Chemokines are best known for their classic leukocyte chemoattractant activity, which is critical for directing the immune response to sites of infection and injury. However, recent studies have suggested that at least some chemokines may also interfere with infectious agents directly. Antimicrobial chemokines tend to contain amphipathic alpha helical secondary structure, and broad-spectrum activity against both Gram-positive and Gram negative bacteria, as well as fungi. Conversely, several bacteria have been identified that possess mechanisms for specifically blocking the antimicrobial activities of chemokines. Although the precise mechanisms by which chemokines and microbes disarm one another in vitro remain unknown, there is now emerging evidence in vivo that such interactions may be biologically significant. More research will be needed to determine whether chemokines with direct antimicrobial activity may be translated into a novel class of antibiotics.

**Keywords:** chemoattractant, immunology, G protein-coupled receptor, mucosa, microbiome
As the Na$^+$olar alveolar lavage (Pacheco-Rodriguez et al., 2009). In general, lower concentrations of chemokine (Berkhout et al., 1997; Siveke 2001; Yang et al., 2003; Maerki et al., 2009; Yung et al., 2011). In general, and CCL2) and in saliva (CXCL8; Jones et al., 1995; Yang et al., 2003). The nutrient poor agar and broth, described above, all contained low ionic concentrations, with most assays having 10 mM of Na$^+$. As the Na$^+$ concentration is increased to 100 mM, the antimicrobial activity of chemokines diminishes or even disappears. Therefore knowing whether antimicrobial chemokines are secreted into these fluids is important. Indeed, several chemokines have been reported in sweat (CXCL8 and CCL2) and in saliva (CXCL8; Jones et al., 1995; Yang et al., 2005). Theoretically, chemokines may inhibit growth of organisms on the skin and mucosal surfaces, provided they are in high enough concentrations for antimicrobial activity.

SALT DEPENDENCE OF CHEMOKINE ANTIMICROBIAL ACTIVITIES

The nutrient poor agar and broth, described above, all contained low ionic concentrations, with most assays having 10 mM of Na$^+$. As the Na$^+$ concentration is increased to 100 mM, the antimicrobial activity of chemokines diminishes or even disappears. Therefore knowing whether antimicrobial chemokines are secreted into these fluids is important. Indeed, several chemokines have been reported in sweat (CXCL8 and CCL2) and in saliva (CXCL8; Jones et al., 1995; Yang et al., 2005). Theoretically, chemokines may inhibit growth of organisms on the skin and mucosal surfaces, provided they are in high enough concentrations for antimicrobial activity.

CONCENTRATION OF CHEMOKINES NEEDED FOR ANTIMICROBIAL ACTIVITY

For most antimicrobial chemokines, micromolar concentrations are needed for pathogen killing (Krijgsvejl et al., 2000; Cole et al., 2001; Yang et al., 2003; Maerki et al., 2009; Yung et al., 2011). In contrast, leukocyte chemotaxis in vitro typically requires 1000-fold lower concentrations of chemokine (Berkhout et al., 1997; Siveke and Hamann, 1998). A non-comprehensive list of chemokine concentrations found constitutively in different body fluids is listed in Table 2. The most extensive survey has been obtained by bronchial alveolar lavage (Pacheco-Rodriguez et al., 2009). In general, naturally occurring chemokine concentrations are in the picomolar to nanomolar range. However, two chemokines produced by platelets are found at micromolar concentrations in serum (Brandt et al., 2000). CXCL4 (also known as platelet factor 4) was the first chemokine ever to be characterized. It binds to the receptor CXCR3B, a splice variant affecting the N-terminus. The other is CXCL7 (also known as neutrophil-activating peptide 2). Both chemokines are released during platelet activation. From the table, it is apparent that many chemokines are made constitutively and secreted into serum/plasma, sweat, tears, saliva, and breast milk.

During infection and inflammation, chemokine concentrations in biological fluids may be greatly increased (Luster, 1998). For example, inflamed tonsils have nanomolar concentrations of CXCL9, CXCL10, and CXCL11 whereas in normal unflamed tonsils these chemokines are not detectable (Egesten et al., 2007). Nanomolar concentrations of CXCL9 are sufficient to inhibit Streptococcus pyogenes growth in vitro. However, in most infection models, the concentrations of chemokines detected in biological fluids are not sufficient for antimicrobial activities in vitro. Therefore, the combined effect of all antimicrobial chemokines present in the infection site may be needed to achieve antimicrobial effects. However, we are not aware of any data showing that antimicrobial chemokines have additive or synergistic effects. In our own unpublished studies, we had problems obtaining consistent inhibition data at low chemokine concentrations to perform combination chemokine experiments. Another possibility is that the concentration of chemokines on mucosal surfaces may be much higher than in secretions because many chemokines can bind glycosaminoglycans on cellular surfaces.

| Class | Names | N |
|-------|-------|---|
| CC    | C……C…………C……C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C…………C……}
### Table 1 | Electrolytes concentration and major components in different human secretions.

| Fluid          | Sodium (mmol/L) | Chloride (mmol/L) | Potassium (mmol/L) | Bicarbonate (mmol/L) | Phosphate (mmol/L) | Creatinine (mmol/L) | Others (mmol/L) |
|----------------|-----------------|-------------------|--------------------|----------------------|--------------------|---------------------|------------------|
| Serum¹         | 136–145         | 98–106            | 3.5–5.0            | 23–28                | 0.97–1.45          | 7–13                |
| Sweat²         | 15–90           | <40               | 0.002              | 2–40                 |                    |                     |
| Saliva³        | 2–50            | 5–40              | 10–36              | 25                   | 1.4–39             |
| Tears⁴         | 120–177         | 87–137            | 13–24              |                      |                    |                     |
| Nasal²         | 100–140         | 100–160           | 10–25              |                      |                    |                     |
| Urine⁵         | 10–200          | 15–200            | 10–0               | <0.1–low             | 133–221 mmol/Kg per 24 h | Taurocholate 100–260 |
| Bile⁷          | 200–260         | 10–60             | 5–8                | 10–60                |                    |                     |
| Gastric juice⁸ | 100             | 280               | 15                 |                      |                    |                     |
| Pancreatic juice⁸ | 150          | 40                | 5                  |                      |                    |                     |
| Intestinal fluid⁸ | 150          | 100               | 5                  |                      |                    |                     |
| Stool⁸         | 44–112          | 29–147            |                    |                      |                    |                     |
| Diarrh⁸        | 3–139           | 15–115            |                    |                      |                    |                     |
| Milk³          | 5–8             | 9–13              | 11–15              |                      |                    | Lactose 193–207    |
| PBS            | 137             | 139.7             | 2.7                | 10                   |                    |                     |

Listed are the major ions found in human secretions. Major compounds such as creatinine, taurocholate, and lactose in urine, bile, and milk are also indicated.

¹MAKSAP 14 Normal Laboratory Values. American College of Physicians, Copyright 2005. All rights reserved
²Bulmer and Forwell (1956), Kaiser et al. (1974).
³Chicharro et al. (1994).
⁴Lev et al. (2005).
⁵Cavaliere et al. (1988), Cavaliere et al. (1989).
⁶Moritz (2008).
⁷Wheeler et al. (1960).
⁸Shiau (1987).
⁹Koo and Gupta (1982), Wack et al. (1997).

### RANGE OF ORGANISMS AFFECTED BY ANTIMICROBIAL CHEMOKINES

Antimicrobial chemokines have been shown to inhibit a broad-spectrum of organisms: Gram-positive bacteria, Gram negative bacteria, and dimorphic fungi. Table 3 summarizes currently available information on this subject. In general, most antimicrobial chemokines have activity against multiple organisms. This suggests that the mechanism for antimicrobial activity is either through a conserved or non-specific pathway. All highly active antimicrobial chemokines have basic or cationic charge, but not all cationic chemokines have antimicrobial properties.

Chemokines have been surveyed most comprehensively for antimicrobial activity in the case of *Staphylococcus aureus* and *Escherichia coli*. Of 45 chemokines tested, 22 were found to have some anti-staphylococcal activity (Yung et al., 2011). For *E. coli*, 25 of 33 chemokines tested had some activity (Yang et al., 2003). A report on *S. pyogenes* showed that CXCL9, CXCL10, and CXCL11 can exert antimicrobial activities even at 150 mM sodium concentration (Egesten et al., 2007). Likewise, the antimicrobial effect of CXCL9, CXCL10, and CXCL11 on *Bacillus anthracis* may also be independent of ionic concentrations (Crawford et al., 2010). These chemokines were able to inhibit both spore germination and bacillus viability in culture media. In addition to bacteria, several chemokines also have antifungal properties against *Cryptococcus neoformans* and *Candida albicans*.

### COMPARISON WITH OTHER ANTIMICROBIAL PROTEINS

Antimicrobial chemokines represent a fraction of known antimicrobial proteins.

In general, antimicrobial proteins can be divided into three different classes. The largest class consists of β-stranded proteins with four to six conserved cysteines linked by disulfide bonds. Defensins and chemokines are members of this class. Whereas chemokines typically have four conserved cysteines that form two disulfide bonds, defensins have six conserved cysteines that form three disulfide bonds to stabilize β-strands. Both β-defensins and chemokines have overall cationic charge.

The antimicrobial effect of defensins occurs at micromolar concentration and in low sodium conditions just as antimicrobial chemokines. For example, micromolar concentrations of human beta defensin two are required for activity against...
Table 2 | Chemokine concentrations in different body fluids.

| Chemokine | Size MW (kD) | Serum or plasma nM | BAL nM | Sweat (nM) | Tears (nM) | Saliva (nM) | Parotid Secr (nM) | Breast milk (nM) |
|-----------|-------------|-------------------|--------|------------|-----------|------------|-----------------|-----------------|
| CXC ELR+  |             |                   |        |            |           |            |                 |                 |
| CCL1      | 78          | 0.01D             | 3.07   |            |           |            |                 |                 |
| CCL2      | 79          | 0.03X             |        |            |           |            |                 |                 |
| CCL3      | 79          | 0.09P             |        |            |           |            |                 |                 |
| CXC ELR-  |             |                   |        |            |           |            |                 |                 |
| CCL4      | 78          | 1100              |        |            |           |            |                 |                 |
| CXC ELR+  |             |                   |        |            |           |            |                 |                 |
| CCL5      | 8.0         | 0.12D             | 0.567  |            |           |            |                 |                 |
| CCL6      | 79          | 0.04R             |        |            |           |            |                 |                 |
| CCL7      | 76          | 3200              |        |            |           |            |                 |                 |
| CCL8      | 8.4         | 0.003L            | 0.227  | 0.02A      | 0.038V    | 0.03O      |                 |                 |
| CCL9      | 11.7        | 0.012M            | 0.327  |            |           |            |                 |                 |
| CCL10     | 8.5         | 0.009K            | 0.227  | 0.358V     | 0.008Y    | 0.08K      |                 |                 |
| CCL11     | 8.3         | 0.005S            | 0.087  |            |           |            |                 |                 |
| CCL12     | 8.0         | 0.3S              | 1.747  |            |           |            | 0.005Y          |                 |
| CCL13     | 10.3        | 0.003L            |        | 0.008Y     | 0.000Y    |            | 0.003F          |                 |
| CCL14     | 9.4         | 0.11V             |        |            |           |            |                 |                 |
| CCL16     | 10.1        | 0.26O             | 0.027  |            |           |            |                 |                 |
| CCL17     | 11.5        | <0.005x           |        |            |           |            |                 |                 |
| CX3C      |             |                   |        |            |           |            |                 |                 |
| CCL1      | 8.5         | 0.1P              | 0.005V |            |           |            |                 |                 |
| C CC      |             |                   |        |            |           |            |                 |                 |
| CCL1      | 8.5         | 0.003P            | 0.047  |            |           |            |                 |                 |
| CCL2      | 8.6         | 0.02E             | 0.557  | 0.01A      | 0.019V    | 0.044V     | 0.003F          |                 |
| CCL3      | 78          | 0.003E            | 0.197  |            | 0.004Y    |            |                 |                 |
| CCL4      | 76          | 0.001P            | 0.177  |            | 0.001V    |            |                 |                 |
| CCL5      | 78          | 1.79E             | 0.057  | 0.009V     | 0.000Y    |            | 0.006F          |                 |
| CCL6      | 9.0         | 0.003P            | 0.03T  |            |           |            |                 |                 |
| CCL7      | 8.9         | 0.002P            | 0.05T  |            |           |            |                 |                 |
| CCL8      | 8.3         | 0.01P             | 0.13T  |            | 0.007V    |            | 0.006F          |                 |
| CCL11     | 8.6         | 0.026P            | 0.03T  |            |           |            |                 |                 |
| CCL12     | 8.4         | 1.31C             |        |            |           |            |                 |                 |
| CCL13     | 10.1        | 0.1P              |        |            |           |            |                 |                 |
| CCL14     | 11.2        | 0.42P             |        |            |           |            |                 |                 |
| CCL15     | 8.0         | 0.02S             | 0.04T  |            |           |            |                 |                 |
| CCL18     | 78          | 4.23J             |        |            |           |            |                 |                 |
| CCL19     | 8.8         | 0.009P            | 0.02T  |            |           |            |                 |                 |
| CCL20     | 8.0         | 0.001S            | 0.11T  |            |           |            |                 |                 |
| CCL21     | 12.2        | 0.01S             | 0.12T  |            |           |            |                 |                 |
| CCL22     | 8.1         | 0.07S             | 0.07T  |            |           |            |                 |                 |
| CCL23     | 11.3        | 0.036P            |        |            |           |            |                 |                 |
| CCL24     | 8.8         | 0.025H            |        |            |           |            |                 |                 |
| CCL25     | 14.2        |                   |        |            |           |            |                 |                 |
| CCL26     | 8.4         | 0.004H            |        |            |           |            |                 |                 |
| CCL27     | 10.2        | 0.038S            |        |            |           |            |                 |                 |
| CCL28     | 12.3        | 0.004N            | 47G    | 149G       | 24G       |            |                 |                 |

Family and members of chemokines are listed on the left. The predicted molecular weights (MW) of mature chemokine forms are denoted in kilodaltons (kD). Reported serum or plasma concentrations of chemokines are grouped together in a single column. Italicized letter next to value designates reference source. BAL, broncholalveolar lavage. All concentrations are reported as nanomolar (nM). Blanks indicate not determined.

Jones et al. (1995), Brandt et al. (2000), Nomiyama et al. (2001), Holven et al. (2002), Parissis et al. (2002), Bottcher et al. (2003), Hieshima et al. (2003), Kagami et al. (2003), Maheshwari et al. (2003), Struyf et al. (2003), Takahata et al. (2003), Antonelli et al. (2005), Chuang et al. (2005), Kagami et al. (2005), Yang et al. (2005), Bauer et al. (2006), Sheikine et al. (2006), Alzoghaibi et al. (2008), Narbutt et al. (2009), Pacheco-Rodriguez et al. (2009), Schiffer et al. (2009), Carreno et al. (2010), Izuuki et al. (2010), Dong et al. (2011), Hernandez-Molina et al. (2011), Burkhardt et al. (2012).
### Table 3 | Antibacterial and antifungal properties of chemokines.

| Chemokine | MRSA | S. coag neg | S. pyogenes | E. faecium | B. subtilis | L. monocytogenes | E. coli | P. aeruginosa | C. neoformans | C. glabrata | C. albicans |
|-----------|------|-------------|-------------|------------|------------|-----------------|--------|---------------|--------------|-------------|-------------|
| MRSA      | 150 mM | 10 mM       | 10 mM       | 10 mM      | 100 mM     | 100 mM          | 10 mM  | 100 mM        | 100 mM       | 100 mM      | 100 mM      |
| S. coag neg | 10 mM | 10 mM       | 10 mM       | 10 mM      | 100 mM     | 100 mM          | 10 mM  | 100 mM        | 100 mM       | 100 mM      | 100 mM      |
| S. pyogenes | 10 mM | 10 mM       | 10 mM       | 10 mM      | 100 mM     | 100 mM          | 10 mM  | 100 mM        | 100 mM       | 100 mM      | 100 mM      |
| E. faecium | 10 mM | 10 mM       | 10 mM       | 10 mM      | 100 mM     | 100 mM          | 10 mM  | 100 mM        | 100 mM       | 100 mM      | 100 mM      |
| B. subtilis | 100 mM | 10 mM       | 10 mM       | 10 mM      | 100 mM     | 100 mM          | 10 mM  | 100 mM        | 100 mM       | 100 mM      | 100 mM      |
| L. monocytogenes | 100 mM | 10 mM       | 10 mM       | 10 mM      | 100 mM     | 100 mM          | 10 mM  | 100 mM        | 100 mM       | 100 mM      | 100 mM      |
| E. coli  | 100 mM | 10 mM       | 10 mM       | 10 mM      | 100 mM     | 100 mM          | 10 mM  | 100 mM        | 100 mM       | 100 mM      | 100 mM      |
| P. aeruginosa | 100 mM | 10 mM       | 10 mM       | 10 mM      | 100 mM     | 100 mM          | 10 mM  | 100 mM        | 100 mM       | 100 mM      | 100 mM      |
| C. neoformans | 100 mM | 10 mM       | 10 mM       | 10 mM      | 100 mM     | 100 mM          | 10 mM  | 100 mM        | 100 mM       | 100 mM      | 100 mM      |
| C. glabrata | 100 mM | 10 mM       | 10 mM       | 10 mM      | 100 mM     | 100 mM          | 10 mM  | 100 mM        | 100 mM       | 100 mM      | 100 mM      |
| C. albicans | 100 mM | 10 mM       | 10 mM       | 10 mM      | 100 mM     | 100 mM          | 10 mM  | 100 mM        | 100 mM       | 100 mM      | 100 mM      |

(Continued)
Yung and Murphy Antimicrobial chemokines

**Table 3 | Continued**

| Family | Member | pI 10 mM 137 mM 10 mM 10 mM 150 mM 10 mM 10 mM 10 mM 100 mM 10 mM 100 mM 10 mM 10 mM 10 mM 10 mM |
|--------|--------|----------------------------------|
|         | CCL27  | 9.07 meeting in 1 mM K+ C− D,H E,F I,J, A,D,E,F.I,J, empty boxes indicate these chemokines have not been tested. Most antimicrobial assays were performed at low salt concentration (10 mM). The CXCL7 cleaved peptide “+” and antimicrobial properties was purified from platelet granules and is a C-terminal processed form. The pI of each chemokine is also shown. |
|         | CCL28  | 10.88 meeting in 1 mM K+ C− D,H E,F I,J, A,D,E,F.I,J, empty boxes indicate these chemokines have not been tested. Most antimicrobial assays were performed at low salt concentration (10 mM). The CXCL7 cleaved peptide “+” and antimicrobial properties was purified from platelet granules and is a C-terminal processed form. The pI of each chemokine is also shown. |

Gram-positive organisms [methicillin-resistant S. aureus (MRSA), Staphylococcus coagulase negative species, S. pyogenes, E. faecium, B. subtilis, L. monocytogenes] are arranged on the left side, followed by Gram negative organisms (E. coli, P. aeruginosa) and fungi (C. neoformans, C. glabrata, C. albicans). Only for assays with MRSA and E. coli do the following symbols of inhibition apply: +++++, 80%; ++++, 40–59%; ++, 20–29%; +, <20%. For all other organisms +, ±, and − denote >50, 20–50, and <20% antimicrobial activity respectively. The “+”, “++”, and “++++” symbols represent discrepancies in antimicrobial properties from published literature. Italicized letter next to symbols denotes references. All MRSA data were taken from Yung et al. (2011). Empty boxes indicate these chemokines have not been tested. Most antimicrobial assays were performed at low salt concentration (10 mM). The CXCL7 cleaved peptide “+” and antimicrobial properties was purified from platelet granules and is a C-terminal processed form. The pI of each chemokine is also shown.

E. coli and P. aeruginosa (Schneider et al., 2005). Most other antimicrobial proteins also exert antimicrobial effects at micromolar concentrations. One exception is bactericidal/permeability increasing protein (BPI), which has activity against Gram negative bacteria at nanomolar concentrations (Wiesner and Vilcinskas, 2010).

The other classes of antimicrobial proteins consist of linear amphipathic helical proteins and peptides with high content of certain amino acids such as histidine, glycine, proline, or tryptophan. Since these groups have little similarity to chemokines, readers can refer to other recent reviews for details (Wiesner and Vilcinskas, 2010).

**DEFINING ANTIMICROBIAL AND CHEMOTACTIC DOMAINS**

There are no mutagenesis studies showing whether the antimicrobial and chemotactic regions of antimicrobial chemokines are linked. The best-studied antimicrobial chemokine is CXCL9. Several groups have identified CXCL9 antimicrobial activities against S. pyogenes, S. aureus, B. anthracis, P. aeruginosa, and E. coli. Initially discovered as the monokine induced by gamma interferon (MIG), CXCL9 is cleaved into several smaller forms endogenously (Liao et al., 1995). These smaller proteins have C-terminal truncations and are able to activate CXCR3, albeit with less potency and efficacy than full-length CXCL9. Mixtures of truncated CXCL9 proteins were separated into a high molecular weight and a low molecular weight group, presumably each group having several truncated forms. However, it is unknown whether these truncated CXCL9 forms have antimicrobial properties. A summary of functional analysis for CXCL9 is shown in Figure 3.

A 27 amino acid synthetic peptide consisting of the C-terminal region of CXCL9 has been reported to be sufficient for antimicrobial activity (red boxed). This peptide is predicted to have an amphipathic alpha helix structure. In contrast, a peptide spanning the N-terminal region of CXCL9 with beta sheet structure had no detectable antimicrobial activity (blue boxed). Both peptides have overall cationic charge. Another group tested the N-terminal (50 amino acids) and C-terminal (19 amino acids, corresponding to the alpha helix) domains of CXCL6 and found that the N-terminal fragment had higher antimicrobial properties than the C-terminal peptide (Linge et al., 2008). It may be that the whole protein is needed for full antimicrobial properties.

Streptococcal cysteine proteinase (SpeB) from S. pyogenes and subtilisin-like serine proteinase (SuA) from Finegoldia magna can degrade full-length CXCL9 to smaller proteins (Egesten et al., 2009; Karlsson et al., 2009). By mass spectrometry, these proteins were identified to have both N-terminal and C-terminal truncations (Figure 3). Interestingly, both proteins retained antimicrobial properties for S. pyogenes. However there is a discrepancy between the peptides in activating CXCR3. SpeB-degraded CXCL9 cannot activate CXCR3, whereas SuA-degraded peptides can. The authors did not individually purify and test each truncated form of CXCL9. Therefore, it is possible that the least processed form of CXCL9 by SuA is responsible for receptor activation. In addition, SuA can also degrade CXCL10 and CXCL11, causing both chemokines to lose both receptor activation and antimicrobial properties. More studies will be needed to precisely determine specific regions necessary and sufficient
for chemotactic and antimicrobial properties, and whether these regions overlap.

**MECHANISM OF ACTIVITY**

By electron microscopy criteria, antimicrobial chemokines appear to induce lysis of the bacterial membrane (Hieshima et al., 2003; Egesten et al., 2007; Linge et al., 2008). Since the outer membrane of bacteria are highly anionic, charge interaction is thought to mediate initial binding (Brogden, 2005). In eukaryotic cell membranes, negatively charged phospholipids are sequestered in the inner leaflet of the lipid bilayer, and the outer leaflet is composed mostly of zwitterionic and uncharged lipids. Therefore, the lipid content of the outer membranes of eukaryotic cells are devoid of electrostatic charge (Wiesner and Vilcinskas, 2010). In contrast, both leaflets of bacterial cell membranes are enriched with acidic phospholipids such as phosphatidylglycerol and cardiolipin, making these membranes negatively charged.

The exact mechanism for antimicrobial killing is not known nor is it known whether all chemokines kill microbial targets by a common mechanism. Defensins, which have been studied in greater detail, are absorbed onto the bacterial membrane by electrostatic attraction and subsequently aggregate there to cause local membrane thinning and ion channel formation (Ganz, 2003). Rupture of bacterial membranes has been demonstrated in *S. pyogenes* for CXCL9, in *E. coli* for CXCL6, and in *P. aeruginosa* for CCL28 (Hieshima et al., 2003; Egesten et al., 2007; Linge et al., 2008).

For the bacterium responsible for anthrax, *B. anthracis*, the membrane protein FtsX has been identified as a unique target for CXCL10 (Crawford et al., 2011). The authors used a transposon-based genetic screen to identify ftsX, and two other genes (*BAS0651* and the cell wall autolysin *lytE*) to be critical for resistance against CXCL10. *B. anthracis* genetically deficient in ftsX could not bind CXCL10 as assessed by transmission electron microscopy. FtsX is a cell division ABC transporter that is conserved among Gram-positive and Gram negative bacteria. Comparative protein analysis of FtsX with CXCR3, the receptor for CXCL10, showed a 27-residue region of FtsX that has 45% similarity with the N-terminal chemokine-binding domain of CXCR3. This is low, and how FtsX actually functions in antimicrobial chemokine resistance remains to be determined.

If charge–charge interaction were the sole means of chemokine-binding and antimicrobial action, chemokines would not be predicted to have antifungal properties since fungi have eukaryotic membranes. Histatin, a known antifungal peptide, inhibits *C. albicans* by binding to fungal mitochondria and inhibiting respiration by inducing reactive oxygen species (ROS) formation (Helmerhorst et al., 2001). The level of induced ROS correlated with fungicidal activity. However, it is not known whether antimicrobial chemokines act through similar pathways. The chemokine CCL28 has been shown to cause membrane disruption in *C. albicans* by scanning electron microscopy (Hieshima et al., 2003). How it binds to the fungal membrane and the domain responsible for antifungal activities has not been determined.

By whatever mechanisms chemokines kill pathogens, they do so rapidly. CXCL6 can disrupt liposome membranes within minutes, and pathogens treated with CCL28 show numerous surface blebs within 1 h (Hieshima et al., 2003; Linge et al., 2008). It is unclear whether gene activation or signal transduction needs to occur for antimicrobial chemokines to kill targets. Unlike traditional antibiotics, which work best on dividing organisms, antimicrobial proteins typically can exert effects on non-dividing organisms (Wiesner and Vilcinskas, 2010).

The salt dependence seen with antimicrobial chemokines is typical of most antimicrobial proteins. With the exception of human beta defensin 3, all human defensins exert antimicrobial properties at ~10 mM Na+. Killing of pathogens is thought to occur in vivo even in host environments with >10 mM Na+ because defensins...
are packed in high concentration within granules. Activation and degranulation of leukocytes by pathogens would produce very high local concentrations of defensins for optimal antimicrobial effects. In addition, two recent reports have shown that the antimicrobial activity of chemokines is not dependent on sodium concentration for certain bacteria. In particular, CXCL9, CXCL10, and CXCL11 are able to inhibit growth of *S. pyogenes* even in 150 mM sodium chloride solutions. The same chemokines could also inhibit *B. anthracis* germination and growth in culture media with serum.

**INHIBITION OF ANTIMICROBIAL CHEMOKINES BY BACTERIA**

In addition to degrading chemokines with SpeB, *S. pyogenes* produces additional factors that inhibit antimicrobial chemokines. Streptococcal interleukin-8 inactivating cell envelope protease (SpyCEP) cleaves CXCL1, 2, 6, and 8 (Zinkernagel et al., 2008), and Streptococcal inhibitor of complement (SIC) binds CXCL9 to inhibit its antimicrobial properties (Egesten et al., 2007). As mentioned previously, the bacterium *F. magna* expresses a subtilisin-like serine-proteinase (SufA) that partially degrades CXCL9, abolishing its antimicrobial activity (Karlsson et al., 2009). Therefore, several bacteria have developed means to degrade antimicrobial chemokines and evade host defense.

**IN VIVO EVIDENCE FOR ANTIMICROBIAL PROPERTIES OF CHEMOKINES**

Many inflammatory chemokines with antimicrobial properties are induced during inflammatory or infectious processes. It is unclear to what extent control of pathogens depends on direct antimicrobial activities of chemokines versus recruitment of leukocytes. One study demonstrated that neutralization of either CXCL9 or CXCL10 but not CXCR3 (the receptor for CXCL9 and CXCL10) increased susceptibility of C57BL/6 mice to *B. anthracis* (Crawford et al., 2011). Increased mortality was shown to arise from extrapulmonary dissemination of bacteria to kidneys, spleen, and liver from toxemia with lethal factor. However, the bacterial load at the site of inoculation, the lung, was similar between treated and untreated mice.

We are not aware of genetic mutations in chemokine genes leading to increased susceptibility to infectious diseases in humans. However, there is persuasive evidence that the chemokine system mediates control of pathogens in mammals, including humans. For example, a form of congenital neutropenia, WHIM syndrome, arises from gain-of-function mutations truncating the C-tail of CXCR4 (Hernandez et al., 2003). Patients with WHIM syndrome have recurrent bacterial infections, especially in the sino-pulmonary tract, apparently caused by neutropenia and hypogammaglobulinemia (Kawai and Malech, 2009). However, this susceptibility appears to be due to leukocyte trafficking defects, not direct antimicrobial effects of chemokines.

**FUTURE DIRECTIONS**

There is no doubt that many chemokines have antimicrobial properties *in vitro*. However, the *in vivo* significance of chemokines as antimicrobial agents is still unclear. Direct antimicrobial activity will depend on the chemokine concentration and the environmental context between host and pathogen. In theory, chemokines in mucosal surfaces might inhibit colonization of certain bacteria in the host since antibiotic usage has clearly been shown to alter normal flora. New studies will be needed to determine whether lack of constitutive chemokines affect skin and mucosal flora.

Studying the domains important for antimicrobial activities might lead to more potent antimicrobial peptides. Cationic charge and amphipathic alpha helix secondary structure appears to be important for antimicrobial properties. However, with the great diversity of antimicrobial peptides there has been neither consensus primary sequence nor a predominant tertiary structure. This makes molecular modeling to generate more potent synthetic antimicrobial proteins difficult. There is also the issue of unwanted side effects. Several potent antimicrobial peptides have systemic toxicity because they lyse red blood cells as well as pathogen membranes (Wiesner and Vilcinskas, 2010). In addition, we have found that many chemokines can induce the release of a virulence factor, protein A, in *S. aureus* (Yung et al., 2011). This potentially would harm the host if bacteria deploy virulence factors in response to chemokines. There is also evidence that subinhibitory concentrations of aminoglycoside antibiotics induce biofilm formation (Hoffman et al., 2005). Antibiotics trigger a protective response by bacteria to form biofilms. Bacteria in biofilms are very difficult to eradicate and are generally thousands of times more resistant to antimicrobial therapy than planktonic bacteria. In the same manner, antimicrobial proteins might trigger a protective response by bacteria to form biofilms.

Currently, there are no antimicrobial proteins in clinical use. The antimicrobial peptide MSI-78 or pexigalan completed a phase III clinical trial for the treatment of diabetic foot ulcers. MSI-78 was originally derived from magainan, an antimicrobial peptide found on the skin of frogs (Zasloff, 1987). However, it failed to receive approval from the FDA on the grounds that efficacy was not sufficiently demonstrated. Few companies are actively developing antimicrobial proteins as therapeutics. Both MBI-226 (a 12 amino acid peptide developed for the prevention of catheter-related bloodstream infections) and Neuprex (a recombinant fragment of bacterialid/permeability increasing protein developed for meningococcal meningitis) finished phase III clinical trials and never made it to market. It is unclear how antimicrobial proteins will be used in the clinics. Topical formulations to treat skin infections may be most applicable but effective topical antiseptics such as chlorhexidine already exist. To treat invasive infections, an injectable or intravenous form would be needed since proteins would most likely be degraded in the gastrointestinal tract. If developed, such potential broad-spectrum antimicrobial peptides might be useful in the setting of life-threatening infections caused by multi-drug resistant organisms.

Even though antimicrobial chemokines have been shown to generally disrupt bacterial membranes, unique targets on pathogens may actually mediate killing. As mentioned previously, the cell membrane protein FtsX in *B. anthracis* binds CXCL10 specifically and mediates antimicrobial chemokine resistance. Understanding this pathway and determining whether the same or related pathways exist in other organisms may lead to new antibiotics.
Genome-wide association studies have not linked increased infection susceptibility to single nucleotide polymorphism in chemokine genes. This could imply either no association, a weak association that has not been analyzed, or redundancy of the antimicrobial protein system. Further genetic studies in mice will be able to address whether genetic deficiencies in chemokines correlate with pathogen control, especially when compared with their respective chemokine receptor deficient controls.

CONCLUSION

In conclusion, the utility of chemokines likely extends beyond leukocyte recruitment. Understanding domains essential for antimicrobial activities in antimicrobial chemokines could lead to more potent antimicrobial peptides. In addition, specific pathways may be involved in pathogen recognition of antimicrobial chemokines. Much work is needed to determine whether antimicrobial chemokines affect susceptibility to infection in humans, how chemokines kill microbes, and whether antimicrobial chemokines can be used to treat infections.

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REFERENCES

Alzoghibi, M. A., Al-Moteleh, I. A., and Al-Ibreem, A. M. (2008). Neutrophil chemokines GCP-2 and GRO-alpha in patients with inflammatory bowel disease. J. Dig. Dis. 9, 144–148.

Antonelli, A., Rotondi, M., Fallahi, P., Romagnani, P., Ferrari, S. M., Ferrannini, E., and Servio, M. (2005). Age-dependent changes in CXCL chemokine ligand 10 serum levels in euthyroid subjects. J. Interferon Cytokine Res. 25, 547–553.

Bauer, I. W., Barchler, E. C., Petri, M., Battliwalla, E. M., Crawford, D., Ortmann, W. A., Espe, K. J., Li, W., Patel, D. D., Gregersen, P. K., Romagnani, P., Ferrari, S. M., Groot, P. H., and Behrens, T. W. (2006). Elevated serum levels of interferon-regulated chemokines are biomarkers for active human systemic lupus erythematosus. PLoS Med. 3, e491. doi:10.1371/journal.pmed.0030491

Berkhout, T. A., Sara, H. M., Moore, K., White, J. R., Elsborg, N., Appelbaum, E., Reape, R. J., Brawner, M., Makwana, J., Foley, J. J., Schmidt, D. B., Imburgeia, C., McNulty, D., Matthews, J., O'Donnell, K., O'Shannessy, J., O'Donnell, N., Appelbaum, E., Reape, R. K., White, J. R., Elshourbagy, A. M., Batliwalla, F. M., Crawford, D., Arterioscler. Thromb. Vasc. Biol. 27, 108, 34, 70–74.

Hernandez-Molina, G., Michel-Peregrina, M., Hernandez-Ramirez, D. F., Sanchez-Guerrero, J., and Llorente, L. (2011). Chemokine saliva levels in patients with primary Sjogren’s syndrome, associated Sjogren’s syndrome, pre-clinical Sjogren’s syndrome and systemic autoimmune diseases. Rheumatology (Oxford) 50, 1288–1292.

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

Hoffman, L. R., D’Argenio, D. A., Mac- coss, M. J., Zhang, Z., Jones, R. A., and Miller, S. I. (2005). Aminoglycoside antibiotics induce bacterial biofilm formation. Nature 436, 1171–1175.

Holven, K. B., Aukrust, P., Holm, T., Ose, L., and Nenes, M. S. (2002). Folic acid treatment reduces chemokine release from peripheral blood mononuclear cells in hyperhomocysteinemic subjects. Arterioscler. Thromb. Vasc. Biol. 22, 699–703.
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Izukuri, K., Ito, S., Norazki, N., Tajima, N., Iwashiba, M., Kawahara, S., Suzuki, K., Kubota, E., and Hata, R. (2010). Determination of serum BRAK/CXCL14 levels in healthy volunteers. Lab. med. 41, 478–482.

Jones, A. P., Webb, L. M., Anderson, A. O., Leonard, E. J., and Rot, A. (1995). Normal human sweat contains interleukin-8. J. Leukoc. Biol. 57, 434–437.

Kagami, S., Kakinuma, T., Saeki, H., Tsunemi, Y., Fujita, H., Nakamura, K., Takakoshi, T., Kishimoto, M., Mitsui, H., Torii, H., Komine, M., Asahina, A., and Tamaki, K. (2003). Significant elevation of serum levels of eotaxin-3/CCL26, but not of eotaxin-2/CCL24, in patients with atopic dermatitis: serum eotaxin-3/CCL26 levels reflect the disease activity of atopic dermatitis. Clin. Exp. Immunol. 134, 309–313.

Kagami, S., Kakinuma, T., Saeki, H., Tsunemi, Y., Fujita, H., Saeki, K., Nakamura, K., Takakoshi, T., Kishimoto, M., Mitsui, H., Komine, M., Asahina, A., and Tamaki, K. (2005). Increased serum CCL28 levels in patients with atopic dermatitis, psoriatic vulgaris and bullous pemphigoid. J. Invest. Dermatol. 124, 1088–1090.

Kaiser, D., Songo-Williams, R., and Drack, E. (1974). Hydrogen ion and electrolyte excetration of the single human sweat gland. Pflugers Arch. 349, 63–72.

Karlsson, C., Eliasson, M., Olin, A. I., Morgelin, M., Karlsson, A., Malmsten, M., Egesten, A., and Frick, I. (2009). SDF-1 as an opportunistic pathogen fengedogia magna modulates actions of the antibacterial chemokine Mig/CXCL9, promoting bacterial survival during epithelial inflammation. J. Biol. Chem. 284, 29499–29508.

Kawai, T., and Malech, H. L. (2009). WHIM syndrome: congenital immune deficiency disease. Curr. Opin. Hematol. 16, 20–26.

Koo, W. W., and Gupta, J. M. (1982). Breast milk sodium. Arch. Dis. Child. 57, 500–502.

Kriegsveld, I., Zaat, S. A., Meeldijk, J., Van Weelen, P. A., Fang, G., Poolman, B., Brandt, E., Ehler, J. E., Remmers, J. A., Engbers, G. H., Feijen, J., and Dankert, J. (2000). Thrombocidins, microbialidal proteins from human blood platelets, are terminal deletion products of CXX chemokines. J. Biol. Chem. 275, 20374–20381.

Liew, H., Yun, Y. S., and Lee, S. Y. (2005). Electrotropes and electrophoretic studies of tear proteins in tears of patients with nasolacrimal duct obstruction. Ophthalmologica 219, 142–146.

Liao, F., Rabin, R. L., Yannelli, J. R., Komarits, L. G., Vanguri, P., and Farber, J. M. (1995). Human Mig chemokine: biochemical and functional characterizations. J. Exp. Med. 182, 1301–1314.

Linge, H. M., Collin, M., Nordenfelt, P., Morgelin, M., Malmsten, M., and Egesten, A. (2008). The human CXC chemokine granulo- cytic chemotactic protein 2 (GCP-2)/CXCL6 possesses membrane-disrupting properties and is an antibacterial. Antimicrob. Agents Chemother. 52, 2599–2607.

Luster, A. D. (1998). Chemokines – chemotactic cytokines that mediate inflammation. N. Engl. J. Med. 338, 436–445.

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

Maheshwari, A., Christensen, R. D., and Calhoun, D. A. (2003). ELR+ CXC chemokines in human milk. Cytokine 24, 91–102.

Mentz, M. L. (2008). Urine sodium composition in ambulatory healthy children: hypotonic or isotonic? Pediatr. Nephrol. 23, 955–957.

Murphy, P. M. (2008). Chemokines and Chemokine Receptors. Philadelphia, PA: Elsevier.

Murphy, P. M., Bagnolini, M., Charo, I. F., Hebert, C. A., Horuk, R., Matsuno, K., Takamori, H., Fujisawa, R., Tanase, S., Nishiura, M., and Hashimoto, K. (2007). Human defensins in vitro bactericidal activities and isotypic expression in bile. Pediatr. Nephrol. 22, 142–146.

Schneider, J. J., Unbolzer, A., Schaller, M., Schaffer-Korting, M., Korting, H. C., Routias, J. K., Karagou- nis, P., Parmulescu, G., Legakis, N. J., and Tsakiris, A. (2005). Human defensins in vitro bactericidal activity of human beta-defensin 2 against nosocomial strains. J. Mol. Med. (Berl.) 83, 587–595.

Sheikine, Y., Nilsson, B., Nilsson, L., Samnegard, A., Hamsten, A., and Oppenheim, I. J. (2003). Many chemokines including CCL20/MIP-3alpha display antimicrobial activity. J. Leukoc. Biol. 74, 448–455.

Yeaman, M. R. (1997). The role of platelets in antimicrobial host defense. Clin. Infect. Dis. 25, 951–968 [quiz 969–970].

Yung, S. C., Parenti, D., and Murphy, P. M. (2011). Host chemokines bind to Staphylococcus aureus and stimulate protein A release. J. Biol. Chem. 286, 5069–5077.

Zaslowski, M. (1987). Cytokine receptors in childhood acute lymphoblastic leukemia. Am. J. Pathol. 163, 2065–2075.

Takahata, Y., Takada, H., Nomura, A., Nakayama, H., Oshikawa, K., and Hata, T. (2003). Detection of interferon-gamma-inducible chemokines in human milk. Acta Paediatr. 92, 659–665.

Tohyama, M., Sayama, K., Komat- susawa, H., Hanakawa, E., Shirakata, Y., Dai, X., Yang, L., Tomokura, S., Nagai, H., Hirakawa, S., Sugai, M., and Hashimoto, K. (2007). CXCL16 is a novel mediator of the innate immunity of epidermal keratinocytes. Int. Immunol. 19, 1095–1102.

Wack, R. P., Lien, E. L., Taft, D., and Roscelli, J. D. (1997). Electrotrop- cyme composition of human breast milk beyond the early postpartum period. Nutrition 13, 774–777.

Wheeler, H. O., Ramos, O. L., and Whitlock, R. T. (1960). Electrolyte excretion in bile. Circulation 21, 988–996.

Wiener, J., and Víckincas, A. (2010). Antimicrobial peptides: the ancient arm of the human immune system. Virulence 1, 440–446.

Yang, C. Y., Brooks, E., Li, Y., Denny, P., Ho, C. M., Qi, F., Shi, W., Wolinsky, L., Wu, B., Wong, D. T., and Mon- termargno, C. D. (2005). Detection of picomolar levels of interleukin-8 in human saliva by SPR. Lab. Chip 5, 1017–1023.

Yang, D., Chen, Q., Hoover, D. M., Staley, P., Tucker, K. D., Lubkowski, I., and Oppenheim, I. J. (2003). Many chemokines including CCL20/MIP-3alpha display antimicrobial activity. J. Leukoc. Biol. 74, 448–455.
cDNA sequence of a precursor. Proc. Natl. Acad. Sci. U.S.A. 84, 5449–5453.
Zinkernagel, A. S., Timmer, A. M., Pence, M. A., Locke, J. B., Buchanan, J. T., Turner, C. E., Mishalian, I., Sriskandan, S., Hanski, E., and Nizet, V. (2008). The IL-8 protease Spy-CEP/ScpC of group A Streptococcus promotes resistance to neutrophil killing. Cell Host Microbe 4, 170–178.

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