsterdam 1996). After exposure to the peptide, wells are inspected for growth to assess the minimum inhibitory concentration, and/or serial dilutions are plated on solidified media and colony-forming units are counted after incubation, to assess the microbicidal concentration. Despite the widespread use of this technique, there is considerable variation in test parameters, such as composition of the incubation solution, period of exposure, and test strains used. Hancock and colleagues have attempted to standardize the methodology (Wiegand et al. 2008). Another test to establish the antimicrobial activity of chemokines and antimicrobial peptides is the radial diffusion assay. In this assay the microorganisms are incorporated in buffered agar or agarose-containing media, solidified in plates. Chemokines are added to wells punched in the agar, and after a defined period in which the compounds diffuse into the agar, a nutrient overlay medium is applied to the plates. The plates are then incubated to allow growth of microorganisms, and zones of inhibition are measured (Lehrer et al. 1991). The chemokines antimicrobial activity collected in Table 1 includes antimicrobial information determined by both assays, solution, and/or solid-phase test.

The antimicrobial function of the chemokines draws attention to the complementary roles that these proteins play in immunity. The capacity to exert a direct and potent antimicrobial activity seems to be an additional mechanism for fighting bacteria and other pathogens (Eliasson and Egesten 2008). Recently the group of chemokines with antimicrobial activity have been called “kinocidins” by some

Table 1 Antimicrobial activity of chemokines

	Synonyms	Antimicrobial activity	References
XC chemokine			
XCL1	Lymphotactin α; SCM-1α	*E. coli; S. aureus; S. typhimurium; C. albicans*	Yount et al. (2007), Yang et al. (2003)
XCL2	Lymphotactin β; SCM-1β	ND	
CC chemokines			
CCL1	I-309; (mouse) TCA-3	*E. coli; S. aureus*	Yang et al. (2003)
CCL2	MCP-1; MCAF; (mouse) JE	*S. aureus; S. typhimurium*	Yount et al. (2007)
CCL3	MIP-1α; MIP-1αS	No activity against *E. coli* and *S. aureus*	Cole et al. (2001), Yang et al. (2003)
CCL4	MIP-1β	No activity against *E. coli*	Cole et al. (2001)
CCL5	RANTES	*E. coli; S. aureus; C. albicans; C. neoformans*	Yount et al. (2007), Tang et al. (2002)
CCL6	(mouse) C10; (mouse) MRP-1	*E. coli; S. enterica*	Kotarsky et al. (2009)
CCL7	MCP-3	No activity against *E. coli* and *S. aureus*	Yang et al. (2003)
CCL8	MCP-2	*E. coli*	Yang et al. (2003)
CCL9	(mouse) MRP-2; (mouse) MIP-1γ	ND	
CCL10		ND	
CCL11	Eotaxin	*E. coli; S. aureus*	Yang et al. (2003)
CCL12	(mouse) MCP-5	ND	
CCL13	MCP-4	*E. coli; P. aeruginosa*	Martínez-Becerra et al. (2007), Yang et al. (2003)
CCL14	CC-1; HCC-1; NCC-2; Ckβ1; MCIF	*E. coli*	Kotarsky et al. (2009)
CCL15	CC-2; HCC-2; NCC-3; MIP-5	*E. coli*	Kotarsky et al. (2009)
CCL16	LEC; HCC-4; NCC-4; LCC-1; LMC	No activity against *E. coli* and *S. aureus*	Yang et al. (2003)
CCL17	TARC	*E. coli; S. aureus*	Yang et al. (2003)
CCL18	PARC; DC-CK-1; MIP-4; AMAC-1	*E. coli; S. aureus*	Yang et al. (2003)
CCL19	MIP-3β; ELC; exodus-3; ckβ11	*E. coli*	Yang et al. (2003)
CCL20	MIP-3α; LARC; exodus-1; (mouse) ST38	*E. coli; S. aureus; P. aeruginosa; M. catarrhalis; S. pyogenes; E. faecium; C. albicans; C. neoformans; S. coag. neg.* spp.*; Propionibacterium* ssp.	Hoover et al. (2002), Yang et al. (2003), Maerki et al. (2009)
CCL21	SLC; 6Ckine; exodus-2; TCA4; ckβ9	*E. coli; S. aureus*	Yang et al. (2003)

(continued)

Table 1 (continued)

	Synonyms	Antimicrobial activity	References
CCL22	MDC; (mouse) abcd-1	*E. coli; S. aureus*	Yang et al. (2003)
CCL23	MIP-3; MPIF-1; ckβ8-1	ND	
CCL24	MPIF-2; eotaxin-2; ckβ6	ND	
CCL25	TECK; ckβ15	*E. coli; S. aureus*	Yang et al. (2003)
CCL26	Eotaxin-3; MIP-4α	ND	
CCL27	CTACK; Eskine; ILC (mouse) ALP	*E. coli; C. albicans*	Hieshima et al. (2003), Maerki et al. (2009)
CCL28	MEC	*P. aeruginosa; K. pneumoniae; S. mutants; P. pyogenes; S. aureus; C. albicans*	Hieshima et al. (2003), Liu and Wilson (2010)
ELR CXC chemokines			
CXCL1	GRO-α; MGSA	*E. coli; S. aureus; S. typhimurium; C. albicans*	Yount et al. (2007), Yang et al. (2003)
CXCL2	GRO-β; MIP-2α	*E. coli; S. aureus*	Yang et al. (2003)
CXCL3	GRO-γ; MIP-2β	*E. coli; S. aureus*	Yang et al. (2003)
CXCL5	ENA-78	*S. pyogenes*	Linge et al. (2008b)
CXCL6	GCP-2	*E. coli; S. pyogenes; S. dysgalactiae; S. aureus; P. aeruginosa; N. gonorrhoeae; E. faecalis*	Collin et al. (2008), Linge et al. (2008b)
CXCL7	NAP-2	*S. pyogenes*	Linge et al. (2008b)
CXCL8	IL-8	*E. coli; S. typhimurium; S. aureus; C. albicans*	Yount et al. (2007), Maerki et al. (2009)
Non-ELR CXC chemokines			
CXCL4	Platelet factor-4 (PF-4)	*E. coli; S. aureus; S. typhimurium; B. subtilis; C. albicans; C. neoformans*	Krijgsveld et al. (2000), Yount et al. (2007), Tang et al. (2002), Yeaman et al. (2007)
CXCL9	Mig	*E. coli; S. aureus; S. pyogenes; L. monocytogenes; N. gonorrhoeae; B. anthracis*	Cole et al. (2001), Yang et al. (2003), Egesten et al. (2007), Crawford et al. (2009), Linge et al. (2008a)
CXCL10	gIP-10; (mouse) CRG-2	*E. coli; S. aureus; S. pyogenes; L. monocytogenes; B. anthracis*	Cole et al. (2001), Yang et al. (2003), Egesten et al. (2007), Crawford et al. (2009)
CXCL11	I-TAC; IP9; H174	*E. coli; S. aureus; S. pyogenes; L. monocytogenes; B. anthracis*	Cole et al. (2001), Yang et al. (2003), Egesten et al. (2007), Crawford et al. (2009)
CXCL12	SDF-1α; SDF-1β; PBSF	*E. coli; S. aureus*	Yang et al. (2003)
CXCL13	BCA-1; BLC	*E. coli; S. aureus*	Yang et al. (2003)

(continued)

Table 1 (continued)

	Synonyms	Antimicrobial activity	References
CXCL14	BRAK; bolekine	*E. coli; S. coag. neg.* spp.; *Propionibacterium* spp.; *S. aureus; C. albicans*	Yang et al. (2003), Maerki et al. (2009)
CXCL16[a]	SR-PSOX	*E. coli; S. aureus*	Tohyama et al. (2007)
CXCL17	DMC	ND	
CX_3C chemokines			
CX_3CL1[a]	Fractalkine; (mouse) neurotactin	No activity against *E. coli* and *S. aureus*	Cole et al. (2001), Yang et al. (2003)

ND not determined
[a]Only the chemokine domains of CXCL16 and CX3CL1 were tested for antimicrobial activity

authors (Yount et al. 2004; Yount and Yeaman 2004). One of the structural elements that may relate this group of chemokines to other disulfide-containing antimicrobial peptides is the presence of a multidimensional signature composed by a so-called γ-core motif, perhaps suggesting a common ancestry for previously unrelated groups of antimicrobial agents (Yeaman and Yount 2007; Yount and Yeaman 2004).

The activity of the majority of the antimicrobial chemokines is markedly dependent on the ionic strength of the incubation media as is also observed for antimicrobial peptides. Intriguingly, some of the antimicrobial chemokines, such as CCL20, CCL28, CXCL9, CXCL10, and CXCL11, are expressed in different epithelial cells, and it is noteworthy that they are secreted into fluids with a relatively low salt concentration which allows them to exhibit their full antimicrobial potency (Moser et al. 2006; Fujiie et al. 2001; Nakayama et al. 2001; Shirane et al. 2004; Starner et al. 2003; Sauty et al. 1999).

The structural requirements for chemokines with antimicrobial activity are not clearly identified, but some studies have tried to uncover the main features involved. A comprehensive study of a group of 30 chemokines has established that almost 80 % of the chemokines can exhibit antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* at neutral pH (Yang et al. 2003). An initial analysis of the biochemical characteristics of the antimicrobial chemokines showed that normally the chemokines with pI values higher than 9.0 possess antibacterial activity, which indicates the importance of cationicity as a major factor for the antimicrobial activity. However, unlike what is observed with many antimicrobial peptides, the potency of this activity was not correlated with the pI value of the chemokines. Other factors, such as the cationicity of the N-terminal tail, and the hydrophobicity of the surface of the chemokines were analyzed, but again there was no direct correlation between these features and the antimicrobial activity. However, the three-dimensional structures of the chemokines and in particular the electrostatic potential surface distribution of the positive charges revealed a common theme. The presence of a large three-dimensional positively charged surface patch in the proteins was a characteristic shared by all the antimicrobial chemokines. Chemokines without antimicrobial activity either do not have such a surface or have negatively charged residues interfering with the cationic

surface patches. Nonetheless, the potency of the antimicrobial activity seemed not related with the size of the positively charged surface patches (Yang et al. 2003). In general, dissecting the structural elements that are responsible for the antimicrobial activity of the chemokines can be performed by studying peptides resembling particular domains of the chemokines (Fig. 1). These domains are normally selected on the basis of biochemical properties, such as cationicity and amphipathicity, which are important properties for many AMPs (Haney et al. 2009a; Epand and Vogel 1999). A limitation to this approach obviously is that conformation-dependent positively charged surface domains of the full-length proteins cannot be mimicked. Rather, these peptides represent parts of the linearized molecules which would arise from reduction of their disulfides. Still, a considerable number of such peptides with antimicrobial activity have been identified.

Interestingly, despite the evidence related to the antimicrobial activity of chemokines, recently it had been reported that antimicrobial chemokines also induce the release of the virulence factor protein A (SPA) by a community-associated methicillin-resistant *S. aureus* (CA-MRSA) (Yung et al. 2011). It is possible that this is an example of how bacteria may utilize host defense system signals (e.g., chemokines) in order to evade the immune response. In addition, the binding of chemokines to the *S. aureus* membrane, which had been reported for CXCL9 and CXCL10, may also contribute to avoid the immune reaction of the host by restricting the amount of free chemokines available for recruitment and activation of the immune cells (Yung et al. 2011).

3 Antimicrobial Activity of CC Chemokines

3.1 Monocyte Chemoattractant Protein-4 (MCP-4)/CCL13

The monocyte chemoattractant protein-4 (MCP-4)/CCL13 contains 75 amino acid residues (Uguccioni et al. 1996). It interacts with the chemokine receptors CCR2, CCR3, and CCR5 (Leach et al. 2007; Uguccioni et al. 1996) and induces the migration of monocytes, T lymphocytes, and eosinophils (Garcia-Zepeda et al. 1996; Uguccioni et al. 1996). This particular chemokine has been shown to be involved in several inflammatory diseases including arthritis and asthma (Rojas-Ramos et al. 2003; Iwamoto et al. 2006; Kalayci et al. 2004). Although the sequence identity for the members of the monocyte chemoattractant protein (MCP) subfamily of CC chemokines (composed of CCL2, CCL7, CCL8, CCL12, and CCL13) is around 60 %, CCL13 is the only chemokine from this subfamily to exhibit antimicrobial activity against Gram-positive and Gram-negative bacteria (Martínez-Becerra et al. 2007). Studies on 19-mer peptides using CCL13 sequence as a template showed that the C-terminal peptide $CCL13_{57–75}$, called CDAP-4, had similar activity as full-length CCL13, against *E. coli* (Martínez-Becerra et al. 2007). The antimicrobial activity of this peptide was further studied against *Pseudomonas aeruginosa* and significant morphological changes were observed by transmission electron microscopy when lethal concentrations were used (Martínez-Becerra et al. 2007). The

antibacterial activity of CDAP-4 was susceptible to the ionic strength of the media, being greatly reduced at NaCl concentrations higher than 100 mM (Martínez-Becerra et al. 2007). The stability of the three-dimensional structure of the CDAP-4 peptide was studied by molecular dynamics simulations. The peptide forms a short amphipathic α-helix with a net positive charge (+5) and a high pI (10.58), characteristics that are normally observed for α-helical AMPs (Martínez-Becerra et al. 2007). In addition, an electrostatic potential analysis revealed a large positively charged surface on the peptide, which may account for its antimicrobial activity (Martínez-Becerra et al. 2007).

The structure of the full-length CCL13 shows the typical central three-stranded antiparallel β-sheet flanked by an extended loop in the N-terminal region and an α-helix in the C-terminal region, which includes residues 57–67, which are part of the CDAP-4 peptide (Barinka et al. 2008). The crystal structure suggests the formation of CCL13 dimers (Barinka et al. 2008), although solution experiments in 100 mM NH_4OAc at pH 6.8 show that CCL13 is a monomer or that it forms heterodimers when combined with other members of the same chemokine family (Crown et al. 2006). Taken together, all these results indicate that the C-terminal region of CCL13 is important for the antibacterial activity of this chemokine and that the full-length molecule may form dimers which can possibly contribute to the antimicrobial activity.

3.2 Human Macrophage Inflammatory Protein-3α (MIP-3α)/CCL20

The human macrophage inflammatory protein-3α (MIP-3α)/CCL20 is made up of 70 amino acid residues (8 kDa) and bears some resemblance with the antimicrobial β-defensin peptides (Hoover et al. 2002). This chemokine plays a role in diseases such as cancer and rheumatoid arthritis, among others (Kleeff et al. 1999; Schutyser et al. 2003; Matsui et al. 2001). CCL20 is responsible for the migration of immature dendritic cells (DC), effector/memory T cells, and B cells, upon interaction with the chemokine receptor CCR6 (Schutyser et al. 2003). The structure of CCL20 resembles that of all CC chemokines (Fig. 1). The N-terminal region, containing the two first cysteine residues, is fairly flexible, and it is connected by a 3.10 helical turn to the central region made up of a three-stranded antiparallel β-sheet, which is followed by the usual C-terminal α-helix (Chan et al. 2008; Hoover et al. 2002; Malik and Tack 2006). The available crystal structures of CCL20 show the protein forming a dimer structure (Hoover et al. 2002; Malik and Tack 2006). Diffusion NMR and pH titration studies established that the dimerization of CCL20 is markedly pH dependent, CCL20 being a dimer at neutral pH and a monomer at lower pH (Chan et al. 2008). The CCR6 receptor only interacts with CCL20, while CCR6 is also the only chemokine receptor for CCL20. This specificity between chemokine ligand and receptor is an interesting characteristic shared with eight more chemokine receptor-ligand couples (Viola and Luster 2008). However, the human β-defensin antimicrobial peptides (hBD 1–3) can also induce the migration

of cells that express either CCR6 or CCR2 (Rohrl et al. 2010a, b; Yang et al. 1999). Like the β-defensins, CCL20 has a direct antimicrobial activity against Gram-positive and Gram-negative bacteria (Hoover et al. 2002; Yang et al. 2003), as well as antifungal (Yang et al. 2003) and antiviral activity (Ghosh et al. 2009; Kim et al. 2007). The activity of CCL20 against *E. coli* is actually higher than the activity of human β-defensin 2 (hBD-2) (Hoover et al. 2002). Structurally, there are no obvious similarities between CCL20 and the β-defensins which could account for the antimicrobial activity of CCL20. The presence of a relatively high number of positive charges in the N-terminal region of CCL20 was initially thought to contribute to its antimicrobial activity, but the existence of chemokines with positively charged N-terminal regions and without antimicrobial activity indicates that other aspects should be also considered. The overlap in antimicrobial activity may arise from the presence of similarly localized positively charged patches on the surfaces of CCL20 and hBD-2 (Fig. 2). These regions are located in the turns between the N-terminal loop and the β1 strand and the turn between the β2 and β3 strands (Hoover et al. 2002). Another important structural characteristic for antimicrobial activity, contributing to possible pore formation in bacterial membranes, is the amphipathicity of the protein. CCL20 lacks a large hydrophobic surface, and the region surrounding Leu-8 is the only recognizable hydrophobic patch on the protein surface (Hoover et al. 2002).

In addition to the antimicrobial activity of the full-length CCL20, antimicrobial activity is also found in some peptides derived from CCL20. During cancer progression, the protease cathepsin D generates a C-terminal 12-residue peptide from CCL20 ($CCL20_{59–70}$) with antimicrobial activity against *E. coli*, which is significantly reduced compared to the activity of intact CCL20 (Hasan et al. 2006). Further analysis of the CCL20 structure revealed that the C-terminal α-helix starts after the Pro-51 residue. It is possible that this segment of the protein is also important for the antimicrobial activity, because of its overall cationicity and amphipathicity. The antimicrobial activity of a synthetic C-terminal CCL20 peptide composed of the last 20 amino acids ($CCL20_{51–70}$) was 60 times higher against *S. aureus* and *E. coli*, and 30 times higher against *Bacillus subtilis*, than the activity of the naturally occurring C-terminal peptide $CCL20_{59–70}$ (Chan et al. 2008; Nguyen et al. 2010). The $CCL20_{51–70}$ peptide has a large number of positive charges and forms an amphipathic α-helix upon binding to membrane mimetics such as SDS micelles (Chan et al. 2008; Nguyen et al. 2010). The shorter 12-residue $CCL20_{59–70}$ peptide has a lower positive net charge and does not form a stable α-helix in the presence of SDS micelles. Additionally its amphipathicity is perpendicular to the length of the peptide instead of parallel, as was found for $CCL20_{51–70}$ (Nguyen et al. 2010). These structural differences account for the large difference in antimicrobial activity, as a high net positive charge and amphipathicity are shared characteristics for most members of the α-helical class of antimicrobial peptides such as the magainins, cecropins, and lactoferrampin (Epand and Vogel 1999; Haney et al. 2007, 2009b). The lack of antimicrobial activity for the N-terminal fragments $CCL20_{1–52}$ and $CCL20_{1–55}$ further indicates that the C-terminal region of CCL20 is mostly responsible for the antimicrobial activity of this chemokine (Chan et al. 2008; Hasan et al. 2006).

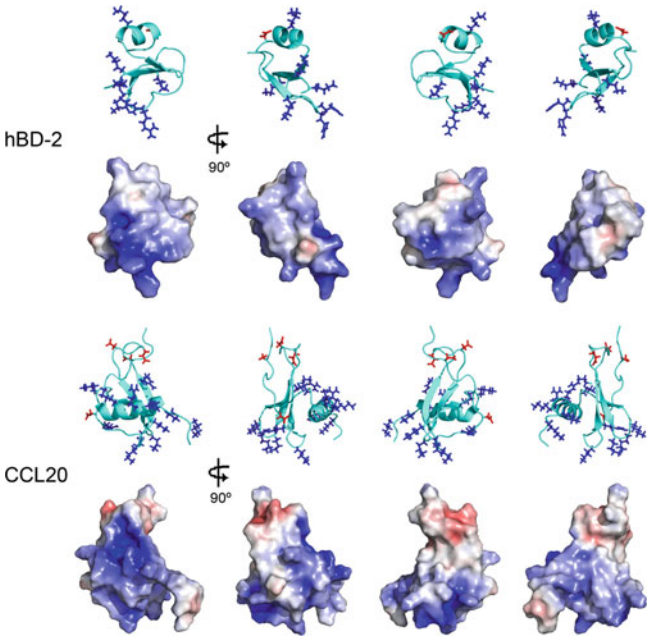

Fig. 2 *Ribbon* representation and electrostatic potential surface of human β-defensin 2 (hBD-2) (PDB code 1FD3) and chemokine CCL20 (PDB code 2JYO), with positively charged side chains in *blue* and negatively charged side chains in *red*. Each image is rotated 90° from the vertical axis. Electrostatic potential surfaces were calculated by adaptive Poisson–Boltzmann Solver (APBS) using PDB2PQR software and depicted by PyMOL software

3.3 Mucosa-Associated Epithelial Chemokine MEC/CCL28

The mucosa-associated epithelial chemokine (MEC)/CCL28 is a protein of 108 amino acids residues (12.3 kDa) that regulates the migration and activation of specific leukocytes, such as cutaneous lymphocytes, antigen-positive (Ag^+) memory T cells, and eosinophils. It interacts with the chemokine receptors CCR10 and CCR3 (Pan et al. 2000). CCL28 is constitutively expressed in human and mouse epithelial cells of tissues such as the mammary glands, the respiratory tract, the colon, and the salivary glands (Pan et al. 2000; Wang et al. 2000). The C-terminal region of CCL28 shares a high sequence identity (53 %) with the antimicrobial peptide histatin-5, which has a potent antifungal activity against *Candida albicans* (Hieshima et al. 2003). This unique feature of CCL28 focused early attention on its possible antimicrobial activity. Human CCL28 (hCCL28) exerts a direct antimicrobial activity against Gram-negative and Gram-positive bacteria, in addition to possessing an antifungal activity (see Table 1). The antimicrobial activity against *C. albicans* and *P. aeruginosa* was more potent at lower salt concentrations (Hieshima et al. 2003; Liu and Wilson 2010). The most closely related chemokine, CCL27 (sequence identity 40 %) (Pan et al. 2000), which lacks the extended C-terminal region of CCL28, does not have bactericidal activity and

only low activity against *C. albicans* at high concentrations (Hieshima et al. 2003; Yang et al. 2003). In line with this, the first two positively charged residues in the 85–89 region (RKDRK) of murine CCL28 (mCCL28) are highly conserved among CCL28 homologues in all studied mammalian species and are essential for the antibacterial activity (Liu and Wilson 2010). These results indicate that the direct antimicrobial and antifungal activities of CCL28 rely on the C-terminal histatin-like region. A 28-residue peptide corresponding to this C-terminal region of the hCCL28 was even more active than histatin-5 against *C. albicans* but showed a low antibacterial activity (Hieshima et al. 2003). In a similar fashion, the 52-residue peptide corresponding to the C-terminal region of mCCL28 had a reduced antimicrobial activity against *S. aureus* and *P. aeruginosa*, while a 56-residue peptide resembling the N-terminal region of mCCL28 had no antibacterial activity (Liu and Wilson 2010). The broader antimicrobial activity of CCL28 compared to its C-terminal CCL28 peptide established that not only the C-terminal region is required for killing the bacteria but in addition the interaction with the rest of the protein is essential for the expression of the full antimicrobial potential, a notion that was further supported by elegant studies with protein chimeras (Liu and Wilson 2010). In addition, the influence of the disulfide bridges that normally stabilize the chemokine structure of CCL28 was studied, showing that the tertiary structure is not required for antimicrobial activity but is essential for its chemotactic activity (Liu and Wilson 2010). Similar results have been obtained for human β-defensin 3 (hBD-3) and tachyplesin I, showing that the cysteine bridges are not mandatory for their antimicrobial activity (Hoover et al. 2003; Wu et al. 2003; Ramamoorthy et al. 2006) but in the case of hBD-3 are again required for the chemotactic function (Hoover et al. 2003; Wu et al. 2003).

In addition to the above-mentioned CC chemokines, other members of this family have been shown to possess antimicrobial activity (see Table 1). Further studies need to be done in order to establish the structural characteristics that confer the antimicrobial activity to these chemokines.

4 Antimicrobial Activity of ELR CXC Chemokines

4.1 Granulocyte Chemotactic Protein-2 (GCP-2)/CXCL6

Granulocyte chemotactic protein-2 (GCP-2)/CXCL6 is a chemokine of the ELR CXC family, made up of 77 amino acids residue (Proost et al. 1993a, b; Froyen et al. 1997; Van Damme et al. 1997). It is mainly involved in chemoattracting neutrophils (Froyen et al. 1997; Wuyts et al. 1997; Viola and Luster 2008) due to its interactions with the chemokine receptors CXCR1 and CXCR2 which are also expressed in monocytes and mast cells (Wuyts et al. 1997, 1998; Wolf et al. 1998). CXCL6 is expressed by epithelial cells, macrophages and mesenchymal cells (Fillmore et al. 2003; Collin et al. 2008; Mine et al. 2003; Prause et al. 2003; Gijsbers et al. 2004;

Wuyts et al. 2003). CXCL6 has been shown to possess an important NaCl-sensitive antimicrobial activity (Collin et al. 2008), which is comparable with the activity of LL-37 (Linge et al. 2008b), a well-known human antimicrobial peptide of the cathelicidin family (Zanetti 2004). Gram-positive and Gram-negative bacteria that are normally involved in infections of dermis and mucosal surfaces are susceptible to the bactericidal action of CXCL6 (see Table 1), which appears to be related to the disruption of the bacterial membranes (Linge et al. 2008b). Interestingly, previous studies did not detect any antimicrobial activity of CXCL6 against *E. coli* and *S. aureus* (Yang et al. 2003). The discrepancies in the antibacterial activity can likely be attributed to the differences in the incubation media used in the different studies. Egesten and coworkers showed that under the same conditions, the antimicrobial activity of CXCL5/ENA-78 and CXCL7/NAP-2 against *Streptococcus pyogenes* was 30 times less than the activity of CXCL6 (Linge et al. 2008b). The structure of CXCL6 has not been determined yet, but a structural model can be predicted based on the known structures of other members of the CXC chemokine family, which showed that the structure resembles the general fold of chemokines. The N-terminal region is devoid of regular secondary structure and it contains two cysteine residues located at positions 12 and 14, which form disulfide bonds with the Cys residues at positions 38 and 54. The central region is formed by three antiparallel β-strands and the C-terminal region is a short α-helix. The cationic charge and amphipathicity of the C-terminal α-helix resembles the biochemical properties and secondary structure of some antimicrobial peptides, pinpointing this region of the chemokine as being possibly responsible for the antibacterial activity. Comparisons of the antimicrobial activity of the full-length CXCL6 protein and peptides resembling the N-terminal or C-terminal region of CXCL6 established that full-length CXCL6 is more active than either of these peptides. Interestingly, the bactericidal activity of the peptide encompassing the N-terminal region was higher than the activity of the C-terminal α-helix peptide, indicating that the C-terminal region alone is not the major determinant for the antimicrobial activity. Instead, the N-terminal region seems to be more relevant for the bactericidal activity in this case. These results also correlate with the higher leakage induced in DOPE/DOPG liposomes by CXCL6 and its N-terminal region peptide. Circular dichroism experiments showed that CXCL6 and its C-terminal region share up to 25 % of helical content in the structure, while the N-terminal region only contains 6 % upon interaction with PGPE liposomes. These results reveal that helical content is not directly correlated with the antimicrobial activity of CXCL6-derived peptides. When the net charge of the CXCL6-derived peptides is compared, the presence of an extra positive charge in the N-terminal peptide appeared related with the higher antibacterial activity (Linge et al. 2008b). It is known that the secreted *Streptococcus pyogenes* cell envelope proteinase (SpyCEP) cleaves CXCL6 in a position affecting the chemokine-induced neutrophil activation (Zingaretti et al. 2010; Sumby et al. 2008). Additionally, in vivo studies showed that the non-SpyCEP expressing bacterial strains gave rise to larger lesions in infected mice (Sumby et al. 2008). Although studies of the direct antimicrobial activity of the CXCL6-derived peptides

resulting from SpyCEP digestion have not yet been reported, it is possible that the action of this protease also impairs the antimicrobial activity of CXCL6 thereby contributing to the large lesions observed in the in vivo studies.

4.2 Neutrophil-Activating Peptide-2 (NAP-2)/CXCL7 and CTAP-3

Neutrophil-activating peptide-2 (NAP-2)/CXCL7 is a 70 amino acid residue chemokine of the ELR CXC family (Brandt et al. 2000). Together with connective tissue-activating protein-3 (CTAP-3), CXCL4/PF-4, and CCL5/RANTES, it forms the major portion of the platelet-derived chemokines (Flad and Brandt 2010). CXCL7 is derived from the β-thromboglobulins which represent a group of homologous α-granule-stored proteins (Brandt et al. 2000). The primary sequences of these proteins are identical except for their N-termini (Fig. 3a). The two main β-thromboglobulins found in human platelets are the platelet basic protein (PBP) and CTAP-3 (Brandt et al. 2000). Although both of these CXCL7 precursors contain the full sequence of the active CXC chemokine (CXCL7) including the ELR motif, they lack detectable chemotactic activity (Walz et al. 1989). PBP and CTAP-3 have minor antimicrobial activity against *E. coli*, *S. aureus*, and *Cryptococcus neoformans* under slightly acidic conditions (Tang et al. 2002). Proteases such as cathepsin G, which is membrane-associated or released from neutrophils and monocytes, can cleave both precursors between a specific Tyr and an Ala residue in the N-terminal region, thereby releasing the active CXCL7 chemokine (Car et al. 1991; Walz and Baggiolini 1990; Cohen et al. 1992; Brandt et al. 1991; Harter et al. 1994). CXCL7 exhibits important chemoattracting activity for neutrophils (Brandt et al. 2000), but its antimicrobial activity is almost negligible (Linge et al. 2008b; Krijgsveld et al. 2000). In addition to the N-terminally extended precursors of CXCL7, C-terminally truncated variants of CXCL7 have been described (Fig. 3a). Proteolytic processing of chemokine proteins occurs quite frequently in vivo and can have a major impact on the biological activity of the proteins (Wolf et al. 2008). C-terminally truncated CXCL7 has increased chemotactic activity, which is attributed to the removal of negatively charged Asp residues that are present in the C-terminal end of the full-length CXCL7 protein (Ehlert et al. 1995, 1998; Brandt et al. 1993, 2000). Regarding the antimicrobial activity of C-terminally truncated CXCL7 variants, a protein with high microbicidal activity, termed thrombocidin-1 (TC-1), has been isolated from human platelets following antimicrobial activity-guided purification. It possesses the same primary sequence as CXCL7, but the last two residues of the C-terminal region have been cleaved off, which causes an important increase in the microbicidal activity of the chemokine, which due to the truncation becomes highly active against *E. coli*, *B. subtilis, S. aureus, Lactococcus lactis*, *and C. neoformans* (Krijgsveld et al. 2000). Additionally, TC-1 is an important factor in the defense against infective endocarditis induced by viridans streptococci (Dankert et al. 2001). The TC-1 protein may

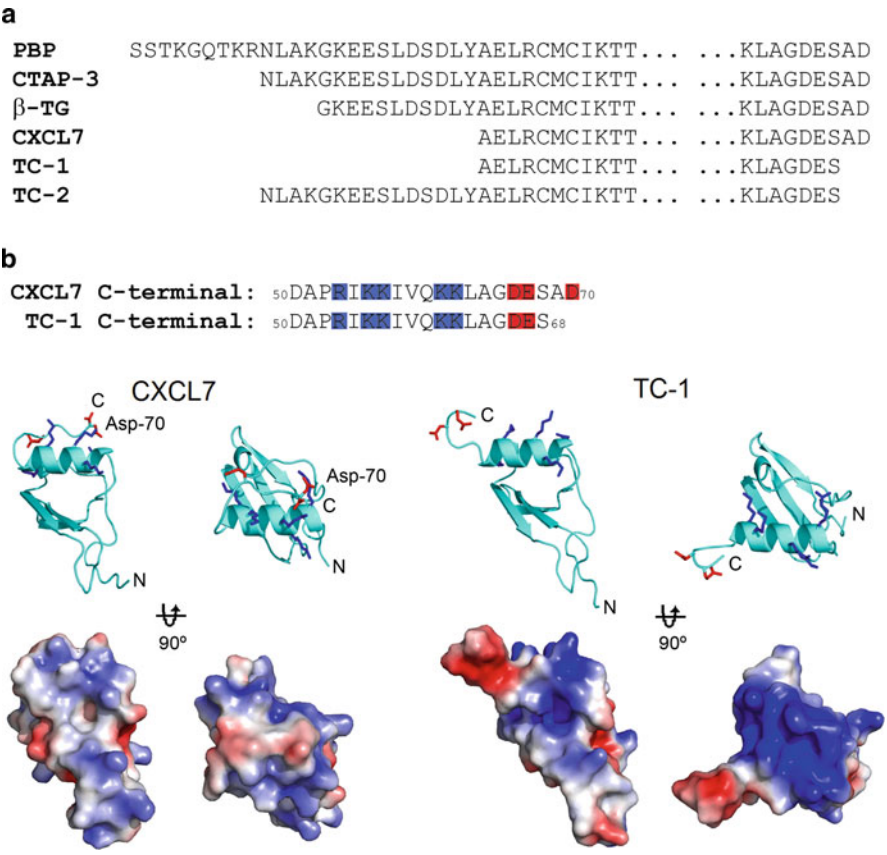

Fig. 3 (**a**) N- and C-terminal primary sequences of platelet basic protein (PBP) and derived ß-thromboglobulin (ß-TG) family members. (**b**) NAP-2/CXCL7 and TC-1 3D structures and electrostatic potential surfaces. Full-length CXCL7 and TC-1 structures were created by Nguyen et al. (2011) based on the combination of CXCL7 crystal structure (PDB code 1NAP) and C-terminal peptides (*upper sequences*) NMR structures. Negatively and positively charged residues are colored in *red* and *blue* respectively. Electrostatic potential surface were calculated by Adaptive Poisson–Boltzmann Solver (APBS) using PDB2QR software and depicted by PyMOL software

not act through membrane perturbation, as indicated by its inability to dissipate the transmembrane potential of *L. lactis* bacteria and of liposomes composed of *E. coli* lipids (Krijgsveld et al. 2000). The structure of CXCL7 has been solved by NMR spectroscopy (Mayo et al. 1994; Yang et al. 1994) and X-ray crystallography (Young et al. 1999). The structures are in agreement and show the classic CXC chemokine topology of an N-terminal loop, a three-stranded antiparallel β-sheet, followed by a C-terminal α-helix which includes residues Arg54-Asp66 (Fig. 3b). Disordered electron density for the last four residues of the crystal structure suggest a high degree of flexibility for the extreme C-terminal region of this chemokine (Young et al. 1999). Recent NMR studies have shown that the overall fold of TC-1 closely resembles that of CXCL7 (Nguyen et al. 2011). One of the truncated

residues in TC-1 is the negatively charged Asp-70, the removal of which seems to account for the emergence of the antibacterial activity. Studies of synthetic C-terminal α-helical peptides of both chemokines (CXCL7 and TC-1) were carried out in order to identify the influence of these parts of the proteins on the antimicrobial activity. Neither of the peptides was antimicrobial, and they showed a helical structure with a low degree of hydrophobicity and amphipathicity. The structure of the CXCL7 C-terminal peptide showed that the side chain of the C-terminal residue Asp-70 actually folds back and interacts with the positively charged residue Arg-61 located in the α-helical region (Nguyen et al. 2010). Subsequent NMR relaxation studies, which studied the flexibility of the protein backbone in CXCL7 and TC1, showed that the Asp-70 residue of CXCL7 is motionally restricted and interacts with the positive surface region of the protein (Fig. 3b). In contrast, in TC-1 the last few residues are highly flexible, and the positively charged surface region is exposed (Fig. 3b), being able to directly interact with negatively charged bacterial membranes to either perturb the membrane or enter the cell (Nguyen et al. 2011), explaining the difference in microbicidal activity with full-length CXCL7.

In the activity-guided isolation of human platelet antimicrobial proteins, a second protein, thrombocidin-2 (TC-2) was identified (Krijgsveld et al. 2000). TC-2 is identical to CTAP-3 except for a truncation of the two C-terminal residues. CTAP-3 has been putatively identified as one of the antimicrobial-active PBP derivatives in monocytes after detection in gel overlay assays with a highly AMP-susceptible *S. typhimurium phoP* mutant strain as the target organism (Schaffner et al. 2004). As mentioned before, the activity as such of CTAP-3 is not high, with only minor activity reported against wild-type strains of *S. aureus*, *E. coli*, and *C. neoformans* in slightly acidic conditions (Tang et al. 2002), and no bactericidal activity against *Bacillus subtilis*, *E. coli*, or *S. aureus* when tested up to 30 μM. A C-terminal two amino acid truncation, yielding TC-2, however strongly increases the microbicidal activity (Krijgsveld et al. 2000). Of note, the truncation is identical to that generating TC-1 from CXCL7 (Fig. 3), suggesting that this is a more general step in generating antimicrobially active derivatives from members of the PBP protein family.

4.3 Interleukin-8 (IL-8)/CXCL8

Interleukin-8 (IL-8)/CXCL8 was one of the first chemokines discovered (Schroder et al. 1987; Walz et al. 1987; Yoshimura et al. 1987b). It contains 72 amino acid residues (8 kDa) and upon stimulation CXCL8 can be produced by a wide variety of cells, including fibroblasts, epithelial and endothelial cells, hepatocytes and monocytes, among others (Baggiolini et al. 1989). CXCL8 is important for the activation and attraction of neutrophils to sites of inflammation, but other cells are also susceptible to its chemotactic activity (Baggiolini et al. 1989). As expected, the chemotactic activity arises through interactions with chemokine receptors, in this case CXCR1 and CXCR2 (Wu et al. 1996; Holmes et al. 1991; Murphy and Tiffany 1991). CXCR1 and CXCR2 and their associated ligands have been related to a large number

of pathologies (Bizzarri et al. 2006). CXCL8 is also important for the promotion of angiogenesis (Li et al. 2005; Matsuo et al. 2009). As described above for CXCL7, a C-terminally truncated variant of CXCL8 lacking the last three residues exhibits higher chemotactic activity than the full-length chemokine (Clark-Lewis et al. 1991). The structure of CXCL8 has been resolved by both NMR spectroscopy and X-ray crystallography (Baldwin et al. 1991; Clore et al. 1989, 1990). These structures are in agreement and show an architecture composed of an extended loop followed by a 3.10 helical turn (involving residues 19–22) and the antiparallel stranded β-sheet connected to a C-terminal α-helix (corresponding to residues 57–72). In solution CXCL8 behaves as a dimer, where the monomers are connected by six backbone hydrogen bonds between residues 25, 27, and 29 (Clore et al. 1989, 1990). The antibacterial activity of CXCL8 has been somewhat controversial. Bylund and colleagues, using a modified inhibition zone assay, were not able to observe any antimicrobial activity for full-length CXCL8 against *E. coli* (Bjorstad et al. 2005). Similarly, no antimicrobial activity for CXCL8 against *E. coli* and *S. aureus* (Cole et al. 2001; Yang et al. 2003) was found using standard colony-forming unit assays (Harder et al. 2001) or radial diffusion assays (Steinberg and Lehrer 1997). In contrast, Yeaman and colleagues observed different levels of pH-dependent antimicrobial activity of CXCL8 against *Salmonella typhimurium* and *S. aureus* in solid-phase assays, but they did not record bactericidal activity in solution-phase assay. Their results suggest that the growth inhibition observed in the solid-phase assay is due to bacteriostatic effects. Additionally, an antifungal activity against *C. albicans* was detected in both solid- and solution-phase assays (Yount et al. 2007). The γ-core motif of CXCL8 (IL-8γ), located in the central β-sheet region, was tested as a separate peptide for antimicrobial and antifungal activity. The peptide showed no activity against *S. typhimurium, S. aureus* or *C. albicans*, in neither the solid-phase nor the solution-phase assay (Yount et al. 2007).

Another interesting structural element of CXCL8 which may exert antimicrobial activity is the C-terminal α-helix. A 19-residue C-terminal peptide, termed IL-8α, proved to be active against *S. typhimurium* and *C. albicans* in solid-phase assays and in solution-phase assays against *C. albicans*. In human blood matrices, the IL-8α 19-mer peptide exerts anti-*E. coli* activity (Yount et al. 2007). Acid hydrolysis of CXCL8 in vitro can generate a 20-residue IL-8α peptide with an extra Pro residue at the N-terminal region compared to the 19-mer peptide (Bjorstad et al. 2005). Antimicrobial assays performed with this IL-8α 20-mer peptide showed high activity against *E. coli* and moderate activity against *Salmonella enterica, Klebsiella pneumoniae*, and *S. pyogenes*, as established by inhibition zone assays (Bjorstad et al. 2005). The same peptide was tested for antibacterial activity in blood agar plates and moderate activity was found for *B. subtilis* and almost no activity could be detected with *S. aureus* and *E. coli* (Nguyen et al. 2010). The differences in the antimicrobial activity for IL-8α and CXCL8 in these studies may have been due to differences in media and incubation conditions. When comparing the SDS-micelle-bound structure of IL-8α 19-mer peptide and the C-terminal α-helix in the full-length CXCL8, there are no significant differences (Bourbigot et al. 2009). The structure of the IL-8α 20-mer peptide, dissolved in a chloform–ethanol–water (4:4:1) solvent mixture (Nguyen et al. 2010), is also in

agreement with the structures of the IL-8α peptide (Bourbigot et al. 2009) and that of the C-terminal α-helix in intact CXCL8 (Baldwin et al. 1991; Clore et al. 1989, 1990). In summary, the C-terminal region of CXCL8 seems to be important for the antimicrobial activity of this particular chemokine. As mentioned before, the *S. pyogenes* SpyCEP protease can cleave ELR CXC chemokines, including human CXCL8 (Edwards et al. 2005; Zinkernagel et al. 2008; Zingaretti et al. 2010; Hidalgo-Grass et al. 2006). The action of SpyCEP on CXCL8 releases a C-terminal peptide composed of residues 60–72 (Edwards et al. 2005). The direct antimicrobial activity of this peptide has not yet been tested in vitro, but in vivo and in vitro studies did show that the SpyCEP protease prevents eradication by neutrophils of the SpyCEP-expressing bacteria and other bacteria at the site of infection (Zinkernagel et al. 2008; Hidalgo-Grass et al. 2006). This effect has been attributed to diminished neutrophil recruitment and migration (Edwards et al. 2005; Zinkernagel et al. 2008) in addition to an inhibition of the formation of neutrophil extracellular traps (NETs) (Zinkernagel et al. 2008), due to digestion of CXCL8.

4.4 Growth-Regulated Oncogene α (GROα)/CXCL1, β (GROβ)/CXCL2 and γ (GROγ)/CXCL3

Among the members of the ELR CXC chemokine family with antimicrobial activity, there are three chemokines with similar characteristics: CXCL1/GROα, CXCL2/GROβ, and CXCL3/GROγ, closely related chemokines that are produced mainly by macrophages (Becker et al. 1994). These proteins have the capacity to attract neutrophils (Clark-Lewis et al. 1995) and monocytes (Smith et al. 2005), through interactions with the chemokine receptor CXCR2 (Katancik et al. 2000). All three chemokines have antibacterial activity against *E. coli* and *S. aureus* (Yang et al. 2003).

In addition to the above-mentioned ELR CXC chemokines, CXCL5 also exhibits antimicrobial activity against *S. pyogenes*. In summary, all members of the ELR CXC chemokine family exhibit a direct antimicrobial activity (see Table 1).

5 Antimicrobial Activity of Non-ELR CXC Chemokines

5.1 Platelet Factor-4 (PF-4)/CXCL4

Although the platelet factor-4 (PF-4)/CXCL4 is the earliest discovered member of the CXC chemokine family (Deuel et al. 1977), its biological function was not known to be related to the chemoattraction of specific cells until recent years.

CXCL4 is expressed mainly in the megakaryocytes and in platelets (Slungaard 2005). While other members of the non-ELR CXC chemokines, e.g., CXCL9–11, induce the migration of lymphocytes (Bonecchi et al. 1998; Sallusto et al. 1998), CXCL4 was initially thought to be devoid of such an activity (Clark-Lewis et al. 1993). A spliced variant of CXCR3 (named CXCR3-B) was described early on as the receptor for CXCL4. However, this receptor did not mediate a chemotactic response but induced an increase in the intracellular cyclic AMP levels instead (Lasagni et al. 2003; Slungaard 2005). A recent study suggests that migration of activated T lymphocytes can be induced by CXCL4 and that this is mediated by the chemokine receptor CXCR3 (Mueller et al. 2008), but these results have not been confirmed by other groups (Flad and Brandt 2010). In addition, the biological activity of CXCL4 is broad and includes roles in coagulation and functions such as inhibition of angiogenesis and hematopoiesis, promotion of neutrophil adhesion and activation, enhancement of oxy-LDL binding to the LDL receptor, and stimulation of anticoagulant activated protein C generation by the thrombomodulin/protein C system (Slungaard 2005). The generation of a CXCL4 knockout mouse has provided support for the role of this chemokine in platelet-dependent thrombosis (Slungaard 2005). CXCL4 also exhibits direct antimicrobial activity under slightly acidic conditions against Gram-positive and Gram-negative bacteria, in addition to antifungal activity (Tang et al. 2002) (see Table 1). The structure of human CXCL4 was solved as a tetramer by X-ray crystallography, showing a three-dimensional structure in agreement with the overall chemokine topology formed by the usual N-terminal loop, three-stranded antiparallel β-sheet, and C-terminal α-helix (Zhang et al. 1994). Based on phylogenetic relatedness, similar sequence motif, and predicted three-dimensional structures, platelet microbicidal protein-1 (PMP-1) was identified as a rabbit analogue of the human CXCL4 (Yount et al. 2004). In earlier studies, prior to their sequence identification, PMP-1 and its variant tPMP-1 (secreted by platelets after thrombin stimulation) were shown to exhibit a pH-dependent antimicrobial activity against Gram- positive and Gram-negative bacteria and fungi (Yeaman et al. 1997). The antimicrobial activities of PMP-1 and human CXCL4 exhibit similar specificities and efficacy (Yeaman et al. 2007). In the case of *S. aureus*, PMP-1 activity has been linked with an inhibition of intracellular macromolecular synthesis (Xiong et al. 2002). A synthetic peptide corresponding to the last 13 residues of the CXCL4 C-terminal region, which constitute an α-helix, is active against *E. coli* in the presence of a sub-MIC concentration of cefepime (a β-lactam antibiotic) in serum bactericidal assays (Darveau et al. 1992). RP-1 and RP-11 are peptides designed based on the α-helical C-terminal region of platelet proteins PMP-1, tPMP-1, and CXCL4, which have antimicrobial activity in in vitro biological matrices (Yeaman et al. 2002). The effects of RP-1 on *S. aureus* resemble the effects induced by tPMP-1, indicating that the antimicrobial mechanism of certain proteins can be reproduced by the synthetic peptides modeled upon relevant structural domains (Xiong et al. 2006). Comparison of the sequences for CXCL4, PMP-1, and their respective variants led to the creation of a consensus PMP sequence (cPMP), which was used to

generate a peptide library constituting of 15 amino acid long peptides, overlapping by 3 residues each, and larger peptides comprising the N or C-terminal half of the cPMP (Yeaman et al. 2007). Among the library, the peptides with the highest antimicrobial activity were $cPMP_{38–74}$, $cPMP_{49–64}$, and $cPMP_{60–74}$, which represent the C-terminal region of the protein (Yeaman et al. 2007). Most of the peptides corresponding to the N-terminal and the β-sheet regions did not exhibit considerable antimicrobial activity (Yeaman et al. 2007). These results suggest that the C-terminal region of CXCL4 is important for the overall antimicrobial activity of this chemokine.

5.2 *Monokine Induced by IFN-γ (MIG)/CXCL9*

The human monokine induced by IFN-γ (MIG)/CXCL9 is a protein of 103 residues of the CXC chemokine family (Liao et al. 1995; Farber 1993). The protein is chemotactic for monocytes, activated T cells, and NK cells (Liao et al. 1995; Lazzeri and Romagnani 2005; Loetscher et al. 1996). The chemotactic activity involves binding to the chemokine receptors CXCR3A and CXCR3B (Loetscher et al. 1996; Lazzeri and Romagnani 2005). The long C-terminal region of CXCL9 undergoes proteolytic processing by monocytes. This truncation has a profound effect on its biological activity (Liao et al. 1995; Farber 1997). Interferon-inducible protein-10 (IP-10)/CXCL10 and interferon-inducible T-cell alpha chemoattractant (I-TAC)/CXCL11 are closely related to CXCL9/MIG, sharing the same receptor and subsequently expressing similar biological activity (Farber 1997; Cole et al. 1998). Full-length CXCL9 displays a broad antimicrobial activity against organisms including *E. coli*, *Listeria monocytogenes, S. aureus* (Cole et al. 2001; Yang et al. 2003), *S. pyogenes* (Egesten et al. 2007), *Neisseria gonorrhoeae* (Linge et al. 2008a), and *Bacillus anthracis* spores and bacilli (Crawford et al. 2009). This antibacterial activity was shown to be inhibited by an increase in the ionic strength of the media in the case of *E. coli, L. monocytogenes*, and *S. pyogenes*. Although the three-dimensional structure of CXCL9 has not yet been solved, a model structure was created using the NMR structure of a truncated CXCL2/GROβ (Qian et al. 1999) as a template (Egesten et al. 2007). Due to the presence of a high number of positive charges in both the N-terminal and C-terminal regions of CXCL9, the antimicrobial activity might be embedded in either of these two sections of the protein. Consequently, peptides resembling the cationic regions of the N-terminal and C-terminal sections of CXCL9 were tested against *S. pyogenes*, and the activity of the C-terminal peptide was similar to the antibacterial activity of the full-length CXCL9. The N-terminal peptide did not have any anti-*S. pyogenes* activity, indicating that the antimicrobial activity of CXCL9 resides in its C-terminal region (Egesten et al. 2007). Streptococcal inhibitor of complement (SIC) is a protein secreted by *S. pyogenes* strains of the M1 serotype, which in addition to interfering with complement function inhibits the activity of human antimicrobial peptides,

such as LL-37 and β-defensins (Frick et al. 2003). Additionally SIC interferes with the antibacterial activity of CXCL9, without disturbing its chemotactic activity (Egesten et al. 2007), suggesting that SIC interacts with the antimicrobial C-terminal region of CXCL9. Although CXCL9, CXCL10, and CXCL11 are interferon-γ-inducible related chemokines that interact with the same CXCR3 receptor and have a similar antibacterial spectrum (Cole et al. 2001; Egesten et al. 2007; Yang et al. 2003; Crawford et al. 2009), the antimicrobial activity of CXCL10 and CXCL11 against *E. coli*, *L. monocytogenes*, and *S. pyogenes* is tenfold less than that of CXCL9 (Cole et al. 2001; Egesten et al. 2007). Analysis of the structures of CXCL10/IP-10 (Swaminathan et al. 2003), IP-10 mutant (NMeLeu27) (Booth et al. 2002), and CXCL11 (Booth et al. 2004) has shown that their C-terminal α-helices are smaller than the predicted C-terminal α-helix in CXCL9, which may account for the difference in the antimicrobial activities (Egesten et al. 2007; Eliasson and Egesten 2008). SpeB from *S. pyogenes* is a cysteine protease that cleaves and inactivates the antimicrobial peptide LL-37 (Schmidtchen et al. 2002). It also inactivates several human chemokines with antibacterial activity (Egesten et al. 2009). SpeB fully degrades CXCL10 and CXCL11, completely abolishing their chemotactic as well as antimicrobial activity. In contrast CXCL9 is only partially digested by SpeB, with cleavage sites in the N-terminal and C-terminal regions, releasing a smaller version of CXCL9 comprising the residues 18–73. This truncated version of CXCL9 has lost its chemotactic activity but retains its antibacterial activity against *S. pyogenes*. The synthetic peptide $CXCL9_{57–83}$ and SpeB-produced $CXCL9_{18–73}$ showed similar antimicrobial activities as the full-length CXCL9 (Egesten et al. 2007, 2009), indicating that not all the residues in the α-helical C-terminal peptide are responsible for the antimicrobial activity. Similarly, the truncation of CXCL10 by the furin protein generates a CXCL10 with four C-terminal residues removed. This variant is as antimicrobial as its precursor, CXCL10, against *E. coli* and *L. monocytogenes* (Hensbergen et al. 2004).

Among the group of non-ELR CXC chemokines, most members exert antimicrobial activity (see Table 1). Although structural elements as the C-terminal α-helical region have emerged as possibly being responsible for the antimicrobial activity in these chemokines, more studies are required.

6 Chemokine Dimerization and Antimicrobial Activity

Although many of the chemokines have the ability to form dimers or even higher-order oligomers in solution or after binding to glycosaminoglycans (Allen et al. 2007; Salanga and Handel 2011), the relationship of these oligomers with the antimicrobial activity of the bactericidal chemokines has not yet been studied much. Oligomerization has been postulated as one of the key steps in the antimicrobial activity mechanism of pore-forming antimicrobial peptides (Shai 1999; Mihajlovic and Lazaridis 2010; Mani et al. 2006). Oligomerization can allow the

formation of transmembrane pores leading to bacterial cell death (Shai 1999; Matsuzaki 1998). For some antimicrobial peptides, such as LLP1 and modified versions of magainin-2, the formation of disulfide-linked dimers has been shown to play an important role in the antimicrobial activity against Gram-positive and Gram-negative bacteria (Tencza et al. 1999; Dempsey et al. 2002). The formation of dimers prior to the interaction with the bacterial membranes increases the attraction for negatively charged membranes, probably due to the increase in the net positive charge of these peptides (Dempsey et al. 2002), generating peptides with 4–8 times higher activity, compared with the monomeric forms (Tencza et al. 1999). Not only the formation of covalently bound dimers can be related with an increase in antimicrobial activity. Protegrin-1 (PG-1) is a potent antimicrobial peptide for which the mechanism of action has been related to the formation of transmembrane pores (Bolintineanu et al. 2010; Langham et al. 2008). Using NMR, the dimerization of PG-1 was established as an essential requirement for the formation of pores in anionic membranes (Mani et al. 2006; Roumestand et al. 1998). Molecular dynamic simulations have confirmed the tendency of PG-1 to form dimers in the aqueous phase, on a membrane surface, or in the membrane core (Vivcharuk and Kaznessis 2010b). It was also established that the PG-1 dimer rather than the PG-1 monomer has a favorable energy for interactions with the membrane (Vivcharuk and Kaznessis 2010a). Other examples of this are seen in the β-defensin family of antimicrobial peptides. The tendency of human β-defensin 3 (hBD-3) to form non-covalent dimers may explain the large difference in the antimicrobial activity among the known human β-defensins (Schibli et al. 2002). When compared with other human β-defensins (hDB-1 and hDB-2), hBD-3 has been reported to possess a more potent and salt-resistant antimicrobial activity against a broader spectrum of pathogens (Pazgier et al. 2006; Taylor et al. 2008). It has been proposed that this increase may be related to the formation of dimers which exhibit a considerable increase in the size of the positively charged surface, thereby increasing the attraction for negatively charged membranes (Schibli et al. 2002). Although crystallography studies of the hDB-2 structure in addition to molecular dynamic studies had shown the possibility of dimerization for this protein (Suresh and Verma 2006; Hoover et al. 2000), static and dynamic light scattering experiments in 0.1 M Tris and pH 8.0 showed that only hDB-3 exists as a dimer in solution (Schibli et al. 2002). Some of the antimicrobial chemokines also have the ability to form non-covalent dimers in solution, which in addition to enhancing the net positive charge of the protein also increases the size of the positively charged surface. The configuration of the chemokines dimers can be divided into two groups. CC-chemokines normally form dimers by interactions between the residues of the first β-strand, forming a two-stranded antiparallel β-sheet with the corresponding β-strand from the other monomer, resulting in a more elongated dimer structure. In contrast, CXC chemokines normally form dimers by interactions between the β-strands forming a continuous six-stranded β-sheet, resulting in a more compact dimer structure (Allen et al. 2007) (Fig. 4a). The chemokine CCL20 is an exception to the previously described dimerization scheme for CC chemokines, as it follows the scheme for CXC chemokines. The CCL20

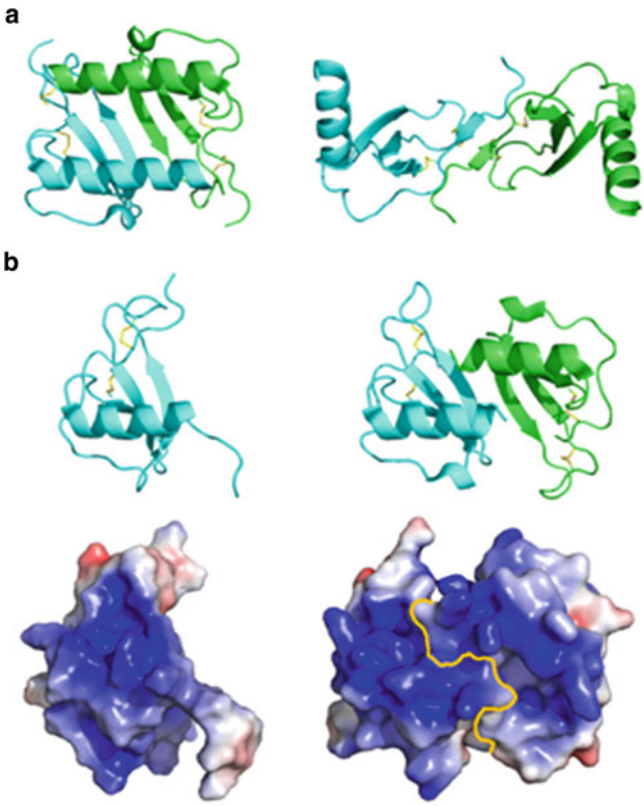

Fig. 4 (**a**) Dimerization schemes for chemokines. CXCL8 chemokines dimer structure (PDB code IL8) (*left*). CCL3 chemokine dimer structure (PDB code 2X69) (*right*). Each monomer is depicted in a different color and the disulfide bonds are represented in *yellow*. (**b**) Chemokine CCL20 (PDB code 2JYO) and dimer (PDB code 1M8A) structure (*upper*) with the electrostatic potential surface distribution (*lower*). The disulfide bonds are represented in *yellow* (*upper*) and the monomer interface is depicted by a *yellow line* (*lower, right*). Electrostatic potential surfaces were calculated by Adaptative Poisson–Boltzmann Solver (APBS) using PDB2PQR software and depicted by PyMOL software

dimer structure characteristics may explain the high antimicrobial activity of this particular chemokine. In a monomeric form, CCL20 already exhibits a large positively charged surface. This is even increased considerably when the dimer is formed by the generation of the six-stranded β-sheet with both C-terminal α-helices located on top, establishing a large contiguous positively charged surface (Fig. 4b). The dimerization of CCL20 is dependent on the protonation state of the His-40 sidechain. At neutral pH, His-40 is unprotonated, allowing dimer formation of CCL20; at lower pH, the two His-40 residues become positively charged, and charge repulsion between these residues prevents the dimer formation (Chan et al. 2008). The formation of an extended positively charged surface under physiological conditions may explain the strong antimicrobial activity of CCL20,

and the same could apply to other chemokines. In a recent study, it was also suggested that the differences in the dimerization behavior of CXCL7 and its truncated TC-1 derivative contributed to the drastically increased antimicrobial activity of the latter (Nguyen et al. 2011). Clearly detailed studies addressing the influence of dimerization on the antimicrobial activity of chemokines are warranted in order to definitively establish its possible role in the activity of these proteins.

In addition to the formation of homodimers, several chemokines are also able to form heterodimers. This has been shown to modulate their biological activities (Allen et al. 2007). For example, the chemotaxis of specific cells has been significantly affected by the formation of heterodimers as observed for the CXCL8-CXCL4 and CXCL4-CCL5 dimers (Nesmelova et al. 2005; von Hundelshausen et al. 2005). Heterodimers are also observed for other members of the innate immune system, such as AMPs. Some AMPs can undergo heterodimerization thereby modifying their antimicrobial activity, membrane permeabilization activity, and/or their resistance to protease digestion. Distinctin is an example of a covalently bound heterodimer antimicrobial peptide isolated from the tree frog *Phyllomedusa distincta* (Batista et al. 2001; Dalla Serra et al. 2008; Raimondo et al. 2005; Resende et al. 2009). Not only covalently bound dimers are observed among AMPs, but it is believed that the peptides magainin-2 and peptidyl-glycylleucine-carboxyamide (PGLa) form a non-covalent dimer upon interaction with membranes increasing the antimicrobial activity and membrane permeabilization activity but also increasing their cytotoxicity (Matsuzaki 1998; Hara et al. 2001). Similar to AMPs, the ability of chemokines to form heterodimers could be related not only with the modulation of their chemotactic function, but it could also regulate their microbicidal activity. Further studies are required in order to establish the effects of chemokine heterodimerization on their direct antimicrobial activity. Finally, it is known that several chemokines can form larger oligomeric structures (Salanga and Handel 2011). Such aggregated structures could markedly influence the antimicrobial activity. The formation of the oligomers is usually promoted by binding of glycosaminoglycans. In a recent study the structure of an oligomeric form of CCL5/RANTES was reported (Wang et al. 2011). This work sets the stage for future studies with other chemokines. It will be interesting to see if the binding of negatively charged glycosaminoglycans promotes or competes with the antimicrobial activities of the chemokines.

7 Activity of Unfolded Chemokines and of Derived Peptides

The antimicrobial chemokines share a large conformational, positively charged surface patch (Yang et al. 2003). In thrombocidin-1 (TC-1), disruption of this positive patch by substitution of the central cationic Lys-17 with an alanine residue substantially reduced the antimicrobial potency (Kwakman et al. 2011), providing direct evidence for its importance. Indeed, lysine substitution of negative or neutral residues bordering the positive patch enhanced the activity. Interestingly, reduced

TC-1 had equal antimicrobial activity as the folded protein, even though the positive patch was disrupted (Kwakman et al. 2011). The structural elements of unfolded TC-1 were likely localized in the N-terminal region of the protein, since linear 15-mer synthetic peptides corresponding to the N-terminal part of TC-1 had very potent antimicrobial activity. This implies that the structural elements involved in antimicrobial activity of native folded TC-1 strongly differ from those of the reduced, unfolded protein.

Similar to TC-1, several cationic antimicrobial peptides with positive patches retain their antimicrobial activity after linearization (Klüver et al. 2006; Liu and Wilson 2010; Mandal et al. 2002; Wu et al. 2003). For instance, variants of hBD-3 lacking disulfides are still antimicrobial (Hoover et al. 2003; Wu et al. 2003) despite the loss of most structural elements of the native protein. A variant of the antimicrobial chemokine CCL28, lacking both cysteines of the CC sequence, has similar antimicrobial activity as the native, folded protein (Liu and Wilson 2010). As CCL28-derived peptides have antimicrobial activity equivalent to the intact, folded protein (Liu and Wilson 2010), the activity of linear CCL28 may well be due to peptide regions in the unfolded protein, similar to the case of linearized TC-1.

The strongest example of the influence of unfolding on antimicrobial activity probably is the case of human β-defensin 1 (hDB-1). This protein is abundantly expressed by all human epithelia but was considered a low-activity AMP. However, recent research showed that reduction and unfolding of hBD-1 turns this protein into a potent antimicrobial (Schroeder et al. 2011). Reduced hBD-1 colocalizes with thioredoxin in several human tissues, suggesting that this enzyme system may be involved in hBD-1 reduction in vivo (Schroeder et al. 2011).

Assuming that linearization of chemokines and antimicrobial proteins does indeed occur in vivo, one may speculate that in addition to the known truncation peptides from folded proteins, additional linear peptides may be generated by proteolytic degradation from the now linearized parts, potentially further contributing to the already impressive role of this class of molecules to host defense.

8 Concluding Remarks

The study of the antimicrobial activity of chemokines is still a relatively unexplored field, which is not surprising as this “moonlighting” activity of chemokines was only first reported a little more than 10 years ago. To date, the activity of a number of CC and CXC chemokines has been dissected in detail, as described in this contribution. While in quite a few cases the characteristic C-terminal α-helix of the chemokines plays a role in the antimicrobial activity, this is certainly not universal, as for selected chemokines the N-terminal region of the proteins seems to be more important. In some instances extensions beyond the usual ~70 residues of the typical chemokine structure play a role in the activity, as in the case of the histatin-like extension of CCL28 and for the 30-residue C-terminal extension of CXCL9. The latter activities seem to be grafted onto the normal chemokine structure. A positively charged patch is essential for the activity of a number of

chemokines in their folded form. In addition, we have drawn attention to the potential effects of proteolysis and protein dimerization, which can further modulate the activity. A new topic is the structure-activity relationship of reduced, unfolded chemokines, with the reduction-activated hBD-1 as a recent revelation. Obviously much work remains to be done, and clearly more surprises will be found in this intriguing family of antimicrobial host defense proteins.

Acknowledgments We are grateful to Dr. Leo Nguyen and Dr. David Chan for providing the PDB files for full-length NAP-2 and TC-1 and to Dr. P.H.S. Kwakman and Dr. J. Krijgsveld for sharing unpublished results on thrombocidin-1.

This work has been funded by an operating grant from the "Novel Alternatives to Antibiotics" program of the Canadian Institutes of Health Research to Dr. Hans J. Vogel. Mauricio Arias and Dr. Hans J. Vogel are the holders of a Studentship and a Scientist award, respectively, of the Alberta Heritage Foundation for Medical Research.

References

Allen SJ, Crown SE, Handel TM (2007) Chemokine: receptor structure, interactions, and antagonism. Annu Rev Immunol 25(1):787–820. doi:10.1146/annurev.immunol.24.021605.090529

Amsterdam D (1996) Susceptibility testing of antimicrobial in liquid media. Antibiotics in laboratory medicine, 4th edn. Williams and Wilkins, Baltimore, MA, pp 61–143

Baggiolini M, Walz A, Kunkel SL (1989) Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. J Clin Invest 84(4):1045–1049

Baldwin ET, Weber IT, St Charles R, Xuan JC, Appella E, Yamada M, Matsushima K, Edwards BF, Clore GM, Gronenborn AM (1991) Crystal structure of interleukin 8: symbiosis of NMR and crystallography. Proc Natl Acad Sci USA 88(2):502–506

Barinka C, Prahl A, Lubkowski J (2008) Structure of human monocyte chemoattractant protein 4 (MCP-4/CCL13). Acta Crystallogr D Biol Crystallogr 64(3):273–278. doi:10.1107/S0907444907066164

Batista CVF, Scaloni A, Rigden DJ, Silva LR, Rodrigues RA, Dukor R, Sebben A, Talamo F, Bloch C (2001) A novel heterodimeric antimicrobial peptide from the tree-frog Phyllomedusa distincta. FEBS Lett 494(1–2):85–89

Becker S, Quay J, Koren HS, Haskill JS (1994) Constitutive and stimulated MCP-1, GRO alpha, beta, and gamma expression in human airway epithelium and bronchoalveolar macrophages. Am J Physiol Lung Cell Mol Physiol 266(3):L278–L286

Bizzarri C, Beccari AR, Bertini R, Cavicchia MR, Giorgini S, Allegretti M (2006) ELR+ CXC chemokines and their receptors (CXC chemokine receptor 1 and CXC chemokine receptor 2) as new therapeutic targets. Pharmacol Ther 112(1):139–149

Bjorstad A, Fu H, Karlsson A, Dahlgren C, Bylund J (2005) Interleukin-8-derived peptide has antibacterial activity. Antimicrob Agents Chemother 49(9):3889–3895. doi:10.1128/aac.49.9.3889-3895.2005

Bolintineanu D, Hazrati E, Davis HT, Lehrer RI, Kaznessis YN (2010) Antimicrobial mechanism of pore-forming protegrin peptides: 100 pores to kill *E. coli*. Peptides 31(1):1–8

Bonecchi R, Bianchi G, Bordignon PP, D'Ambrosio D, Lang R, Borsatti A, Sozzani S, Allavena P, Gray PA, Mantovani A, Sinigaglia F (1998) Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. J Exp Med 187(1):129–134. doi:10.1084/jem.187.1.129

Booth V, Clark-Lewis I, Sykes BD (2004) NMR structure of CXCR3 binding chemokine CXCL11 (ITAC). Protein Sci 13(8):2022–2028

Booth V, Keizer DW, Kamphuis MB, Clark-Lewis I, Sykes BD (2002) The CXCR3 binding chemokine IP-10/CXCL10: structure and receptor interactions. Biochemistry 41(33):10418–10425

Bourbigot S, Fardy L, Waring AJ, Yeaman MR, Booth V (2009) Structure of chemokine-derived antimicrobial peptide interleukin-8a and interaction with detergent micelles and oriented lipid bilayers. Biochemistry 48(44):10509–10521

Brandt E, Petersen F, Flad HD (1993) A novel molecular variant of the neutrophil-activating peptide NAP-2 with enhanced biological activity is truncated at the C-terminus: identification by antibodies with defined epitope specificity. Mol Immunol 30(11):979–991

Brandt E, Petersen F, Ludwig A, Ehlert JE, Bock L, Flad HD (2000) The beta-thromboglobulins and platelet factor 4: blood platelet-derived CXC chemokines with divergent roles in early neutrophil regulation. J Leukoc Biol 67(4):471–478

Brandt E, Van Damme J, Flad H-D (1991) Neutrophils can generate their activator neutrophil-activating peptide 2 by proteolytic cleavage of platelet-derived connective tissue-activating peptide III. Cytokine 3(4):311–321

Car BD, Baggiolini M, Walz A (1991) Formation of neutrophil-activating peptide 2 from platelet-derived connective-tissue-activating peptide III by different tissue proteinases. Biochem J 275(3):581–584

Clark-Lewis I, Dewald B, Geiser T, Moser B, Baggiolini M (1993) Platelet factor 4 binds to interleukin 8 receptors and activates neutrophils when its N terminus is modified with Glu-Leu-Arg. Proc Natl Acad Sci USA 90(8):3574–3577

Clark-Lewis I, Kim KS, Rajarathnam K, Gong JH, Dewald B, Moser B, Baggiolini M, Sykes BD (1995) Structure-activity relationships of chemokines. J Leukoc Biol 57(5):703–711

Clark-Lewis I, Schumacher C, Baggiolini M, Moser B (1991) Structure-activity relationships of interleukin-8 determined using chemically synthesized analogs. Critical role of NH2-terminal residues and evidence for uncoupling of neutrophil chemotaxis, exocytosis, and receptor binding activities. J Biol Chem 266(34):23128–23134

Clore GM, Appella E, Yamada M, Matsushima K, Gronenborn AM (1989) Determination of the secondary structure of interleukin-8 by nuclear magnetic resonance spectroscopy. J Biol Chem 264(32):18907–18911

Clore GM, Appella E, Yamada M, Matsushima K, Gronenborn AM (1990) Three-dimensional structure of interleukin 8 in solution. Biochemistry 29(7):1689–1696

Cohen AB, Stevens MD, Miller EJ, Atkinson MA, Mullenbach G (1992) Generation of the neutrophil-activating peptide-2 by cathepsin G and cathepsin G-treated human platelets. Am J Physiol Lung Cell Mol Physiol 263(2):L249–L256

Cole AM, Ganz T, Liese AM, Burdick MD, Liu L, Strieter RM (2001) Cutting edge: IFN-inducible ELR- CXC chemokines display defensin-like antimicrobial activity. J Immunol 167(2):623–627

Cole KE, Strick CA, Paradis TJ, Ogborne KT, Loetscher M, Gladue RP, Lin W, Boyd JG, Moser B, Wood DE, Sahagan BG, Neote K (1998) Interferon-inducible T cell alpha chemoattractant (I-TAC): a novel non-ELR CXC chemokine with potent activity on activated T cells through selective high affinity binding to CXCR3. J Exp Med 187(12):2009–2021. doi:10.1084/jem.187.12.2009

Collin M, Linge HM, Bjartell A, Giwercman A, Malm J, Egesten A (2008) Constitutive expression of the antibacterial CXC chemokine GCP-2/CXCL6 by epithelial cells of the male reproductive tract. J Reprod Immunol 79(1):37–43

Crawford MA, Zhu Y, Green CS, Burdick MD, Sanz P, Alem F, O'Brien AD, Mehrad B, Strieter RM, Hughes MA (2009) Antimicrobial effects of interferon-inducible CXC chemokines against Bacillus anthracis spores and bacilli. Infect Immun 77(4):1664–1678. doi:10.1128/iai.01208-08

Crown SE, Yu Y, Sweeney MD, Leary JA, Handel TM (2006) Heterodimerization of CCR2 chemokines and regulation by glycosaminoglycan binding. J Biol Chem 281(35):25438–25446. doi:10.1074/jbc.M601518200

Chan DI, Hunter HN, Tack BF, Vogel HJ (2008) Human macrophage inflammatory protein 3a: protein and peptide nuclear magnetic resonance solution structures, dimerization, dynamics, and anti-infective properties. Antimicrob Agents Chemother 52(3):883–894. doi:10.1128/aac.00805-07

Dalla Serra M, Cirioni O, Vitale RM, Renzone G, Coraiola M, Giacometti A, Potrich C, Baroni E, Guella G, Sanseverino M, De Luca S, Scalise G, Amodeo P, Scaloni A (2008) Structural features of distinctin affecting peptide biological and biochemical properties. Biochemisty 47(30):7888–7899

Dankert J, Krijgsveld J, van der Werff J, Joldersma W, Zaat Sebastian AJ (2001) Platelet microbicidal activity is an important defense factor against Viridans streptococcal endocarditis. J Infect Dis 184(5):597–605. doi:10.1086/322802

Darveau RP, Blake J, Seachord CL, Cosand WL, Cunningham MD, Cassiano-Clough L, Maloney G (1992) Peptides related to the carboxyl terminus of human platelet factor IV with antibacterial activity. J Clin Invest 90(2):447–455

Dempsey CE, Ueno S, Avison MB (2002) Enhanced membrane permeabilization and antibacterial activity of a disulfide-dimerized magainin analogue. Biochemistry 42(2):402–409

Deuel TF, Keim PS, Farmer M, Heinrikson RL (1977) Amino acid sequence of human platelet factor 4. Proc Natl Acad Sci USA 74(6):2256–2258

Edwards RJ, Taylor GW, Ferguson M, Murray S, Rendell N, Wrigley A, Bai Z, Boyle J, Finney Simon J, Jones A, Russell Hugh H, Turner C, Cohen J, Faulkner L, Sriskandan S (2005) Specific C-terminal cleavage and inactivation of interleukin-8 by invasive disease isolates of Streptococcus pyogenes. J Infect Dis 192(5):783–790. doi:10.1086/432485

Egesten A, Eliasson M, Johansson Helena M, Olin Anders I, Morgelin M, Mueller A, Pease James E, Frick I-M, Bjorck L (2007) The CXC chemokine MIG/CXCL9 is important in innate immunity against Streptococcus pyogenes. J Infect Dis 195(5):684–693. doi:10.1086/510857

Egesten A, Olin AI, Linge HM, Yadav M, Morgelin M, Karlsson A, Collin M (2009) SpeB of Streptococcus pyogenes differentially modulates antibacterial and receptor activating properties of human chemokines. PLoS One 4(3):e4769

Ehlert JE, Gerdes J, Flad H-D, Brandt E (1998) Novel C-terminally truncated isoforms of the CXC chemokine b-thromboglobulin and their impact on neutrophil functions. J Immunol 161(9):4975–4982

Ehlert JE, Petersen F, Kubbutat MHG, Gerdes J, Flad H-D, Brandt E (1995) Limited and defined truncation at the C terminus enhances receptor binding and degranulation activity of the neutrophil-activating peptide 2 (NAP-2). J Biol Chem 270(11):6338–6344. doi:10.1074/jbc.270.11.6338

Eliasson M, Egesten A (2008) Antibacterial chemokines - actors in both innate and adaptive immunity. In: Egesten A, Schmidt A, Herwald H (eds) Trends in innate immunity, vol 15. Karger Publisher, Basel, pp 101–117. doi:10.1159/000136317

Epand RM, Vogel HJ (1999) Diversity of antimicrobial peptides and their mechanisms of action. Biochim Biophys Acta 1462(1–2):11–28

Farber JM (1993) HuMig: a new human member of the chemokine family of cytokines. Biochem Biophys Res Commun 192(1):223–230

Farber JM (1997) Mig and IP-10: CXC chemokines that target lymphocytes. J Leukoc Biol 61(3):246–257

Fillmore RA, Nelson SE, Lausch RN, Oakes JE (2003) Differential regulation of ENA-78 and GCP-2 gene expression in human corneal keratocytes and epithelial cells. Invest Ophthalmol Vis Sci 44(8):3432–3437. doi:10.1167/iovs.03-0095

Flad H-D, Brandt E (2010) Platelet-derived chemokines: pathophysiology and therapeutic aspects. Cell Mol Life Sci 67(14):2363–2386

Frick I-M, Akesson P, Rasmussen M, Schmidtchen A, Bjorck L (2003) SIC, a secreted protein of Streptococcus pyogenes that inactivates antibacterial peptides. J Biol Chem 278(19):16561–16566. doi:10.1074/jbc.M301995200

Froyen G, Proost P, Ronsse I, Mitera T, Haelens A, Wuyts A, Opdenakker G, van Damme J, Billiau A (1997) Cloning, bacterial expression and biological characterization of recombinant human granulocyte chemotactic protein-2 and differential expression of granulocyte chemotactic protein-2 and epithelial cell-derived neutrophil activating peptide-78 mRNAs. Eur J Biochem 243(3):762–769

Fujiie S, Hieshima K, Izawa D, Nakayama T, Fujisawa R, Ohyanagi H, Yoshie O (2001) Proinflammatory cytokines induce liver and activation-regulated chemokine/macrophage inflammatory protein-3a/CCL20 in mucosal epithelial cells through NF-kB. Int Immunol 13(10):1255–1263. doi:10.1093/intimm/13.10.1255

Garcia-Zepeda EA, Combadiere C, Rothenberg ME, Sarafi MN, Lavigne F, Hamid Q, Murphy PM, Luster AD (1996) Human monocyte chemoattractant protein (MCP)-4 is a novel CC chemokine with activities on monocytes, eosinophils, and basophils induced in allergic and nonallergic inflammation that signals through the CC chemokine receptors (CCR)-2 and −3. J Immunol 157(12):5613–5626

Ghosh M, Shen Z, Schaefer TM, Fahey JV, Gupta P, Wira CR (2009) CCL20/MIP3α is a novel anti-HIV-1 molecule of the human female reproductive tract. Am J Reprod Immunol 62 (1):60–71

Gijsbers K, Van Assche G, Joossens S, Struyf S, Proost P, Rutgeerts P, Geboes K, Van Damme J (2004) CXCR1-binding chemokines in inflammatory bowel diseases: down-regulated IL-8/CXCL8 production by leukocytes in Crohn's disease and selective GCP-2/CXCL6 expression in inflamed intestinal tissue. Eur J Immunol 34(7):1992–2000

Haney EF, Hunter HN, Matsuzaki K, Vogel HJ (2009a) Solution NMR studies of amphibian antimicrobial peptides: linking structure to function? Biochim Biophys Acta 1788(8):1639–1655

Haney EF, Lau F, Vogel HJ (2007) Solution structures and model membrane interactions of lactoferrampin, an antimicrobial peptide derived from bovine lactoferrin. Biochim Biophys Acta 1768(10):2355–2364

Haney EF, Nazmi K, Lau F, Bolscher JGM, Vogel HJ (2009b) Novel lactoferrampin antimicrobial peptides derived from human lactoferrin. Biochimie 91(1):141–154

Hara T, Mitani Y, Tanaka K, Uematsu N, Takakura A, Tachi T, Kodama H, Kondo M, Mori H, Otaka A, Nobutaka F, Matsuzaki K (2001) Heterodimer formation between the antimicrobial peptides magainin 2 and PGLa in lipid bilayers: A cross-linking study. Biochemistry 40(41):12395–12399

Harder J, Bartels J, Christophers E, Schroder J-M (2001) Isolation and characterization of human defensin-3, a novel human inducible peptide antibiotic. J Biol Chem 276(8):5707–5713. doi:10.1074/jbc.M008557200

Harter L, Petersen F, Flad HD, Brandt E (1994) Connective tissue-activating peptide III desensitizes chemokine receptors on neutrophils. Requirement for proteolytic formation of the neutrophil-activating peptide 2. J Immunol 153(12):5698–5708

Hasan L, Mazzucchelli L, Liebi M, Lis M, Hunger RE, Tester A, Overall CM, Wolf M (2006) Function of liver activation-regulated chemokine/CC chemokine ligand 20 is differently affected by cathepsin B and cathepsin D processing. J Immunol 176(11):6512–6522

Hensbergen PJ, Verzijl D, Balog CIA, Dijkman R, van der Schors RC, van der Raaij-Helmer EMH, van der Plas MJA, Leurs R, Deelder AM, Smit MJ, Tensen CP (2004) Furin is a chemokine-modifying enzyme. J Biol Chem 279(14):13402–13411. doi:10.1074/jbc.M312814200

Hidalgo-Grass C, Mishalian I, Dan-Goor M, Belotserkovsky I, Eran Y, Nizet V, Peled A, Hanski E (2006) A streptococcal protease that degrades CXC chemokines and impairs bacterial clearance from infected tissues. EMBO J 25(19):4628–4637

Hieshima K, Ohtani H, Shibano M, Izawa D, Nakayama T, Kawasaki Y, Shiba F, Shiota M, Katou F, Saito T, Yoshie O (2003) CCL28 has dual roles in mucosal immunity as a chemokine with broad-spectrum antimicrobial activity. J Immunol 170(3):1452–1461

Holmes WE, Lee J, Kuang WJ, Rice GC, Wood WI (1991) Structure and functional expression of a human interleukin-8 receptor. Science 253(5025):1278–1280. doi:10.1126/science.1840701

Hoover DM, Boulegue C, Yang D, Oppenheim JJ, Tucker K, Lu W, Lubkowski J (2002) The structure of human macrophage inflammatory protein-3alpha/CCL20. J Biol Chem 277 (40):37647–37654. doi:10.1074/jbc.M203907200

Hoover DM, Rajashankar KR, Blumenthal R, Puri A, Oppenheim JJ, Chertov O, Lubkowski J (2000) The structure of human b-defensin-2 shows evidence of higher order oligomerization. J Biol Chem 275(42):32911–32918. doi:10.1074/jbc.M006098200

Hoover DM, Wu Z, Tucker K, Lu W, Lubkowski J (2003) Antimicrobial characterization of human b-defensin 3 derivatives. Antimicrob Agents Chemother 47(9):2804–2809. doi:10.1128/aac.47.9.2804-2809.2003

Iwamoto T, Okamoto H, Iikuni N, Takeuchi M, Toyama Y, Tomatsu T, Kamatani N, Momohara S (2006) Monocyte chemoattractant protein-4 (MCP-4)/CCL13 is highly expressed in cartilage from patients with rheumatoid arthritis. Rheumatology (Oxford) 45(4):421–424. doi:10.1093/rheumatology/kei209

Kalayci O, Sonna LA, Woodruff PG, Camargo CA, Luster AD, Lilly CM (2004) Monocyte chemotactic protein-4 (MCP-4; CCL-13): a biomarker of asthma. J Asthma 41(1):27–33. doi:10.1081/JAS-120024590

Katancik JA, Sharma A, de Nardin E (2000) Interleukin 8, neutrophil-activating peptide-2 and GRO-alpha bind to and elicit cell activation via specific and different amino acid residues of CXCR2. Cytokine 12(10):1480–1488

Kim BE, Leung DYM, Streib JE, Boguniewicz M, Hamid QA, Howell MD (2007) Macrophage inflammatory protein 3a deficiency in atopic dermatitis skin and role in innate immune response to vaccinia virus. J Allergy Clin Immunol 119(2):457–463

Kleeff J, Kusama T, Rossi DL, Ishiwata T, Maruyama H, Friess H, Büchler MW, Zlotnik A, Korc M (1999) Detection and localization of MIP-3α/LARC/exodus, a macrophage proinflammatory chemokine, and its CCR6 receptor in human pancreatic cancer. Int J Cancer 81(4):650–657

Klüver E, Adermann K, Schulz A (2006) Synthesis and structure–activity relationship of β-defensins, multi-functional peptides of the immune system. J Pept Sci 12(4):243–257. doi:10.1002/psc.749

Kotarsky K, Sitnik KM, Kotarsky H, Schmidtchen A, Koslowski M, Wehkamp J, Agace WW (2009) A novel role for constitutively expressed epithelial-derived chemokines as antibacterial peptides in the intestinal mucosa. Mucosal Immunol 3(1):40–48. doi:10.1038/mi.2009.115

Krijgsveld J, Zaat SAJ, Meeldijk J, van Veelen PA, Fang G, Poolman B, Brandt E, Ehlert JE, Kuijpers AJ, Engbers GHM, Feijen J, Dankert J (2000) Thrombocidins, microbicidal proteins from human blood platelets, are C-terminal deletion products of CXC chemokines. J Biol Chem 275(27):20374–20381. doi:10.1074/jbc.275.27.20374

Kwakman PHS, Krijgsveld J, De Boer L, Nguyen LT, Boszhard L, Vreede J, Dekker HL, Speijer D, Drijfhout JW, te Velde AA, Crielaard W, Vogel HJ, Vandenbroucke-Grauls CMJE, Zaat SAJ (2011) Native thrombocidin-1 and unfolded thrombocidin-1 exert antimicrobial activity via distinct structural elements. J Biol Chem 286(50):43506–43514

Langham AA, Ahmad AS, Kaznessis YN (2008) On the nature of antimicrobial activity: a model for protegrin-1 pores. J Am Chem Soc 130(13):4338–4346

Lasagni L, Francalanci M, Annunziato F, Lazzeri E, Giannini S, Cosmi L, Sagrinati C, Mazzinghi B, Orlando C, Maggi E, Marra F, Romagnani S, Serio M, Romagnani P (2003) An alternatively spliced variant of CXCR3 mediates the inhibition of endothelial cell growth induced by IP-10, Mig, and I-TAC, and acts as functional receptor for platelet factor 4. J Exp Med 197(11):1537–1549. doi:10.1084/jem.20021897

Lazzeri E, Romagnani P (2005) CXCR3-binding chemokines: novel multifunctional therapeutic targets. Curr Drug Targets 5(1):109–118

Leach K, Charlton SJ, Strange PG (2007) Analysis of second messenger pathways stimulated by different chemokines acting at the chemokine receptor CCR5. Biochem Pharmacol 74(6):881–890

Lehrer RI, Rosenman M, Harwig SSSL, Jackson R, Eisenhauer P (1991) Ultrasensitive assays for endogenous antimicrobial polypeptides. J Immunol Methods 137(2):167–173. doi:10.1016/0022-1759(91)90021-7

Li A, Varney ML, Valasek J, Godfrey M, Dave BJ, Singh RK (2005) Autocrine role of interleukin-8 in induction of endothelial cell proliferation, survival, migration and MMP-2 production and angiogenesis. Angiogenesis 8(1):63–71

Liao F, Rabin RL, Yannelli JR, Koniaris LG, Vanguri P, Farber JM (1995) Human Mig chemokine: biochemical and functional characterization. J Exp Med 182(5):1301–1314. doi:10.1084/jem.182.5.1301

Linge HM, Collin M, Giwercman A, Malm J, Bjartell A, Egesten A (2008a) The antibacterial chemokine MIG/CXCL9 is constitutively expressed in epithelial cells of the male urogenital tract and is present in seminal plasma. J Interferon Cytokine Res 28(3):191–196. doi:10.1089/jir.2007.0100

Linge HM, Collin M, Nordenfelt P, Morgelin M, Malmsten M, Egesten A (2008b) The human CXC chemokine granulocyte chemotactic protein 2 (GCP-2)/CXCL6 possesses membrane-disrupting properties and is antibacterial. Antimicrob Agents Chemother 52(7):2599–2607. doi:10.1128/aac.00028-08

Liu B, Wilson E (2010) The antimicrobial activity of CCL28 is dependent on C-terminal positively-charged amino acids. Eur J Immunol 40(1):186–196

Loetscher M, Gerber B, Loetscher P, Jones SA, Piali L, Clark-Lewis I, Baggiolini M, Moser B (1996) Chemokine receptor specific for IP10 and mig: structure, function, and expression in activated T-lymphocytes. J Exp Med 184(3):963–969. doi:10.1084/jem.184.3.963

Maerki C, Meuter S, Liebi M, Muhlemann K, Frederick MJ, Yawalkar N, Moser B, Wolf M (2009) Potent and broad-spectrum antimicrobial activity of CXCL14 suggests an immediate role in skin infections. J Immunol 182(1):507–514

Malik ZA, Tack BF (2006) Structure of human MIP-3a chemokine. Acta Crystallogr Sect F Struct Biol Cryst Commun 62(7):631–634. doi:10.1107/S1744309106006890

Mandal M, Jagannadham MV, Nagaraj R (2002) Antibacterial activities and conformations of bovine [beta]-defensin BNBD-12 and analogs: structural and disulfide bridge requirements for activity. Peptides 23(3):413–418. doi:10.1016/S0196-9781(01)00628-3

Mani R, Tang M, Wu X, Buffy JJ, Waring AJ, Sherman MA, Hong M (2006) Membrane-bound dimer structure of a b-hairpin antimicrobial peptide from rotational-echo double-resonance solid-state NMR. Biochemistry 45(27):8341–8349

Martínez-Becerra F, Silva D-A, Domínguez-Ramírez L, Mendoza-Hernández G, López-Vidal Y, Soldevila G, García-Zepeda EA (2007) Analysis of the antimicrobial activities of a chemokine-derived peptide (CDAP-4) on Pseudomonas aeruginosa. Biochem Biophys Res Commun 355(2):352–358

Matsui T, Akahoshi T, Namai R, Hashimoto A, Kurihara Y, Rana M, Nishimura A, Endo H, Kitasato H, Kawai S, Takagishi K, Kondo H (2001) Selective recruitment of CCR6-expressing cells by increased production of MIP-3α in rheumatoid arthritis. Clin Exp Immunol 125(1):155–161

Matsuo Y, Ochi N, Sawai H, Yasuda A, Takahashi H, Funahashi H, Takeyama H, Tong Z, Guha S (2009) CXCL8/IL-8 and CXCL12/SDF-1α co-operatively promote invasiveness and angiogenesis in pancreatic cancer. Int J Cancer 124(4):853–861

Matsuzaki K (1998) Magainins as paradigm for the mode of action of pore forming polypeptides. Biochim Biophys Acta 1376(3):391–400

Mayo KH, Yang Y, Daly TJ, Barry JK, La Rosa GJ (1994) Secondary structure of neutrophil-activating peptide-2 determined by 1 H-nuclear magnetic resonance spectroscopy. Biochem J 304(2):371–376

Mihajlovic M, Lazaridis T (2010) Antimicrobial peptides in toroidal and cylindrical pores. Biochim Biophys Acta 1798(8):1485–1493

Mine S, Nasu K, Fukuda J, Sun B, Miyakawa I (2003) Secretion of granulocyte chemotactic protein-2 by cultured human endometrial stromal cells. Fertil Steril 79(1):146–150

Moser B, Letts G, Neote K, Yoshie O (2006) Antimicrobial and related activities of chemokines. In: Parnham MJ (ed) Chemokine biology—basic research and clinical application. Progress in Inflammation Research, Birkhäuser Basel, pp 151–164

Mueller A, Meiser A, McDonagh EM, Fox JM, Petit SJ, Xanthou G, Williams TJ, Pease JE (2008) CXCL4-induced migration of activated T lymphocytes is mediated by the chemokine receptor CXCR3. J Leukoc Biol 83(4):875–882. doi:10.1189/jlb.1006645

Murphy PM, Baggiolini M, Charo IF, Hebert CA, Horuk R, Matsushima K, Miller LH, Oppenheim JJ, Power CA (2000) International union of pharmacology. XXII. Nomenclature for chemokine receptors. Pharmacol Rev 52(1):145–176

Murphy PM, Tiffany HL (1991) Cloning of complementary DNA encoding a functional human interleukin-8 receptor. Science 253(5025):1280–1283. doi:10.1126/science.1891716

Nakayama T, Fujisawa R, Yamada H, Horikawa T, Kawasaki H, Hieshima K, Izawa D, Fujiie S, Tezuka T, Yoshie O (2001) Inducible expression of a CC chemokine liver- and activation-regulated chemokine (LARC)/macrophage inflammatory protein (MIP)-3a/CCL20 by epidermal keratinocytes and its role in atopic dermatitis. Int Immunol 13(1):95–103. doi:10.1093/intimm/13.1.95

Nesmelova IV, Sham Y, Dudek AZ, van Eijk LI, Wu G, Slungaard A, Mortari F, Griffioen AW, Mayo KH (2005) Platelet factor 4 and interleukin-8 CXC chemokine heterodimer formation modulates function at the quaternary structural level. J Biol Chem 280(6):4948–4958

Nguyen LT, Chan DI, Boszhard L, Zaat SAJ, Vogel HJ (2010) Structure-function studies of chemokine-derived carboxy-terminal antimicrobial peptides. Biochim Biophys Acta 1798 (6):1062–1072

Nguyen LT, Kwakman PHS, Chan DI, Liu Z, de Boer L, Zaat SAJ, Vogel HJ (2011) Exploring platelet chemokine antimicrobial activity: NMR backbone dynamics studies of NAP-2 and TC-1. Antimicrob Agents Chemother 55(5):2074–2083. doi:10.1128/AAC.01351-10

Pan J, Kunkel EJ, Gosslar U, Lazarus N, Langdon P, Broadwell K, Vierra MA, Genovese MC, Butcher EC, Soler D (2000) Cutting edge: a novel chemokine ligand for CCR10 and CCR3 expressed by epithelial cells in mucosal tissues. J Immunol 165(6):2943–2949

Pazgier M, Hoover D, Yang D, Lu W, Lubkowski J (2006) Human β-defensins. Cell Mol Life Sci 63(11):1294–1313

Prause O, Laan M, Lötvall J, Lindén A (2003) Pharmacological modulation of interleukin-17-induced GCP-2-, GRO-a- and interleukin-8 release in human bronchial epithelial cells. Eur J Pharmacol 462(1–3):193–198

Proost P, De Wolf-Peeters C, Conings R, Opdenakker G, Billiau A, Van Damme J (1993a) Identification of a novel granulocyte chemotactic protein (GCP-2) from human tumor cells. In vitro and in vivo comparison with natural forms of GRO, IP-10, and IL-8. J Immunol 150(3):1000–1010

Proost P, Wuyts A, Conings R, Lenaerts JP, Billiau A, Opdenakker G, Van Damme J (1993b) Human and bovine granulocyte chemotactic protein-2: complete amino acid sequence and functional characterization as chemokines. Biochemistry 32(38):10170–10177

Proudfoot AEI (2002) Chemokine receptors: multifaceted therapeutic targets. Nat Rev Immunol 2(2):106–115

Qian YQ, Johanson KO, McDevitt P (1999) Nuclear magnetic resonance solution structure of truncated human GROb [5–73] and its structural comparison with CXC chemokine family members GROa and IL-8. J Mol Biol 294(5):1065–1072

Raimondo D, Andreotti G, Saint N, Amodeo P, Renzone G, Sanseverino M, Socchi I, Molle G, Motta A, Scaloni A (2005) A folding-dependent mechanism of antimicrobial peptide resistance to degradation unveiled by solution structure of distinctin. Proc Natl Acad Sci USA 102(18):6309–6314. doi:10.1073/pnas.0409004102

Ramamoorthy A, Thennarasu S, Tan A, Gottipati K, Sreekumar S, Heyl DL, An FYP, Shelburne CE (2006) Deletion of all cysteines in tachyplesin I abolishes hemolytic activity and retains antimicrobial activity and lipopolysaccharide selective binding. Biochemistry 45(20):6529–6540

Resende JM, Mendonca MC, Munhoz VHO, Aisenbrey C, Verly RM, Bertani P, Cesar A, Pilo-Veloso D, Bechinger B (2009) Membrane structure and conformational changes of the antibiotic heterodimeric peptide distinctin by solid-state NMR spectroscopy. Proc Natl Acad Sci USA 106(39):16639–16644. doi:10.1073/pnas.0905069106

Rohrl J, Yang D, Oppenheim JJ, Hehlgans T (2010a) Human b-defensin 2 and 3 and their mouse orthologs induce chemotaxis through interaction with CCR2. J Immunol 184(12):6688–6694. doi:10.4049/jimmunol.0903984

Rohrl J, Yang D, Oppenheim JJ, Hehlgans T (2010b) Specific binding and chemotactic activity of mBD4 and its functional orthologue hBD2 to CCR6-expressing cells. J Biol Chem 285(10):7028–7034. doi:10.1074/jbc.M109.091090

Rojas-Ramos E, Avalos AF, Pérez-Fernandez L, Cuevas-Schacht F, Valencia-Maqueda E, Terán LM (2003) Role of the chemokines RANTES, monocyte chemotactic proteins-3 and −4, and eotaxins-1 and −2 in childhood asthma. Eur Respir J 22(2):310–316. doi:10.1183/09031936.03.00084802

Roumestand C, Louis V, Aumelas A, Grassy G, Calas B, Chavanieu A (1998) Oligomerization of protegrin-1 in the presence of DPC micelles. A proton high-resolution NMR study. FEBS Lett 421(3):263–267

Salanga CL, Handel TM (2011) Chemokine oligomerization and interactions with receptors and glycosaminoglycans: the role of structural dynamics in function. Exp Cell Res 317(5):590–601. doi:10.1016/j.yexcr.2011.01.004

Sallusto F, Lenig D, Mackay CR, Lanzavecchia A (1998) Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. J Exp Med 187(6):875–883. doi:10.1084/jem.187.6.875

Sauder DN, Mounessa NL, Katz SI, Dinarello CA, Gallin JI (1984) Chemotactic cytokines: the role of leukocytic pyrogen and epidermal cell thymocyte-activating factor in neutrophil chemotaxis. J Immunol 132(2):828–832

Sauty A, Dziejman M, Taha RA, Iarossi AS, Neote K, Garcia-Zepeda EA, Hamid Q, Luster AD (1999) The T cell-specific CXC chemokines IP-10, Mig, and I-TAC are expressed by activated human bronchial epithelial cells. J Immunol 162(6):3549–3558

Schaffner A, King CC, Schaer D, Guiney DG (2004) Induction and antimicrobial activity of platelet basic protein derivatives in human monocytes. J Leukoc Biol 76(5):1010–1018. doi:10.1189/jlb.0404261

Schibli DJ, Hunter HN, Aseyev V, Starner TD, Wiencek JM, McCray PB, Tack BF, Vogel HJ (2002) The solution structures of the human b-defensins lead to a better understanding of the potent bactericidal activity of HBD3 against Staphylococcus aureus. J Biol Chem 277(10):8279–8289. doi:10.1074/jbc.M108830200

Schmidtchen A, Frick IM, Andersson E, Tapper H, Björck L (2002) Proteinases of common pathogenic bacteria degrade and inactivate the antibacterial peptide LL-37. Mol Microbiol 46(1):157–168

Schroder JM, Mrowietz U, Morita E, Christophers E (1987) Purification and partial biochemical characterization of a human monocyte-derived, neutrophil-activating peptide that lacks interleukin 1 activity. J Immunol 139(10):3474–3483

Schroeder BO, Wu Z, Nuding S, Groscurth S, Marcinowski M, Beisner J, Buchner J, Schaller M, Stange EF, Wehkamp J (2011) Reduction of disulphide bonds unmasks potent antimicrobial activity of human [beta]-defensin 1. Nature 469(7330):419–423. doi:10.1038/nature09674

Schutyser E, Struyf S, Van Damme J (2003) The CC chemokine CCL20 and its receptor CCR6. Cytokine Growth Factor Rev 14(5):409–426

Shai Y (1999) Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by [alpha]-helical antimicrobial and cell non-selective membrane-lytic peptides. Biochim Biophys Acta 1462(1–2):55–70

Shirane J, Nakayama T, Nagakubo D, Izawa D, Hieshima K, Shimomura Y, Yoshie O (2004) Corneal epithelial cells and stromal keratocytes efficiently produce CC chemokine-ligand 20

(CCL20) and attract cells expressing its receptor CCR6 in mouse herpetic stromal keratitis. Curr Eye Res 28(5):297–306. doi:10.1076/ceyr.28.5.297.28682
Slungaard A (2005) Platelet factor 4: a chemokine enigma. Int J Biochem Cell Biol 37(6):1162–1167
Smith DF, Galkina E, Ley K, Huo Y (2005) GRO family chemokines are specialized for monocyte arrest from flow. Am J Physiol Heart Circ Physiol 289(5):H1976–H1984. doi:10.1152/ajpheart.00153.2005
Starner TD, Barker CK, Jia HP, Kang Y, McCray PB Jr (2003) CCL20 is an inducible product of human airway epithelia with innate immune properties. Am J Respir Cell Mol Biol 29(5):627–633. doi:10.1165/rcmb.2002-0272OC
Steinberg DA, Lehrer RI (1997) Designer assays for antimicrobial peptides: disputing the "one-size-fits-all". In: Shafer WM (ed) Antibacterial peptide protocols. Methods in molecular biology, vol 78. Humana Press, Totowa, NJ pp 169–186
Sumby P, Zhang S, Whitney AR, Falugi F, Grandi G, Graviss EA, DeLeo FR, Musser JM (2008) A chemokine-degrading extracellular protease made by group A Streptococcus alters pathogenesis by enhancing evasion of the innate immune response. Infect Immun 76(3):978–985. doi:10.1128/iai.01354-07
Suresh A, Verma C (2006) Modelling study of dimerization in mammalian defensins. BMC Bioinformatics 7(Suppl 5):S17
Swaminathan GJ, Holloway DE, Colvin RA, Campanella GK, Papageorgiou AC, Luster AD, Acharya KR (2003) Crystal structures of oligomeric forms of the IP-10/CXCL10 chemokine. Structure 11(5):521–532
Tang Y-Q, Yeaman MR, Selsted ME (2002) Antimicrobial peptides from human platelets. Infect Immun 70(12):6524–6533. doi:10.1128/iai.70.12.6524-6533.2002
Taylor K, Barran PE, Dorin JR (2008) Structure–activity relationships in β-defensin peptides. Pept Sci 90(1):1–7
Tencza SB, Creighton DJ, Yuan T, Vogel HJ, Montelaro RC, Mietzner TA (1999) Lentivirus-derived antimicrobial peptides: increased potency by sequence engineering and dimerization. J Antimicrob Chemother 44(1):33–41. doi:10.1093/jac/44.1.33
Tohyama M, Sayama K, Komatsuzawa H, Hanakawa Y, Shirakata Y, Dai X, Yang L, Tokumaru S, Nagai H, Hirakawa S, Sugai M, Hashimoto K (2007) CXCL16 is a novel mediator of the innate immunity of epidermal keratinocytes. Int Immunol 19(9):1095–1102. doi:10.1093/intimm/dxm083
Uguccioni M, Loetscher P, Forssmann U, Dewald B, Li H, Lima SH, Li Y, Kreider B, Garotta G, Thelen M, Baggiolini M (1996) Monocyte chemotactic protein 4 (MCP-4), a novel structural and functional analogue of MCP-3 and eotaxin. J Exp Med 183(5):2379–2384. doi:10.1084/jem.183.5.2379
Van Damme J, Wuyts A, Froyen G, Van Coillie E, Struyf S, Billiau A, Proost P, Wang JM, Opdenakker G (1997) Granulocyte chemotactic protein-2 and related CXC chemokines: from gene regulation to receptor usage. J Leukoc Biol 62(5):563–569
Viola A, Luster AD (2008) Chemokines and their receptors: drug targets in immunity and inflammation. Annu Rev Pharmacol Toxicol 48(1):171–197. doi:10.1146/annurev.pharmtox.48.121806.154841
Vivcharuk V, Kaznessis Y (2010a) Free energy profile of the interaction between a monomer or a dimer of protegrin-1 in a specific binding orientation and a model lipid bilayer. J Phys Chem B 114(8):2790–2797
Vivcharuk V, Kaznessis YN (2010b) Dimerization of protegrin-1 in different environments. Int J Mol Sci 11(9):3177–3194
von Hundelshausen P, Koenen RR, Sack M, Mause SF, Adriaens W, Proudfoot AEI, Hackeng TM, Weber C (2005) Heterophilic interactions of platelet factor 4 and RANTES promote monocyte arrest on endothelium. Blood 105(3):924–930. doi:10.1182/blood-2004-06-2475

Walz A, Baggiolini M (1990) Generation of the neutrophil-activating peptide NAP-2 from platelet basic protein or connective tissue-activating peptide III through monocyte proteases. J Exp Med 171(2):449–454. doi:10.1084/jem.171.2.449

Walz A, Dewald B, von Tscharner V, Baggiolini M (1989) Effects of the neutrophil-activating peptide NAP-2, platelet basic protein, connective tissue-activating peptide III and platelet factor 4 on human neutrophils. J Exp Med 170(5):1745–1750. doi:10.1084/jem.170.5.1745

Walz A, Peveri P, Aschauer H, Baggiolini M (1987) Purification and amino acid sequencing of NAF, a novel neutrophil-activating factor produced by monocytes. Biochem Biophys Res Commun 149(2):755–761

Wang W, Soto H, Oldham ER, Buchanan ME, Homey B, Catron D, Jenkins N, Copeland NG, Gilbert DJ, Nguyen N, Abrams J, Kershenovich D, Smith K, McClanahan T, Vicari AP, Zlotnik A (2000) Identification of a novel chemokine (CCL28), which binds CCR10 (GPR2). J Biol Chem 275(29):22313–22323. doi:10.1074/jbc.M001461200

Wang X, Watson C, Sharp JS, Handel TM, Prestegard JH (2011) Oligomeric structure of the chemokine CCL5/RANTES from NMR, MS and SAXS data. Structure 19(8):1138–1148. doi:10.1016/j.str.2011.06.001

Wiegand I, Hilpert K, Hancock REW (2008) Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc 3(2):163–175. doi:10.1038/nprot.2007.521

Wolf M, Albrecht S, Märki C (2008) Proteolytic processing of chemokines: implications in physiological and pathological conditions. Int J Biochem Cell Biol 40(6–7):1185–1198

Wolf M, Belen Delgado M, Jones SA, Dewald B, Clark-Lewis I, Baggiolini M (1998) Granulocyte chemotactic protein 2 acts via both IL- 8 receptors, CXCR1 and CXCR2. Eur J Immunol 28(1):164–170

Wu L, Ruffing N, Shi X, Newman W, Soler D, Mackay CR, Qin S (1996) Discrete steps in binding and signaling of interleukin-8 with its receptor. J Biol Chem 271(49):31202–31209. doi:10.1074/jbc.271.49.31202

Wu Z, Hoover DM, Yang D, Boulegue C, Santamaria F, Oppenheim JJ, Lubkowski J, Lu W (2003) Engineering disulfide bridges to dissect antimicrobial and chemotactic activities of human b-defensin 3. Proc Natl Acad Sci USA 100(15):8880–8885. doi:10.1073/pnas.1533186100

Wuyts A, Proost P, Lenaerts J-P, Ben-Baruch A, Van Damme J, Wang JM (1998) Differential usage of the CXC chemokine receptors 1 and 2 by interleukin-8, granulocyte chemotactic protein-2 and epithelial-cell-derived neutrophil attractant-78. Eur J Biochem 255(1):67–73

Wuyts A, Struyf S, Gijsbers K, Schutyser E, Put W, Conings R, Lenaerts JP, Geboes K, Opdenakker G, Menten P, Proost P, Van Damme J (2003) The CXC chemokine GCP-2/CXCL6 is predominantly induced in mesenchymal cells by interleukin-1beta and is down-regulated by interferon-gamma: comparison with interleukin-8/CXCL8. Lab Invest 83(1):23–34

Wuyts A, Van Osselaer N, Haelens A, Samson I, Herdewijn P, Ben-Baruch A, Oppenheim JJ, Proost P, Van Damme J (1997) Characterization of synthetic human granulocyte chemotactic protein 2: usage of chemokine receptors CXCR1 and CXCR2 and in vivo inflammatory properties. Biochemistry 36(9):2716–2723

Xiong Y-Q, Bayer Arnold S, Yeaman Michael R (2002) Inhibition of intracellular macromolecular synthesis in Staphylococcus aureus by thrombin-induced platelet microbicidal proteins. J Infect Dis 185(3):348–356. doi:10.1086/338514

Xiong YQ, Bayer AS, Elazegui L, Yeaman MR (2006) A synthetic congener modeled on a microbicidal domain of thrombin- induced platelet microbicidal protein 1 recapitulates staphylocidal mechanisms of the native molecule. Antimicrob Agents Chemother 50(11):3786–3792. doi:10.1128/aac.00038-06

Yang D, Biragyn A, Hoover DM, Lubkowski J, Oppenheim JJ (2004) Multiple roles of antimicrobial defensins, cathelicidins, and eosinophil-derived neurotoxin in host defense. Annu Rev Immunol 22(1):181–215

Yang D, Chen Q, Hoover DM, Staley P, Tucker KD, Lubkowski J, Oppenheim JJ (2003) Many chemokines including CCL20/MIP-3a display antimicrobial activity. J Leukoc Biol 74(3):448–455. doi:10.1189/jlb.0103024

Yang D, Chertov O, Bykovskaia SN, Chen Q, Buffo MJ, Shogan J, Anderson M, Schroder JM, Wang JM, Howard OMZ, Oppenheim JJ (1999) Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. Science 286(5439):525–528. doi:10.1126/science.286.5439.525

Yang Y, Mayo KH, Daly TJ, Barry JK, La Rosa GJ (1994) Subunit association and structural analysis of platelet basic protein and related proteins investigated by 1 H NMR spectroscopy and circular dichroism. J Biol Chem 269(31):20110–20118

Yeaman MR, Gank KD, Bayer AS, Brass EP (2002) Synthetic peptides that exert antimicrobial activities in whole blood and blood-derived matrices. Antimicrob Agents Chemother 46(12):3883–3891. doi:10.1128/aac.46.12.3883-3891.2002

Yeaman MR, Tang YQ, Shen AJ, Bayer AS, Selsted ME (1997) Purification and in vitro activities of rabbit platelet microbicidal proteins. Infect Immun 65(3):1023–1031

Yeaman MR, Yount NY (2007) Unifying themes in host defence effector polypeptides. Nat Rev Microbiol 5(9):727–740

Yeaman MR, Yount NY, Waring AJ, Gank KD, Kupferwasser D, Wiese R, Bayer AS, Welch WH (2007) Modular determinants of antimicrobial activity in platelet factor-4 family kinocidins. Biochim Biophys Acta 1768(3):609–619

Yoshimura T, Matsushima K, Oppenheim JJ, Leonard EJ (1987a) Neutrophil chemotactic factor produced by lipopolysaccharide (LPS)- stimulated human blood mononuclear leukocytes: partial characterization and separation from interleukin 1 (IL 1). J Immunol 139(3):788–793

Yoshimura T, Matsushima K, Tanaka S, Robinson EA, Appella E, Oppenheim JJ, Leonard EJ (1987b) Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines. Proc Natl Acad Sci USA 84(24):9233–9237

Young H, Roongta V, Daly TJ, Mayo KH (1999) NMR structure and dynamics of monomeric neutrophil-activating peptide 2. Biochem J 338(3):591–598

Yount NY, Gank KD, Xiong YQ, Bayer AS, Pender T, Welch WH, Yeaman MR (2004) Platelet microbicidal protein 1: structural themes of a multifunctional antimicrobial peptide. Antimicrob Agents Chemother 48(11):4395–4404. doi:10.1128/aac.48.11.4395-4404.2004

Yount NY, Waring AJ, Gank KD, Welch WH, Kupferwasser D, Yeaman MR (2007) Structural correlates of antimicrobial efficacy in IL-8 and related human kinocidins. Biochim Biophys Acta 1768(3):598–608

Yount NY, Yeaman MR (2004) Multidimensional signatures in antimicrobial peptides. Proc Natl Acad Sci USA 101(19):7363–7368. doi:10.1073/pnas.0401567101

Yung SC, Parenti D, Murphy PM (2011) Host chemokines bind to Staphylococcus aureus and stimulate protein A release. J Biol Chem 286(7):5069–5077. doi:10.1074/jbc.M110.195180

Zanetti M (2004) Cathelicidins, multifunctional peptides of the innate immunity. J Leukoc Biol 75(1):39–48. doi:10.1189/jlb.0403147

Zhang X, Chen L, Bancroft DP, Lai CK, Maione TE (1994) Crystal structure of recombinant human platelet factor 4. Biochemistry 33(27):8361–8366

Zingaretti C, Falugi F, Nardi-Dei V, Pietrocola G, Mariani M, Liberatori S, Gallotta M, Tontini M, Tani C, Speziale P, Grandi G, Margarit I (2010) Streptococcus pyogenes SpyCEP: a chemokine-inactivating protease with unique structural and biochemical features. FASEB J 24(8):2839–2848. doi:10.1096/fj.09-145631

Zinkernagel AS, Timmer AM, Pence MA, Locke JB, Buchanan JT, Turner CE, Mishalian I, Sriskandan S, Hanski E, Nizet V (2008) The IL-8 protease SpyCEP/ScpC of group A Streptococcus promotes resistance to neutrophil killing. Cell Host Microbe 4(2):170–178