

Chemokines, receptors and virus infection

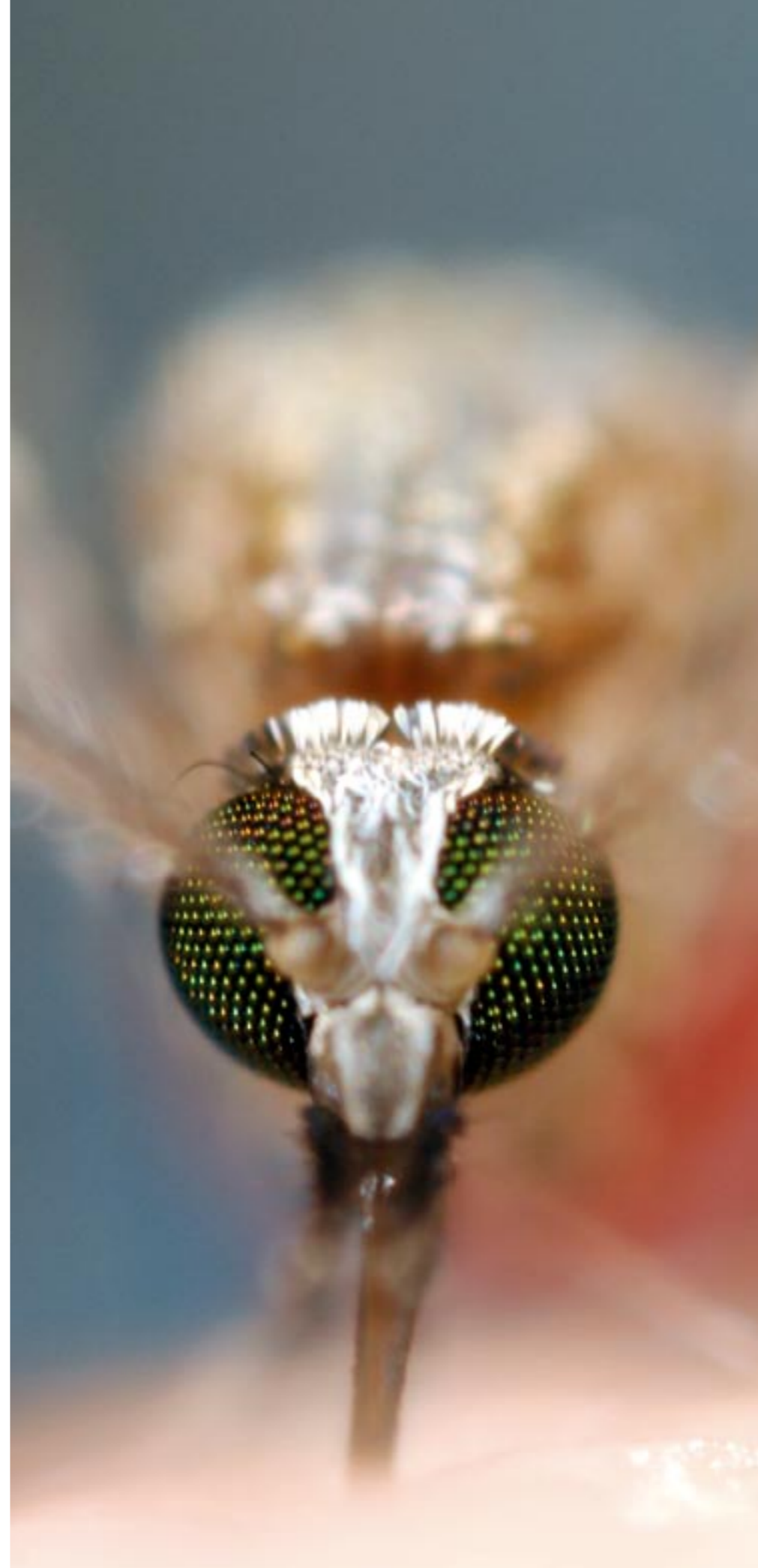
Since the discovery of interferon there has been a rapid increase in the number of cytokines identified. This extensive group of secreted proteins has been shown to regulate many different cellular processes. Some of these signalling molecules are constitutively expressed, being involved in homeostatic and cell migration functions, while others are expressed to high concentrations at sites of infection or tissue damage. The burst of cytokines, released in response to the presence of foreign pathogens and injury, results in the production of a cytokine gradient that is primarily established by a select group comprising nearly 50 *chemotactic cytokines*, or chemokines. This 'chemokine trail' enables the migration of immune cells to the required site where they release further inflammatory chemokines that in turn stimulate both innate and acquired immune responses, guide more immune cells to combat the infection and promote wound healing. While the major role of chemokines is to direct the movement of immune cells to sites of infection, some also have a role in immune surveillance, ensuring there is a constant presence of circulating lymphocytes in the blood. Both inflammatory

and homeostatic chemokines are produced and secreted by many different cell types, including immune and muscle cells, and are closely linked to other cytokines having very similar functions.

Signalling

To elicit their effect on target cells, chemokines bind specific receptors on the cell surface. Attachment is a two-step process with the initial recognition and binding causing a conformational change in the chemokine before the final binding process can occur. As highlighted later, this two-step mechanism has been mimicked by some pathogens in order to hijack chemokine receptors as a point of cellular entry. While chemokines bind to different receptors, these are all anchored within the lipid bilayer and have seven transmembrane domains. As a result, four regions of the receptor are exposed to the extracellular environment that

► A mosquito (*Anopheles stephensi*) feeding on human blood. This mosquito is well known for transmitting malaria. Sinclair Stammers / Science Photo Library



A delicate balance exists between host chemokines, receptors and infections. **Edward Wright** discusses how these interactions show that host genetic factors play an important role in susceptibility to infections.

act in concert to bind the chemokine ligand. Once bound this stimulates a conformational change in the receptor, which itself causes the activation of a G-protein coupled to the intracellular domain of the receptor and a consequent signalling cascade ensues.

Similar chemokines have been identified in vertebrates such as fish, birds and amphibians, suggesting that chemokine signalling occurs by the same mechanism as that observed in mammals (described above). However, of the 24 mammalian chemokine receptors isolated, three, CCBP2/D6, Duffy Antigen Receptor for Chemokines (DARC) and CCX-CKR, are unable to induce downstream signalling as they lack an associated intracellular G-protein. Instead they target the chemokine for endosomal destruction by

internalizing it once bound to the receptor (hence they are termed 'interceptors'). These receptors are therefore thought to be more important in the control of circulating concentrations of chemokines than immune signalling.

Interfering with infection

It is not only chemokines that are able to bind chemokine receptors. Through co-evolution with their hosts, foreign pathogens have adapted to exploit these receptors in order to unlock cells and initiate infection. The first example of such an interaction was presented in the mid-1970s; it is well known that vivax malaria is endemic in most tropical and subtropical areas of the world; however, it was noted that more than 95 % of the population in West Africa is completely resistant to infection by the malarial parasite. Further investigation revealed that the malaria parasite *Plasmodium vivax*, along with its respective simian and murine relatives, *P. knowlesi* and *P. yoelii*, use DARC for attachment and infection of erythrocytes, and that protection from malarial infection correlates with the absence of DARC on red cells. It has since been shown that the lack of DARC expression on the surface of erythrocytes is due to a single nucleotide polymorphism that is differentially distributed across populations, but is at particularly high prevalence in West Africans.

HIV and AIDS

Since then, with the ever-expanding number of emerging infections and newly identified chemokine receptors, the number of pathogens that have been found to modulate the chemokine network has increased markedly. Six years after the DARC–malaria interaction was resolved, the first cases of possibly the best known infectious agent to use chemokine receptor entry was identified, human immunodeficiency virus (HIV). In 1984, within one year of HIV being confirmed as the aetiological agent causing acquired immunodeficiency syndrome (AIDS), its primary receptor, CD4, had been identified. However, it was quickly determined that another, unknown receptor was required for HIV to infect its target cells. Twelve years later this receptor was identified as a chemokine receptor and HIV is now known to be able to use more than 15 chemokine receptors in conjunction with CD4. Of these, two are used by the majority of circulating strains, CC receptor 5 (CCR5) and CXC receptor 4 (CXCR4), so named due to the arrangement of two cysteine residues in the chemokines they bind. As is the case when a chemokine binds its native, cellular receptor, the envelope of HIV must undergo a conformational change to bind CCR5 or CXCR4, but this is primarily seen as an immune evasion strategy since it keeps the major viral epitopes hidden from immune surveillance until the final moments.

HIV does not have it all its own way; genetic polymorphisms in chemokine receptors result in the inability of HIV to attach and subsequently cause infection. One of the most prevalent

is the CCR5 $\Delta 32$ deletion, thought to have been selected due to the protection it conferred against smallpox and Black Death pandemics that swept across Europe until Medieval times. The deletion of 32 bp in the CCR5 gene causes the protein to remain cytosolic and to not be expressed on the cell surface. Without CCR5, HIV is unable to infect the cells and because strains that use CCR5 are transmitted at a much higher frequency than those that use CXCR4, individuals with this mutation are highly resistant to infection. This deletion is found only in Caucasian populations and at highest frequencies amongst Scandinavians (~15 % of the population) due to smallpox and Black Death infections persisting in that area longer than any other. However, it is not only chemokine receptor polymorphisms that affect HIV disease. Increased levels of CCR5 and CXCR4 ligands, known as anti-HIV chemokines, can also result in decreased risk of infection and longer survival. Higher circulating concentrations of these proteins will lead to less receptor without bound ligand and therefore fewer targets for HIV to bind. Whilst many stimuli can cause fluctuations in chemokine production, differences in the number of copies of genes encoding these chemokines (brought about by selective, segmental duplications in the human genome that enriches for immune genes) is known to be especially important in controlling HIV infection. Similar to the two genetic polymorphisms described above, the number of genes expressing anti-HIV chemokines is also distributed differently between populations. On average Africans have between five and six copies of a potent CCR5 agonist, macrophage inflammatory protein-1 α P (MIP-1 α P). However, Africans with more than six copies are less likely to become infected and progress more slowly to AIDS. The converse is seen in Africans with less than five copies of the MIP-1 α P gene. In light of this and with resistance to current HIV drugs increasing, the possibility of complete saturation of CCR5 and CXCR4 has long been thought of as a novel target for an anti-HIV drug. Earlier this year Maraviroc, the first CCR5 small molecule antagonist, was licensed for use in Europe and America, having been shown to reduce HIV viral loads in clinical trials.

Other viruses

Other viruses known to use chemokine receptors to productively infect cells include respiratory syncytial virus and the poxvirus myxoma virus. However, a handful of pathogens have either captured host genes or evolved genes that allow them to express chemokines and receptor homologues, so called 'virokines' and 'viroceptors'. The majority of these are encoded by herpes- and poxviruses, which may be expected, given their genomes are amongst the largest of known viruses, encoding 100–250 genes. These virally encoded foreign proteins are used primarily for immune evasion and to establish the ideal replication environment for the pathogen. While the chemokine homologues only share 35–70 % identity with their host counterparts, they are still

able to stimulate the same functions. Human herpesvirus-8 encodes several vMIPs that can promote cell maturation and other homeostatic processes, while human cytomegalovirus vCXCL1 protein is able to initiate the migration of cells. A chemokine homologue has more recently even been isolated from a bacterium able to attract cells of the myeloid lineage.

I have highlighted some examples that demonstrate the delicate balance that exists between host chemokines, receptors and infections. It is noteworthy that these interactions demonstrate that host genetic factors play an important role in susceptibility to infections and subsequent clinical outcomes. Taken together with the fact that malaria influences the spread of HIV infection, it becomes apparent that the interplay between host and pathogen, and different pathogens themselves can greatly alter chemokine responses and thereby significantly affect morbidity and mortality.

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Further reading

- Alcami, A. (2003). Viral mimicry of cytokines, chemokines and their receptors. *Nat Rev Immunol* 3, 36–50.
- Este, J.A. & Telenti, A. (2007). HIV entry inhibitors. *Lancet* 370, 81–88.
- Laing, K.J. & Secombes, C.J. (2004). Chemokines. *Dev Comp Immunol* 28, 443–460.
- Cytokines & Cells Online Pathfinder Encyclopaedia* (www.copewithcytokines.de/cope.cgi)

► False-coloured SEM of a T cell (green) infected with HIV (pink particles). HIV uses chemokine receptors expressed on the surface of immune cells to achieve attachment and infection. *Scott Camazine / CDC / Science Photo Library*

The interplay between host and pathogen can greatly alter chemokine responses and thereby significantly affect morbidity and mortality.

