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COMMENTARY

The yin and yang of chemokine receptor activation

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Chemokines represent a class of cytokines that control the migration of leucocytes. The human chemokine system comprises 44 ligands and 21 receptors that have evolved to control leucocyte migration. Although chemokines are an attractive therapeutic target for anti-inflammatory intervention, clinical trials of small molecule receptor antagonists have failed to demonstrate efficacy. One often cited explanation for this is the apparent redundancy within the chemokine system, wherein several ligands bind and activate each receptor. The work of Scholten *et al.* and Nedjai *et al.* reported in this issue of the *British Journal of Pharmacology* demonstrates that this redundancy does not exist at the molecular level and provides a powerful insight into the complex nature of chemokine receptor activation.

LINKED ARTICLES
This article is a commentary on Scholten *et al.*, pp. 898–911 of this issue and Nedjai *et al.*, pp. 912–923 of this issue. To view Scholten *et al.* visit http://dx.doi.org/10.1111/j.1476-5381.2011.01648.x and to view Nedjai *et al.* visit http://dx.doi.org/10.1111/j.1476-5381.2011.01660.x

Introduction

Chemokine-driven leucocyte recruitment plays a central role in the functioning of the immune system. The human chemokine system is complex, with some 44 ligands and 21 GPCRs identified to date (Pease and Williams, 2006). These ligands can be functionally divided into two major groups: the homeostatic chemokines, constitutively expressed and involved in leucocyte homing, and inflammatory chemokines. Some inflammatory chemokines such as CXCL8 are stored pre-formed in the vascular endothelium; these are rapidly released following tissue injury and direct rapid, site-specific, migration of innate immune cells such as CXCR2-expressing neutrophils into the inflammatory site. Other inflammatory chemokines such as CXCL11 are expressed later in the immune response and promote the migration of CXCR3-expressing antigen-specific lymphocytes into the tissue (Newton *et al.*, 2009).

The interaction between inflammatory chemokines presented by the inflamed endothelium and receptors expressed by tissue infiltrating leucocytes suggests a target for therapeutic intervention in inflammatory diseases. Disappointingly, clinical trials using specific chemokine receptor blocking strategies have failed either due to lack of efficacy or off-target toxicity (Proudfoot *et al.*, 2010). Existing strategies have employed neutralizing antibodies targeted at receptors or ligands, or small molecule receptor antagonists (Pease and Williams, 2006; Horuk, 2009; Proudfoot *et al.*, 2010). It is increasingly unclear whether such approaches, which have cost many millions of dollars to develop, will ever prove effective. It is clear that further research is required to understand chemokine receptor biology in order to inform the design of new therapeutic interventions that will prove both safe and effective in the future (Schall and Proudfoot, 2011).

One confounding factor in chemokine-targeted drug discovery is the multiplicity with the chemokine system. The inflammatory chemokines tend to be promiscuous and can bind several receptors expressed on a variety of cell types (Horuk, 2009). For example, the chemokine CCL7 can bind to the receptors CCR1, CCR2, CCR3 and CCR5. In turn, most inflammatory chemokine receptors are able to bind and direct migration in response to multiple ligands; the receptor CCR1 binds at least nine ligands. This overlap of ligand and receptor specificities has been long regarded as biological redundancy; however, this *a priori* assumption is being increasingly challenged (Schall and Proudfoot, 2011).

Two papers in this issue of the *British Journal of Pharmacology* (Nedjai *et al*., 2012; Scholten *et al*., 2012) closely examine the biology of the chemokine receptor CXCR3. CXCR3 is an attractive and druggable target for anti-inflammatory intervention as its expression is largely restricted to activated T lymphocytes (Newton *et al*., 2009). Preventing the chemokine-driven migration of these cells may result in patient benefit in a range of inflammatory conditions, such as allograft rejection, multiple sclerosis, rheumatoid arthritis and inflammatory bowel disease, where CXCR3 is thought to be important (Sporici and Issekutz, 2011). Three endogenous ligands for CXCR3, CXCL9 (monokine induced by IFN-γ, MIG), CXCL10 (IFNγ-induced protein 10, IP-10) and CXCL11 (IFN-inducible T-cell α chemoattractant, ITAC) have been described that have previously been described to bind with different affinities to the receptor. As their original names suggest, all of these chemokines are inducible by IFN-γ, and hence, they are often co-expressed.

Scholten *et al*. (2012) employed a previously described, synthetic small molecule, agonist to demonstrate ligand-biased signalling at CXCR3. The small-molecule agonist applied to CXCR3 by Scholten *et al*. is a true agonist as it activates both G-protein-dependent and -independent signalling. In the wider GPCR field, ligand-biased responses have previously been recognized (Kramp *et al*., 2011); however, it is only recently that individual ligand-biased responses have been observed in chemokine receptors (O'Boyle *et al*., 2007). Scholten *et al*. (2012) demonstrate that in some assays, the synthetic small-molecule agonist is less potent than the endogenous chemokine ligand, whereas in other assays, the converse is seen. This suggests that the agonist and the chemokine activate distinct conformations of CXCR3. The consequences of this observation for drug development are clear. It is possible that the receptor conformations activated by the many different chemokine ligands for inflammatory receptors may have differential susceptibility to the small molecule antagonists that have proceeded to clinical trial. A successful antagonist targeted at an inflammatory receptor must disrupt the ligand-induced activation state of each ligand to elicit therapeutic efficacy.

The paper by Nedjai *et al*. (2012) describes an important comparison of the biology of the three CXCR3 ligands in an elegant study that employs point mutations in the receptor and then examines binding of the endogenous ligands CXCL10 and CXCL11, alongside two small molecule synthetic agonists. The small molecule agonists applied to CXCR3 by Nedjai *et al*. allowed the authors to precisely map the ligand-interacting domains of this powerful inflammatory receptor. Such important data will provide a crucial tool for understanding receptor biology and pharmacological modelling, particularly when partnered with the recent explosion in GPCR crystallization, including CXCR4, the first chemokine receptor to have had its structure solved (Wu *et al*., 2011).

Together, the studies by Scholten *et al*. and Nedjai *et al*. provide an example and a mechanism underpinning ligand-specific responses of CXCR3 in a reductionist, single receptor / single ligand system. However, the contribution of these observations to inflammation remains unclear. A further level of complexity exists *in vivo* as chemokines and chemokine receptors are capable of forming homo- and heterodimers (Kramp *et al*., 2011). Such multimeric interactions represent a powerful strategy to regulate inflammation beyond the existing, simple model that suggests that inflammatory chemokines are deleterious in inflammatory disorders, and that single receptor blockade will result in amelioration of disease course.

Whilst knockout animals for specific receptors or ligands can provide information on the importance of specific ligand–receptor axes during the genesis of inflammatory disease, the importance of any single ligand or receptor in ongoing disease is less apparent. For example, in rheumatoid arthritis, over a dozen chemokines are found in the synovial fluid of inflamed joints (Pease and Williams, 2006). Any of these may continue to direct cell migration into the joint in spite of the efficient blockade of a single chemokine–receptor axis. This concept is apparent in other contexts, such as cardiac allograft rejection. Once this inflammation is an ongoing memory response, blocking a single axis such as CXCR3 or CCR5 is not effective at limiting further damage (Oberbarnscheidt *et al*., 2011).

It is clear that existing pre-clinical animal disease models do not accurately estimate efficacy prior to clinical testing (Horuk, 2009). Variations between animal and human receptor interactions can significantly obscure crucial pharmacological information; strikingly demonstrated at Northwick Park in the phase I trial of TGN1412. This CD28 agonist antibody resulted in catastrophic systemic organ failure at a 500-fold lower dose than that proven safe in murine and primate models (Eastwood *et al*., 2010; Pallardy and Hünig, 2010). It is clear that agents targeting inflammatory cell recruitment should be examined *in vivo*, wherever possible, for their effect on human drug targets. The crucial effects of small inter-species changes was illustrated by the finding that, as a consequence of one amino acid change (Q196E) between human and murine CXCR3, the synthetic small molecule CXCR3 agonist employed by these two groups was an agonist only for the human chemokine receptor (Nedjai *et al*., 2012).

One potential strategy to examine human chemokine receptor biology *in vivo* is site-directed replacement of the murine chemokine receptor with the equivalent human sequence (Horuk, 2009). Such transgenic animals have been generated for a number of chemokine receptors such as CCR1 (Gladue *et al*., 2006). This approach does not account for differential coupling to signal transduction, GPCR heterodimerization or receptor trafficking that may occur. Indeed, Nedjai *et al*. (2012) report that the same ligand was an order of magnitude less potent when used to stimulate murine L1.2 cells expressing human CXCR3 than human H9 cells expressing human CXCR3 (Horuk, 2009).

The work of Scholten *et al*. and Nedjai *et al*. has illustrated, at a molecular level, that biological redundancy is not a feature of the chemokine system. The complex network of 44 chemokine ligands and 21 receptors has evolved to control leucocyte migration and the multiplicity of possible interactions confers considerable subtlety on the responses elicited. Furthering our understanding of the biology of inflammatory chemokine receptors should be regarded as a prerequisite for more successful strategies for anti-inflammatory intervention.

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Conflicts of interest

None to declare.

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