

“Neutralizing endogenous chemokines with small molecules. Principles and potential therapeutic applications”

GALZI Jean-Luc*, HACHET-HAAS Muriel*, BONNET Dominique[€], DAUBEUF Francois[€], LECAT Sandra*, HIBERT Marcel[€], HAIECH Jacques[€], FROSSARD Nelly[€]

*IREBS, FRE3211, Ecole Supérieure de Biotechnologie de Strasbourg, Boulevard Sébastien Brant, 67412 Illkirch, France

[€]Laboratoire d’Innovation Thérapeutique, UMR7200, Faculté de Pharmacie, 74 route du Rhin, 67401 Illkirch, France

Corresponding author:

Dr Jean-Luc Galzi, galzi@unistra.fr

Tel +33 368 85 47 59

Keywords:

chemokines, cytokines, GPCRs, decoy receptors, neutralizing ligands, small molecule

Abstract:

Regulation of cellular responses to external stimuli such as hormones, neurotransmitters, or cytokines is achieved through the control of all steps of the complex cascade starting with synthesis, going through maturation steps, release, distribution, degradation and/or uptake of the signalling molecule interacting with the target protein. One possible way of regulation, referred to as scavenging or neutralization of the ligand, has been increasingly studied, especially for small protein ligands. It shows innovative potential in chemical biology approaches as well as in disease treatment. Neutralization of protein ligands, as for example cytokines or chemokines can lead to the validation of signalling pathways under physiological or pathophysiological conditions, and in certain cases, to the development of therapeutic molecules now used in autoimmune diseases, chronic inflammation and cancer treatment. This review explores the field of ligand neutralization and tries to determine to what extent small chemical molecules could substitute for neutralizing antibodies in therapeutic approaches.

Table of contents

1. Introduction

Chemokines and chemokine receptors

2. Natural cytokine and chemokine neutralization

2.1. Endogenous chemokine interceptors

2.2 Avoidance strategies: examples of neutralizing molecules produced by pathogens

3. Potential therapeutic interest of soluble decoy proteins

4. Validation of chemokines in signalling pathways and pathology: Importance of anti chemokine antibodies

5. Neutralizing cytokines and chemokines with small chemical compounds

5.1. The small molecule competitively binds to the same site as the receptor

5.2. The small molecule alters quaternary structure of the protein ligand

5.3. The protein ligand undergoes tertiary structural changes

5.4. The small molecule interferes with ligand bioavailability

6. Conclusions

Acknowledgments

References

Abbreviations

BSA: Bovine serum albumin

DARC: Duffy antigen receptor for chemokines

GAG: glycosaminoglycans

GPCR: G Protein Coupled Receptor

IL-: Interleukin

LPS: lipopolysaccharide

MS: Multiple sclerosis

1. Introduction:

Deciphering biological signalling pathways makes use of convergent approaches including direct gene manipulation or downstream information processing intermediates such as messenger RNAs, proteins or signalling small molecules/hormones or their metabolites. Gene manipulation, in particular gene deletion/invalidation, is one of the most widely used approaches to determine the function of a gene and of its products. It presents the major advantage of selectively altering one gene structure or expression so that a given phenotype, when observed, is generally closely associated with the gene of interest and to its products. On the other hand, gene deletion or over expression can be induced, but not yet in a reversible manner, so that control experiments must be carried out on wild type animals in which developmental or compensatory effects may not have taken place in a comparable manner (Auwerx et al., 2004; S. D. Brown et al., 2005; Chensue et al., 2001; M. Yang et al., 2006). Chemical biology approaches, i.e. methods that use chemical tools to elucidate the function of a protein in a given signalling pathway, and are at the frontier between pharmacology, chemistry and biophysics, are useful too and show complementarity with genetic approaches. They also offer the possibility to transpose small molecule tools into drugs when pathological issues are coming into play during the assessment of the protein function. The advantages and drawbacks of the chemical biology approach are mirror images of the genomic approach. Reversibility of the effect of a molecule can be studied on the same living individual upon cessation of molecule administration. On the other hand, molecules are rarely specific for a given target protein, and the claimed selectivity of a compound generally follows an inverse relationship with the extent of side effects (Wermuth, 2006). Antibodies, and in particular monoclonal antibodies, have arisen as potential substitutes to both the genetic and the chemical biology approaches in the sense that they exhibit quasi-exclusive selectivity for a protein target and that the interruption of treatment leads to reversal of their effects. Antibodies offer in addition the possibility of target interactions, especially large protein-protein interactions, that are difficult to perturb with small molecules. This has led to the exponential development of antibodies or antibody fragments (Chames et al., 2009; Nelson & Reichert, 2009; Wesolowski et al., 2009) for therapeutic purposes. Antibodies are powerful tools in laboratory research because they can be developed much faster than small chemical molecules (see below).

They have thus been largely used to validate the involvement of proteins in signal transduction pathways, and as potential target for drug development. On the other hand, antibodies have intrinsic limitations that constrain their use for biological systems exploration. With some exceptions, antibodies and antibody fragments do not cross biological barriers, such as the intestinal or blood brain barriers. The consequence is that antibodies must be injected and most central nervous system proteins will not be reached. Also, antibodies cannot reach intracellular target proteins unless they cycle to the plasma membrane.

For all these reasons, the chemical biology approach using small molecules as tools or drugs remains a useful and valid strategy. In this article, we review examples of small chemical molecules that can be used to neutralize small signalling proteins such as chemokines or cytokines. The reader should appreciate that only a few examples are known to date. The reason for this is that all neutralizing molecule discoveries that are presented here were serendipitous, and specifically designed experimental approaches are only just entering starting blocks. Small molecules are being searched to inhibit protein-protein interactions, with a focus on intracellular compartments and cancer related interactions, or brain functions exploration (M. Arkin, 2005; M. R. Arkin & Wells, 2004; Berg, 2003; Blazer & Neubig, 2009; J. A. Wells & McClendon, 2007). These will not be reviewed here. We will focus mainly on the family of small signalling proteins, the chemokines, which constitute a well adapted biological system to develop neutralizing small molecules. Examples from other cytokines will be discussed as well.

Chemokines and chemokine receptors

Chemokines are small secreted chemotactic cytokines endowed with multiple activities. Their main function is chemical attraction of leukocytes, but they also contribute to the regulation of organ development during ontogeny. In inflammation, the chemotactic signal given by chemokines leads to egress of leukocytes from the blood circulation across the walls of small blood vessels. To do this, chemokines that are produced on the site of inflammation cross the endothelial cell wall and remain immobilized on the luminal surface of the endothelium. Circulating leukocytes, depending on their chemokine receptor expression will then be attracted and directed towards the inflamed site along the

chemotactic gradient. Chemokines, in addition to attracting cells, contribute to the regulation of gene expression on target cells and help to control cell proliferation and apoptosis, for instance in angiogenesis.

Chemokines are also subdivided into several functional groups depending on whether their expression is constitutive or inducible by inflammatory signals, and also on their capacity to stimulate or inhibit angiogenesis, especially in tumors (Vandercappellen et al., 2008). The CXC chemokines in particular exert angiogenic or angiostatic activities depending on the presence of an ELR (Glu-Leu-Arg) motif in their N terminal portion (Addison et al., 2000). As important regulators of cell migration, therapeutic intervention of the chemokine system(s) include(s) infectious diseases, intra-organism alert systems possibly leading to autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, psoriasis or lupus erythematosus, as well as allergic disorders such as asthma, inflammatory bowel disease, transplant rejection, neuropathies or dermatitis.

More than 50 chemokines are known (T. N. Wells et al., 2006). The chemokine structure (Figures 2 and 3) comprises an N-terminal loop region, three-strand antiparallel beta sheets forming the typical core fold of the chemokines and a C-terminal alpha helix which overlays the beta-sheet. CC, CXC and CX3C chemokines comprise in addition two disulfide bridges linking the N terminal domain with the loop separating sheet 1 and sheet 2 and the N-terminal domain with the end of sheet 3. In order to allow gradients to be formed at the vicinity of the site of release, chemokines bind to extracellular matrix components, i.e. the negatively charged glycosaminoglycans (GAGs), by means of their positively charged amino acids. These positive amino acids form distinct clusters at the surface of the chemokine depending on whether the chemokine belongs to the CC, CXC or CX3C group (Laguri et al., 2008). In the CXC chemokine group, the GAG binding area is on the side of the protein that does not interact with the receptor (Amara et al., 1999; J. W. Murphy et al., 2007; Santiago et al., 2006) and, mutation of the positive amino acids that bind to GAGs does not alter chemokine binding to the receptor (Amara et al., 1999; Proudfoot et al., 2001), and interaction with heparan sulfates does not change the equilibrium binding affinity of the chemokine for its receptor (Valenzuela-Fernandez et al., 2001). In the CC group of chemokines, in contrast, there is significant overlap between receptor binding and GAG

binding areas which, in the case of CCL5 for instance, has influence on receptor subtypes-specific interactions (Proudfoot et al., 2001).

Chemokines signal through G-proteins coupled to seven transmembrane receptors which are classified according to the chemokines they bind (CXCR, CCR, CX3CR and XCR) (P. M. Murphy, 2002). The chemokine receptor family groups twenty G protein-coupled receptors (GPCRs) and covers extremely diverse physiological responses. As a general rule, structural promiscuity between GPCRs accounts for frequently observed problems of ligand selectivity among subtypes. Reciprocally, GPCR ligands, in particular chemokines, are grouped in small chemical families, so that neutralizing the ligand rather than the receptor may allow good focus on a subset of targeted signalling pathways.

Along with several other signalling proteins (Alcami & Smith, 1992; Bamias et al., 2008; Bezerra et al., 2005; Colotta et al., 1993; de Moura et al., 2009; Fili et al., 2009; Funke et al., 2009; Mueller et al., 2009; Pitti et al., 1998; Rahaman et al., 2002; Scola et al., 2009), chemokines are subject to natural modulation of their concentrations by proteins to which they bind (Figure 1) without leading to typical signalling (Alcami, 2003; Graham, 2009; Graham & McKimmie, 2006; Mantovani et al., 2006; J. W. Murphy et al., 2007; P. M. Murphy, 2000; Pruenster et al., 2009). These proteins may be endogenously encoded to modulate chemokine functions or expressed by exogenous sources like pathogens or parasites with the aim of escaping the host immune system (see below). These naturally occurring "scavenger" or "decoy" proteins act as "interceptors" – i.e. intercepting receptors - that neutralize the action of the chemokine. We shall briefly review these systems because they validate the concepts of ligand neutralization, before considering approaches to unnatural neutralization.

2. Natural cytokine and chemokine neutralization

Besides metabolic regulation of hormone or peptide production such as enzymatic degradation, transport (Mortier et al., 2008), a captivating aspect of response regulation is scavenging of ligands by molecules that bind to it and modulate its biological function. This

has been illustrated in the past 25 years with the identification of endogenous receptor-like structures that do not lead to conventional signalling in response to small protein ligands but rather seem to contribute to their blockade or elimination (Bamias et al., 2008; Bezerra et al., 2005; Bonecchi, Borroni, Savino et al., 2008; Colotta et al., 1995; Colotta et al., 1993; de Moura et al., 2009; Mantovani et al., 2008; Mantovani et al., 2006; Mantovani et al., 2007; Mueller et al., 2009; Scola et al., 2009; Thelen & Thelen, 2008). These receptor-like molecules, which can be soluble (Colotta et al., 1993; de Moura et al., 2009; Funke et al., 2009) or membrane-bound (Mantovani et al., 2006; Scola et al., 2009), have been termed "decoy" or scavenger" proteins. They however serve physiological as well as pathophysiological functions.

Decoy proteins for interleukins IL-1 (Colotta et al., 1993), IL-22 (de Moura et al., 2009), IL-13 (Caput et al., 1996; Rahaman et al., 2002), death ligands TRAIL (Bellail et al., 2009) and CD95L (Pitti et al., 1998), activators of NF- κ B -RANK (Khosla, 2001; Simonet et al., 1997) or complement (Cain & Monk, 2002; Scola et al., 2009) generally exhibit ligand selectivity and/or specificity. Those for chemokines (Graham, 2009; Mantovani et al., 2006) display poor ligand selectivity.

2.1. Endogenous chemokine interceptors

There are three, possibly four, endogenous proteins that belong to the structural family of G protein-coupled receptors, bind chemokines with limited to low selectivity, do not signal toward G protein-dependent pathways but keep the capacity to internalize and transport the bound chemokine across the plasma membrane. These proteins, DARC, D6, CCX-CKR and possibly CXCR7, act as uptake or re-uptake proteins that trap the ligand, internalize it and direct it towards degradation, possibly also towards transcytosis. These proteins play important roles in inflammation, development, and chemokine-associated diseases such as cancer (Graham & McKimmie, 2006; Mantovani et al., 2006).

DARC: Duffy antigen receptor for chemokines, DARC, binds both CC (CCL-2, -5, -7, -11, -13) and CXC (CXCL-1, -3, -5, -6, -8, -11) inflammatory chemokines as well as the homeostatic chemokine CCL14. It is a G protein-coupled receptor-like protein that lacks the capacity to stimulate G proteins.

DARC is expressed at high levels in the cell membrane of erythrocytes where it was shown to contribute to clearing circulating chemokines (Darbonne et al., 1991). Supporting this role in chemokine clearance, lack of DARC protein is associated with *an* exaggerated inflammatory response to lipopolysaccharide LPS (Dawson et al., 2000), while overexpression of the protein leads to diminished angiogenesis (Bonecchi, Borroni, Anselmo et al., 2008). Further supporting the importance of DARC in chemokine clearance, circulating CXCL1 is mostly associated with red blood cells in wild type mice, while it is found in the plasma in DARC *-/-* mice. Accordingly, in a mouse model of acute lung injury, LPS-induced polymorphonuclear leukocytes migration in the alveolar space is elevated two-fold in knock-out animals (Reutershan et al., 2009). The chemokine sequestering function of DARC is also clinically validated as preventing/reducing tumor cell growth, as was demonstrated in breast cancer (J. Wang et al., 2006).

DARC is also expressed on endothelial cells, but under normal conditions, this expression is restricted to postcapillary venules (Fra et al., 2003). It may extend to other types of blood vessels (arteries, capillaries) during infection, inflammation or graft rejection (Gardner et al., 2006; Segerer et al., 2000). In endothelial cells, DARC protein serves as a transporter that allows chemokine transcytosis, thus leading to efficient exposure of tissue-derived inflammatory chemokines to the lumen of vessels and subsequent leukocyte recruitment and extravasation (Pruenster et al., 2009).

D6 binds and suppresses inflammatory chemokines

Like DARC, D6 exhibits poor selectivity towards chemokines. It binds 12 different chemokines out of which none belong to the CXC group and 8 are CC pro-inflammatory molecules, implicating D6 as a probable regulator of inflammation. Noteworthy also is the fact that DARC and D6 bind 7 identical chemokines (Bonecchi, Borroni, Anselmo et al., 2008). D6 is expressed in lymphatic endothelial cells from non-inflamed skin, gut and lung. Upon inflammation, D6 is expressed in leukocytes, especially in those that invade inflamed tissues (Graham & McKimmie, 2006). In contrast to DARC, D6 does not promote chemokine transcytosis, but rather contributes to their degradation by directing them towards endosomes. D6 is predominantly localized in recycling endosomes capable of trafficking to and from the cell surface in the absence of ligand. In the presence of ligand, D6 can rapidly

internalize chemokines; however, D6-internalized chemokines are more effectively retained intracellularly because they more readily dissociate from the receptor during vesicle acidification. These chemokines are then degraded while the receptor recycles to the cell surface (Fra et al., 2003; Galliera et al., 2004; Weber et al., 2004). The most likely physiological role of D6 thus is to clear tissues from remaining chemokines in order to prevent an excessive response, and eventually terminate the inflammatory response (Graham & McKimmie, 2006). In support of the lack of D6 expression, *in vivo* results in an amplified chemokine-mediated inflammatory response (Jamieson et al., 2005; Martinez de la Torre et al., 2005). D6^{-/-} mice show high levels of inflammatory chemokines in the lymph nodes. By contrast, overexpression of D6 reduces leukocyte responses in inflammation models (Nibbs et al., 2007).

As a result of deregulated expression of chemokine receptors and chemokines in cancer, a role of D6 in carcinogenesis has been proposed (Nibbs et al., 2007; Wu et al., 2008). Due to its capacity to sequester chemokines, D6 protects from tumorigenesis in chemical treatment-evoked skin tumors (Nibbs et al., 2007). In another study, D6 has been reported to reduce intratumor levels of CCL2 and CCL5 chemokines, and consequently to inhibit proliferation and invasion of breast cancer cells *in vitro* as well as tumorigenesis and metastasis *in vivo* (Wu et al., 2008).

CCX-CKR binds and suppresses homeostatic chemokines

CCX-CKR is, like DARC and D6, derived from a G protein coupled structure, and is devoid of signalling capacity towards G proteins. It is expressed in various organs such as spleen, lymph nodes, heart, kidney, placenta, trachea and brain (Gosling et al., 2000), and in various cell types like T cells, immature dendritic cells, stromal cells, astrocytes (Dorf et al., 2000), ciliated bronchial epithelial cells in pulmonary sarcoidosis (Kriegova et al., 2006), and endothelial cells surrounding cancer cells in tumors (L. Y. Feng et al., 2009).

CCX-CKR binds the homeostatic chemokines CCL19 and CCL21 which control trafficking of naive T cells, CCL25, CXCL13 which mediate B cells and helper T cells migration (Comerford et al., 2006; Gosling et al., 2000; Townson & Nibbs, 2002), and directs them towards degradation. *In vitro*, cells expressing CCX-CKR deplete large quantities of these chemokines (Comerford et al., 2006; Gosling et al., 2000; Townson & Nibbs, 2002). Inflammation promoting signals, such as interleukin 1beta, tumor necrosis factor TNF-alpha or interferon

IFN gamma attenuate CCX-CKR mRNA levels, supporting a potential link of this interceptor with inflammation. *In vivo*, in particular in mice harbouring CCX-CKR transfected xenografts, reduced tumor growth, neo-vascularization and metastasis are detected (L. Y. Feng et al., 2009). Also, a clinical study in breast cancer shows the natural level of CCX-CKR expression to correlate with longer survival of the patients (L. Y. Feng et al., 2009).

Chemokine receptors as temporary interceptors: The debated case of CXCR7.

The capacity of G protein-coupled receptors to endocytose together with their ligands makes it likely that at least some of them behave as interceptors. The expression of CCR5, for example, is up regulated in T cells responding to anti-inflammatory lipids. Such modification represents a mechanism by which chemokines can be trapped and inflammation terminated (Ariel et al., 2006).

The second receptor for CXCL12 and for CXCL11, namely CXCR7, is another example of an atypical receptor. Indeed, although the receptor sequence contains the canonical DRY sequence required for coupling receptors to G proteins and, attempts to detect signalling through G proteins, activation of MAP-kinases or stimulation of PI3-kinase were unsuccessful to date (Balabanian et al., 2005; Burns et al., 2006; Dambly-Chaudiere et al., 2007) with the exception of one report on signalling through Akt (J. Wang et al., 2008) which may itself result from beta-arrestin recruitment (Kalatskaya et al., 2009; K. E. Luker et al., 2009 a; Zabel et al., 2009). Which ever the way CXCR7 signals, it appears important during development, and in particular in heart valve formation (Sierra et al., 2007) and for stabilization of cell adhesion after migration towards CXCL12 gradients (Boldajipour et al., 2008; Dambly-Chaudiere et al., 2007). During development of the zebrafish sensory system, formation of the sensory organ, the lateral line, requires long distance migration of primordial germ cells. Dambly-Chaudière et al. (2007) and Boldajipour et al. (2008) showed that this migration of germ cells involves a chemokinetic response to CXCL12, mediated by CXCR4, that leads to the migration of germ cells. Directionality of the migration is provided by trailing cells that express the second CXCL12 receptor, CXCR7, which prevents backward migration by depleting CXCL12, in the rear of the migrating group of cells. Consistent with this, Mazzinghi and colleagues reported that human renal progenitor cells use both CXCR4 and CXCR7 receptors for transendothelial migration, but that CXCR7 is a major contributor for cell adhesion to endothelial cells and progenitor cell survival (Mazzinghi et al., 2008). CXCR7-

mediated or -enhanced adhesiveness is also clearly established in prostate cancer cells, together with improved cell survival and invasiveness (J. Wang et al., 2008). In the cases reported above, the contribution of CXCR7 to the physiological responses could be chemokine interception and termination of the subsequent migratory response or at least its modulation. CXCR7 indeed shows significantly higher capacity than CXCR4 to increase cell-association of CXCL12 (K. Luker et al., 2009 b).

Another probable physiological function of CXCR7 is related to its capacity to heterodimerize (Levoye et al., 2009; Sierro et al., 2007), as can be detected by bioluminescence- or fluorescence- energy transfer in heterologous expression systems. In the more recent work by Levoye and colleagues (2009), CXCR7 is shown to exhibit an apparent paradoxical effect interfering with CXCR4 responses to CXCL12. CXCR7 indeed reduces responses to low CXCL12 concentrations while leaving responses to high concentrations unchanged, as compared to CXCR4 alone. This effect is also detected in isolated human T lymphocytes. The presence of CXCR7 in cells expressing CXCR4 is to render dose-response relationships steeper than expected from the law of mass action. The net result of that interference is a conversion of CXCR4 responses to CXCL12 into an almost all or nothing type of response (Levoye et al., 2009; Sierro et al., 2007) with triggering versus non-triggering CXCL12 doses differing by only three to five fold. This physiological effect supports the concept open by the structural demonstrations of CXCR7 heterodimerization. Modelling of the behaviour of CXCR7 as a chemokine scavenger could be of interest to discriminate among direct receptor-receptor interactions and the indirect effects of CXCR7 regulating the level of chemokine that would be available to CXCR4.

2.2 Avoidance strategies: examples of neutralizing molecules produced by pathogens.

Pathogenic viruses, bacteria or parasites, have set up several strategies to escape host detection and defence systems. They use cytokine or chemokine signalling molecules (receptors and ligands) to infect host cells (Chitnis & Sharma, 2008; Hughes & Nelson, 2009). They also block cytokine signalling by producing antagonists (Damon et al., 1998) which allow to escape alert systems or to redirect them to their own benefit (Alcami, 2003; Andreasen & Carbonetti, 2008; Mantovani et al., 2006; McFadden et al., 1998; Rosenkilde, 2005; Sozzani et al., 1998). The parasite *Leishmania* infecting macrophages, for example, expresses functional chemokine receptors. These are used as chemoreceptors to promote

chemokinesis toward chemokine-producing macrophages. This leads to an efficient *Leishmania* internalization (Roychoudhury et al., 2006) that takes place before an efficacious immune response is set up to clear the pathogen. A second example is taken from the bacterium *Bordetella pertussis*. The pathogen is reported to delay neutrophil recruitment, by slowing down chemokine production by the host (Andreasen & Carbonetti, 2008), through production of *pertussis* toxin the well known inhibitor of Gi protein mediated signalling. Also, as a third illustration, human herpesvirus 6 (HHV-6) produces a chemokine, U83A, that binds to CCR5 to modify its internalisation-recycling fate (Catusse et al., 2009; Catusse et al., 2007). Indeed, at variance to other CCR5 chemokines, U83A is a CCR5 agonist that does not drive the receptor towards a clathrin-mediated endocytosis but to a delayed and long lasting caveolin-linked pathway. Combined to the fact that U83A is not recognized by DARC and D6, the viral chemokine thus facilitates clearance of all other CCR5 chemokines which can no longer activate the receptor but remain capable of being trapped by interceptors.

Relevant to the present article are the neutralizing molecules produced by viruses and multicellular pathogens that are used to neutralize the immune response of the host. Several articles and reviews describe the production by viruses, of soluble proteins able to bind chemokines sometimes simultaneously with cytokines such as interferon gamma, interleukine-1 β or tumor necrosis factor α (Alcami, 2003; Mantovani et al., 2006; McFadden et al., 1998; P. M. Murphy, 2000; Rosenkilde, 2005), all of which are implicated in the host immune response to pathogens. Two major mechanisms of action are depicted: the inhibition of cytokine – cytokine receptor interaction and the inhibition of cytokine – extracellular matrix interaction (McFadden et al., 1998), both of which being associated with improvement of cytokine clearance by elimination and/or degradation. The biologically active scavenging molecule can be a soluble protein, often mimicking the extracellular binding domain of the host cytokine receptor. It may also be a membrane-bound protein, like the decoy receptors mimicking the chemokines receptors which do not contain any soluble portions.

Representatives of soluble proteins that inhibit the interaction between chemokines and glycosaminoglycans (GAGs) from the extracellular matrix are M-T1 and M-T7 produced by the rabbit-infecting *myxoma* virus. M-T7 binds interferon gamma together with chemokines from the CC-, CXC- and C- groups (Lalani et al., 1997). The herpes virus homodimeric protein

M3 and the glycoprotein G also belong to the group of soluble proteins inhibiting chemokine binding to GAGs (Martin et al., 2006; van Berkel et al., 2000). All exhibit original tridimensional structures that do not resemble chemokine receptors. The mechanisms by which they neutralize the immune system may be two-fold. On the one hand, GAGs are well known to contribute to the setting up and maintenance of chemokine gradients close to their sites of production. The inhibition of chemokines binding to GAGs might thus result in chemokine gradient collapses. Altered immune response that could derive from that could be attenuation of signalling intensity or unsuited, or even absence of, leukocyte targeting (T. N. Wells et al., 2006). On the other hand, the large size of soluble chemokine binding proteins could hinder the interaction with the chemokine receptor. Thus, although the targeted domain of the chemokine is the GAG binding domain, the remainder of the large soluble protein might simultaneously prevent interactions with the cognate chemokine receptors. The use of small molecules mimicking the effects of GAG binding proteins would help to determine the mechanism of action likely to take place.

Another example of soluble proteins produced by parasites is highlighted by recent research developments. Ticks are bloodsucking parasites that transmit the spirochete *Borrelia burgdorferi* responsible for Lyme disease (Behera et al., 2006; Deruaz et al., 2008; Guerau-de-Arellano & Huber, 2005; Hajnicka et al., 2001; Hirschfeld et al., 1999; Vancova et al., 2007). In order to survive, ticks attach and remain feeding on the host for several days-weeks. A particularity of the host-parasite interaction is the absence of an inflammatory response to ticks. This was investigated by several groups who realized that the parasite produces anti-haemostatic, anti-inflammatory and immunomodulatory substances, and secretes them in the host (Valenzuela et al., 2000; Waxman et al., 1990). Anti-chemokine molecules acting against CXCL8 (Hajnicka et al., 2001), CCL2, CCL3, CCL5 and CXCL11 (Vancova et al., 2007) were detected although their identity was not elucidated. Using an expression cloning strategy, the group of Proudfoot identified a family of small proteins, the evasins, that similar to soluble viral chemokine binding proteins, recognize and bind chemokines with various degrees of selectivity, and intercept their signalling to the host immune and anti-inflammatory systems (Deruaz et al., 2008; Frauenschuh et al., 2007). Three identified evasins bind CC (evasins-1 and 4) and CXC (evasin-3) chemokines. The fourth one, evasin-2, is still without a known ligand. The interest in these small proteins resides in

their extreme efficacy to delude the immune system, and to their very small size (60-70 amino acids) that inspires searches for chemokine neutralizing motifs with the potential to become drugs.

The second general mode of action of pathogens is reminiscent of intercepting receptors described above. Human and mouse cytomegalovirus, Kaposi-associated herpesvirus and capripoxvirus produce seven transmembrane segment proteins (ORF74, US28, M33, Q2/3L) which are analogous to G protein-coupled receptors (Alcami, 2003; Rosenkilde, 2005). These proteins are expressed at the surface of infected cells and act, similarly to DARC or CCX-CKR, as decoy proteins that internalize chemokines and drive them towards degradation.

3. Potential therapeutic interest of soluble decoy proteins

Soon after the discovery of 50 different chemokines, the number of receptors grew to 20 members, all belonging to the G protein-coupled receptor family for which it should be noted that one given chemokine may activate several receptor subtypes. The chemokine CXCL8 for instance activates two receptors (CXCR1 and CXCR2) and the chemokine CCL5 activates three receptors (CCR1, CCR3, CCR5). On the other hand, a large number of chemokines may activate a single receptor subtype. This is the case for CXCR2, which is activated by CXCL-1,-2,-3,-5,- 6,- 7 and -8, for CCR5 that is activated by CCL-3, -4, -5, -6, -8, -12, as well as for many other receptors (CXCR3, CCR1, CCR2, CCR3) reviewed in (T. N. Wells et al., 2006). The question then arose as to which receptor and which chemokine should be targeted to decipher physiological signalling pathways and predict therapeutic approaches for disease treatment. Many research groups could help solving this problem by showing that despite chemokines and cytokines cooperate to increase inflammatory responses, knock out or neutralization of one chemokine or chemokine receptor will induce significant attenuation of inflammation (see table 1). Several chemokine gene disruptions result in a clear effect, as for instance knock out of CCL3 that reduces the inflammatory response to viruses such as influenza A and cytomegalovirus (Salazar-Mather et al., 1998). Similarly, the knock out approach indicates the importance of CCL2 and its receptor in chemoattraction of neural progenitors to inflamed neural sites (Belmadani et al., 2006), that

of CCL5 in glial cell activation (El-Hage et al., 2008), of CCL21 in thymus development (C. Liu et al., 2005) or that of CXCL12 in haematopoiesis (Nagasawa et al., 1996). Very convincing results are also obtained by using proteins or antibodies neutralizing the chemokine ligand. The Lucas and Mc Fadden groups have exploited the neutralizing effect of the *myxoma* virus M-T7 soluble protein to reduce post-operative responses in murine models of tissue engrafting (Bedard et al., 2003; L. Liu et al., 2004; L. Liu et al., 2000). They show that intravenous injection of M-T7 protein, that binds all types of chemokines (see § 2.2) in rats after angioplasty-induced injury diminishes atherosclerosis and restenosis (L. Liu et al., 2000). This is in good agreement with the phenotype of CCL2^{-/-} mice (Gosling et al., 1999), and with the reported prevention of renal allograft rejection (Bedard et al., 2003) or reduction of aortic allograft vasculopathy through inhibition of chemokine mediated responses (L. Liu et al., 2004).

Finally, the newly identified evasin proteins from ticks also display potent anti-inflammatory properties *in vivo* in animal models (Deruaz et al., 2008). Evasin-1, which binds CCL3 and CCL4, significantly attenuates recruitment of pro inflammatory cells in phorbol ester-inflamed skin of D6^{-/-} mice and fibrosis in bleomycin-induced lung injury. Evasin-3 recognizes CXCL8 and its mouse homolog KC, as well as CXCL11. It inhibits neutrophil chemotaxis *in vitro*, as well as neutrophil recruitment to the peritoneal cavity in mice in a model of BSA-induced arthritis.

4. Validation of chemokines in signalling pathways and pathology: Importance of anti-chemokine antibodies

Chemokines are directly implicated in many physiological processes including surveillance of organism integrity, elimination of damaged cells and tissues or host defence against pathogens. To this end, they recruit the most adapted cell types on the site where intervention is needed, and promote a controlled reaction generally associated with limited inflammation. Under abnormal conditions, the inflammatory response escapes control, thus leading to pathological states such as inflammatory bowel disease, multiple sclerosis, and probably Alzheimer's disease, among others. In this case, abnormally elevated levels of chemokines, or over-expression of their receptors, lead to permanent recruitment of

immune cells and to tissue damage. In a comparable manner, abnormal recognition of antigen initiates autoimmune diseases (myasthenia gravis, lupus erythematosus, Type I diabetes, rheumatoid arthritis...), where abnormally elevated levels of chemokines are detected (Kong et al., 2009; Matin et al., 2002; A. Wang et al., 2009). The cytokine and chemokine systems are also used by cancer cells to promote cell proliferation, tumor survival and neovascularization, or to establish metastases at distant but non random places (Vandercappellen et al., 2008). Chemokines also contribute to tissue development (Mahabaleshwar et al., 2008; Nagasawa et al., 1996; Raz & Mahabaleshwar, 2009) by forming gradients of morphogens for migrating cells.

Table 1 summarizes all efforts made to investigate the role of the various chemokines, using approaches targeting the chemokine as directly as possible. The two major approaches, namely gene disruption and anti-chemokine antibodies, do generally lead to convergent observations, although the same phenomenon has only rarely been studied using the two approaches.

Deletion of one of the CCL3 and CCL5 receptors, the CCR5 receptor (CCR5 Δ 32 allele found in humans) is associated with protection from HIV infection in humans (Kindberg et al., 2008; Lim et al., 2008; Samson et al., 1996) while deletion of a second receptor, the CCR1 receptor, results in protection from an excessive response to systemic inflammation in mouse models (Gerard et al., 1997). Deletion of the CCR1 and CCR5 ligand, CCL3 (MIP-1 α), results in a weaker inflammatory responses to viral pathogens (Cook et al., 1995), and deletion of CCL5 (RANTES) to a reduced glial cell inflammatory response (El-Hage et al., 2008). Therefore, the absence of perfect matching between chemokines and their receptors is a cause of difficulties encountered when signalling pathways are to be traced, and molecules targeting the function of one receptor do not systematically match the effects of molecules targeting the ligand (Horuk, 2009).

Rather than knocking out chemokine or chemokine receptor genes, neutralizing antibodies, which are rapidly obtained, have been very useful in particular for chemokines. Chemokines are small proteins with a highly stable structure (Figure 3), which renders them amenable to the development of neutralizing antibodies (Table 1).

In the case of CCL5 for instance, neutralizing antibodies allow the functional role of this chemokine in autocrine proliferation of leukemia cells (Aldinucci et al., 2008; Boring et al., 1998; Izikson et al., 2000) to be demonstrated as well as neuroinflammation in models of

multiple sclerosis (Glass et al., 2004). It is interesting to note that although CCL5 binds to the same subset of chemokine receptors as CCL3, the anti-CCL5 neutralizing effect is specific because CCL3 is expressed in other cell types.

Anti-CCL1 antibodies have been used to demonstrate the contribution of CCL1/CCR8 autocrine activation of peritoneal macrophages in the formation of peritoneal adhesion, which constitutes complications in visceral surgery and inflammation (Hoshino et al., 2007).

Also antibodies to CXCL8, CCL2 or CCL5 block the antiapoptotic and proliferative effects of the corresponding tumor-derived cell lines obtained from lung, melanoma, breast ovarian or leukemia cancers (Levina et al., 2008)

Besides their functions in the immune system, the role of chemokines in cancer initiation and progression as well as in tumor survival has been confirmed with neutralizing antibody strategies. Hence, antibodies against chemokines or chemokine receptors block tumor growth and/or migration as well as invasiveness. Various examples may be given, like i) anti-CXCL12/CXCR4 antibodies in ovarian and breast cancer (Kwong et al., 2009; Muller et al., 2001; Scotton et al., 2002), ii) anti-CXCL1 and anti-CXCL2/CXCR2 in lung cancer (B. Wang et al., 2006), iii) anti-CXCL13/CXCR5 in cell lines from pancreatic or colon cancers (Meijer et al., 2006), iv) anti-CCL2/CCR2 and -CCL5/CCR5 (Vaday et al., 2006), and -CCL11/CCR2 in ovarian cancer (Levina et al., 2009), and v) anti-CCL21/CCR7 in thyroid tumor cells (Sancho et al., 2006).

5. Neutralizing cytokines and chemokines with small chemical compounds

The study of protein-protein interactions is important to understand major regulatory pathways, especially in the intracellular compartment, which is not reached by neutralizing antibodies. The difficulty associated with the study of protein-protein interactions is not only that most of the time, interacting partners are both intracellular, and thus not easily accessible for biophysical or pharmacological manipulations, but also that protein-protein interactions generally involve contact areas that are much larger than small molecules. These contact areas, in addition, are quite featureless in terms of the number of attachment

points that can be exploited by medicinal chemists to develop small molecules with high affinity. It follows that small molecules at most bind with modest affinities and frequently hardly compete efficiently to inhibit the interaction between two proteins. Still, favourable cases exist in which neutralization of a protein function can be obtained with a small molecule (M. Arkin, 2005; M. Arkin & Moasser, 2008; M. R. Arkin & Wells, 2004; Blazer & Neubig, 2009). When the approach works, questions related to the mode of action of the small molecule must be addressed, in order to generalize the principles and extend the approach to other specific cases. The different mechanisms of ligand neutralization are numerous and diverse. In terms of chemical biology, the aim thus being to develop small chemical molecules blocking the function of the protein ligand, we shall not discuss molecules that inhibit synthesis, maturation or release of the protein ligand, nor molecules that modulate its catabolism, already reviewed elsewhere (Foxwell et al., 2003; Mortier et al., 2008; Vergote et al., 2006). Rather we will focus on small organic molecules that bind to the protein ligand and prevent its signalling.

Four main modes of action are encountered (Figure 4):

- i) The small molecule competitively binds to the same site as the receptor;
- ii) The small molecule alters the quaternary structure of the protein ligand;
- iii) The protein ligand undergoes structural changes that regulate its activity: the small molecule alters tertiary structure of the protein ligand;
- iv) The small molecule interferes with ligand bioavailability.

These different modes of chemokine / cytokine neutralization will now be illustrated, and the methods to identify them discussed. These mechanisms of action have been validated, (M. Arkin, 2005; M. R. Arkin & Wells, 2004; Berg, 2003; Blazer & Neubig, 2009) and may be extended to chemokine neutralization.

5.1. The small molecule competitively binds to the same site as the receptor

Based upon structure-function relationship studies, many examples of peptides mimicking receptor domains and acting as inhibitors are available. In the family of chemokines and their receptors, the importance of the extracellular parts of the chemokine receptor (Duma et al., 2007; Zoffmann et al., 2002), and in particular of its amino-terminal domain, for ligand-receptor interactions has been extensively documented (Bannert et al., 2001;

Blanpain et al., 1999; Fong et al., 2002; Gayle et al., 1993; Monteclaro & Charo, 1996, 1997; Pease et al., 1998; Prado et al., 2007; Rajagopalan & Rajarathnam, 2004; Veldkamp et al., 2008; Ye et al., 2000). This has led to the identification of peptide fragments capable of binding to the chemokine in a manner thought to mimic the mode of interaction of the chemokine with the receptor. Peptide fragments corresponding to the 1-35 N-terminal residues from CCR3 (Mayer & Stone, 2000; Ye et al., 2000), 1-40 from CXCR1 (Clubb et al., 1994) or 2-19 from CX3CR1 (Kokkoli et al., 2005; Mizoue et al., 1999) interact with CCL24, CXCL8 and CX3CL1, respectively, with millimolar to micromolar affinities. In all three examples, the interaction area of the chemokine is located in the helical turn of the N-loop and the β 1- β 2 and β 2- β 3 hairpin domains, in regions of greatest flexibility and structural variability of the chemokine (Mizoue et al., 1999). These peptides have not been further used to investigate the *in vitro* or *in vivo* functions of chemokines. Their discovery however suggested that neutralizing antibodies-based approaches (table 1) are not the only possible tools with which to inhibit chemokine functions, and paved the way to find high affinity peptides capable of neutralizing chemokines. One approach starts from natural peptides targeting chemokines as illustrated by the work on evasins (Deruaz et al., 2008) described above. Although peptidic in nature, evasins are not immunogenic, at least when secreted by feeding ticks. They are thus expected to represent valuable scaffolds to study, to make analogs and to use as non peptidic drugs. Another approach consists in identifying new chemokine binding molecules from collections of peptides and peptidomimetics (J. A. Burger & Peled, 2009). Such peptides or peptidomimetics may either exhibit selectivity towards a single chemokine or, in contrast, poorly discriminate among chemokines such as CXCL9, CCL2, CXCL8, CXCL12 or CCL11 (Peled, A., Eizenberg, O. Vaizel-Ohayon, D. US patent 7488717). The anti-CXCL12 peptide, BKT 140, identified by surface plasmon resonance and ELISA, is currently in clinical phase I for neutropenia and anemia.

Chemokine neutralizing ligands may also not be peptidic at all. A screening campaign of an academic library of small molecules (Boeglin et al., 2007; Hibert, 2009) was designed, using a fluorescence resonance energy transfer assay (Valenzuela-Fernandez et al., 2001; Vollmer et al., 1999) in order to identify inhibitors of CXCL12 - CXCR4 interactions. A chalcone molecule (4'-phenyl, 3-methoxy, 4-hydroxy chalcone) was found to be very effective ($K_i = 50$ nM) at inhibiting CXCL12 binding to CXCR4 and CXCR7, and signalling through CXCR4, including chemotaxis *in vitro* and *in vivo* (Hachet-Haas et al., 2008). The molecule however was unable

to block cell fusion in an *in vitro* model (Chanel et al., 2002) of HIV entry. The proposed model of chalcone binding to the chemokine rather than to its receptor could be demonstrated using tryptophan fluorescence and microcalorimetry. This was reminiscent of earlier studies describing a natural derivative of chalcones, the flavone baicalin, isolated from *Scutellaria baicalinensis*, which binds to the chemokines CXCL8, CXCL12, CCL4 and CCL8, although affinities were about four orders of magnitude lower (Li et al., 2000). The compound 4'-phenyl, 3-methoxy, 4-hydroxy chalcone, modestly inhibits signalling through CXCL8, a chemokine from the same structural subgroup as CXCL12, but is not active on CCL5, at least not in the micromolar concentration range. Finally, the chalcone compound shows efficacy, *in vivo*, in a mouse model of allergic hypereosinophilic airway inflammation where it is as powerful as neutralizing antibodies to either CXCL12 or CXCR4 (Hachet-Haas et al., 2008). Although the structure of the chemokine-chalcone has not been solved, molecular modelling and preliminary NMR data (C. Veldkamp, Milwaukee University, personal communication) support the idea that the chalcone binds to the same chemokine area as do chemokine receptor-derived peptides, i.e. in the groove delineated by the N-loop hairpin and β -strands 2 and 3 of the chemokine (Figure 5). Yet, whether chalcone perturbs the state of chemokine oligomerization remains an open question. Other chalcone molecules are reported to act as inhibitors of allergic inflammatory diseases (Meng et al., 2007). The question as to whether the chalcone backbone acts as a chemical platform for biologically active molecules is open, and the mechanism of action of the molecules *in vivo* might rely on multiple interactions with different target proteins.

The example of the IL-2 neutralizing small molecules, SP4206 and SP4160, illustrates that flexible regions of small protein ligands can be targeted by high affinity molecules binding at protein-protein interfaces that are poorly druggable, but can adjust their structure to accommodate the ligand (M. R. Arkin & Wells, 2004; Thanos et al., 2006). The IL-2 receptor mediates T-helper cell maturation and is a drug target for transplant rejection (Berard et al., 1999; Waldmann & O'Shea, 1998) and autoimmune diseases (Schippling & Martin, 2008). Its ligand, interleukin-2 is a 15 kDa four helix bundle protein that promotes T cell growth. In a drug design program aiming at mimicking the IL-2 part that binds to the IL-2R, a peptidomimetic molecule, Ro26-4550, was discovered (Tilley et al., 1997). The molecule could inhibit IL-2 binding to its receptor with micromolar affinity, and careful

characterization of the mode of action led to the identification of the interleukin-2 itself being the receptor for Ro26-4550, not the IL-2R receptor protein (Emerson et al., 2003). Detailed structural analysis of Ro26-4550 interaction with IL-2, in particular using X-ray crystallography and NMR (M. R. Arkin et al., 2003) described in detail the binding area. The small chemical compound binds to a pocket that does not pre-exist as such in its absence. In other words, the binding area is the complex result of the adaptation of the local protein folds around the chemical. This particular case illustrates the difficulty to use the structure of the "empty" binding site, *a priori*, to predict Ro26-4550 structure or binding. However, optimization of the compounds, using X-ray crystallography of complexes, fragment-based approaches and tethering techniques, allowed the affinity of the IL-2 binding molecules to be lower to a few tens of nanomolar (Raimundo et al., 2004; Thanos et al., 2006).

Chemokines are present in the brain in glial cells as well as in neurons (Rostene et al., 2007). Concordant with their known role in the periphery, chemokines are upregulated during inflammation, and contribute to brain immunity and neuroprotection (Cartier et al., 2005; Glass et al., 2005; Madrigal et al., 2009; Omari et al., 2009). They are also involved in progenitor cell migration towards brain tumors (Magge et al., 2009), and participate in brain development and neuronal cell differentiation (Park et al., 2009; Zou et al., 1998). They are even distributed in neurons as neuromodulators are, and modify release of neurotransmitters or neuropeptides (Rostene et al., 2007). These findings, together with the development of pharmacological tools for chemokine receptors offers the possibility to better study chemokine signalling in the brain. Not only agonists or antagonists of the receptors, but also small neutralizing molecules directed against chemokines could be extremely valuable, in particular if they cross the blood brain barrier (BBB) to reach their target cytokines. One example is the conversion of the chemokine CXCL12, an agonist of CXCR4 and ligand of CXCR7, into an antagonist of CXCR3 (Vergote et al., 2006). The chalcone molecule mentioned above is probably a good pharmacological agent to study neuronal toxicity associated with CXCL12 catabolism.

Another application for potential neutralizing ligands is exemplified by the effect of anti-CCL5 antibodies. They are administered to fight the leukocyte infiltration that takes place in the brain as a result of virus-driven CCL5 production in mice. This infiltration is followed by destruction of myelin and appearance of neurological impairment (Glass et al., 2004).

Antibodies to CCL5 significantly decrease macrophage accumulation in the brain and demyelination. CCL5 neutraligands should thus prove useful to investigate the incidence of CCL5 in multiple sclerosis.

5.2. The small molecule alters quaternary structure of the protein ligand

Chemokines show a natural propensity to form dimers or oligomers depending on medium composition (Veldkamp et al., 2005), biochemical environment in particular glycosaminoglycans (Crown et al., 2006), or interaction with chemokine receptors (Veldkamp et al., 2008). Homodimerization / oligomerization is well established for CC chemokines such as CCL2, CCL4, CCL5 and CCL14 (Blain et al., 2007; H. Jin et al., 2007; Proudfoot et al., 2003), and heterodimerization is also experimentally supported for instance in CCL2-CCL13, CCL2-CCL11 or CCL8-CCL13 heterodimers (Crown et al., 2006). Up to now, the functional consequences of these homologous or heterologous interactions have not been well understood, but the oligomerization process may be interesting to interfere with when attempting to neutralize chemokine actions (Figure 4). Indeed, CC chemokine dimers do not correspond to the quaternary structure that interacts with the receptor, because residues critical for receptor binding are buried in the dimer (H. Jin et al., 2007), and mutations can be made to abolish dimerization (H. Jin et al., 2007; Proudfoot et al., 2003). In the case of CCL2, it could be demonstrated that certain responses, in particular leukocyte attraction *in vivo*, was abolished. This selective suppression of certain responses which leaves other responses unaffected is reminiscent of previously described multiple active states of G protein-coupled receptors (Palanche et al., 2001) that can be differentially activated by distinct agonists, or modulated by allosteric effectors (Maillet et al., 2007). If prevention of dimer formation is *per se* sufficient to block chemoattraction *in vivo*, then the mechanism of action of the interceptor M3 (from herpesvirus) is probably exquisitely optimized. Decoy receptor M3 (vCKBP3) binds two monomers of CCL2 per M3 homodimer (Alexander et al., 2002). Thus, even though M3 recognizes and binds the GAG binding domain of CCL2, its aptitude to "dissolve" dimers is thus likely to represent a mechanism of inhibition of CCL2 signalling *in vivo* (Handel et al., 2008).

In the case of CXC chemokines, dimerization also takes place spontaneously, but the dimer structure differs from that of CC chemokine dimers in that residues important for receptor interaction are not buried in the dimer. CXC chemokines can thus interact with their

receptors either as monomers or as dimers/oligomers. At variance with what happens when CC chemokines interact with their receptors, it was demonstrated that a CXC chemokine receptor may itself be responsible for chemokine dimerization (Veldkamp et al., 2008). Tyrosine sulfation of the N-terminal domain of CXCR4 on tyrosines 7 and 12 is indeed a key determinant of receptor-mediated dimer formation since sulfated tyrosine 7 interacts with a CXCL12 monomer, while sulfated tyrosine 12 interacts with the second monomer. Two lines of evidence support the importance of CXCL12 dimerization in signalling: i) aminoacids from CXCL12 are involved both in the interaction with heparan sulfates and with CXCR4; heparan sulfates can thus negatively affect CXCL12-evoked chemotaxis *in vitro* (J. W. Murphy et al., 2007), and ii) tethering CXCL12 monomers to obtain permanent CXCL12 dimers results in a partial loss of function of CXCL12. The permanent dimer indeed shows unaltered capacity to promote intracellular calcium elevation in CXCR4 expressing cells, but is no longer capable of triggering *in vitro* chemotaxis (Veldkamp et al., 2008). Although these results are not yet totally interpreted in terms of structure-function relationships, modulation of chemokine oligomerization represents a promising way to change cellular responses with possible important consequences *in vivo*. The unsaturated heparin disaccharide used to perturb CXCL12 dimer structure (J. W. Murphy et al., 2007) is a plausible starting chemical platform to exploit. Virtual and experimental screening of collections of molecules on chemokine dimers could lead to the discovery of neutralizing molecules, the mechanism of action of which would be prevention of dimer formation. The critical step in this kind of project would be the definition of primary and secondary assays allowing qualitative and quantitative description of the new compound effects.

TNF α is produced in response to pathogens through toll-like receptor activation, and promotes expression of many immune system effectors, including cytokines and chemokines (Balkwill, 2009) that will recruit leukocytes to the site of inflammation. If production of TNF α is excessive, chronic inflammation can develop as in rheumatoid arthritis, Crohn's disease, severe asthma or psoriasis, all diseases in which a prominent role of TNF α has been demonstrated. Anti-inflammatory therapies have been developed, based on inhibition of either the production of TNF α or the neutralization of TNF α itself (Foxwell et al., 2003). The neutralizing monoclonal antibodies, etanercept, infliximab and adalimumab have led to successful treatment in rheumatoid arthritis, and show that neutralizing antibodies can prove valuable not only in acute, but also in chronic human diseases. Yet, two problems

remain after several runs of antibodies optimization for human use: the mode of administration associated with dosing difficulties, and the elevated cost of treatment have motivated the search for alternative therapeutic tools. In the search for small molecules from collections of combinatorial fragments capable of inhibiting TNF α binding to its TNF-R1 receptor, He and colleagues (He et al., 2005) discovered a small molecule inhibitor exhibiting a micromolar affinity constant. When trying to identify by X-ray crystallography the binding site of the small molecule on the large trimeric structure of TNF α , the authors realized that soaking TNF α crystals led to their destruction. Crystallization of the complex was then obtained. It revealed that the mode of action of the small molecule antagonist of TNF α is to dissociate its trimeric quaternary structure into inactive inhibitor-bound dimers with a stoichiometry of one molecule *per* dimer (Berg, 2006; He et al., 2005). This illustrates one of the modes of action of small molecule inhibitors that, following description of CCL2 monomer failure to signal (Handel et al., 2008), could be applied to the family of chemokine proteins, using detection of quaternary structure as a primary screen.

5.3. The protein ligand undergoes tertiary structural changes that regulate its activity and the small molecule prevents the active conformer

Soluble proteins may undergo three dimensional structural changes, either spontaneously, in a regulated manner (Monod et al., 1965) or as a mechanism of pathogenesis (Dobson, 1999). Lymphotoxin is a chemokine with unusual properties. First, in contrast to most chemokines its structure is stabilized by a single disulfide bridge. Second, it has the singular property of existing as two unrelated protein folds (Kuloglu et al., 2002; Tuinstra et al., 2008). One of the two protein folds resembles the canonical chemokine structure with 3 anti-parallel beta strands and one carboxy terminal helix. This conformation binds to and activates its receptor XCR1 but does not interact with glycosaminoglycans. The second protein fold exhibits 4 anti-parallel beta strands but has no helix (Tuinstra et al., 2008). This second structure binds glycosaminoglycans with high affinity but is unable to activate the XCR1 receptor. As this example is unique to date in the field of proteins and protein ligands, its generality is questionable. It is an extreme case of the general field of change in protein conformational equilibrium, which has been tackled by many different laboratories on many different regulatory proteins to select new pharmacological tools and active drugs. Ligands

that would stabilize the glycosaminoglycan binding state could reveal neutralizing molecules capable of modulating acute allograft rejection response (J. D. Wang et al., 1998) or attenuate inflammatory bowel disease (Boismenu et al., 1996; Middel et al., 2001), as two examples of mucosal immunity in which lymphotactin is involved.

5.4. The small molecule interferes with ligand bioavailability

Chemokines activate G-protein coupled receptors to recruit leukocytes during organogenesis, immunosurveillance, and inflammation. An important component of this process is the formation of a chemotactic gradient by immobilization of chemokines on the extracellular matrix of cells, in particular on glycosaminoglycans. Analysis of the role played by glycosaminoglycans has been carried out using mutants of CC (Ali et al., 2005; H. Jin et al., 2007; Johnson et al., 2004; Proudfoot et al., 2003) or CXC / XCL (Peterson et al., 2004; Sadir et al., 2004) chemokines devoid of key residues known to bind to negatively charged sugar moieties. Suppression of GAG interactions for CCL2, CCL4, CCL5, CCL7 or XCL1 chemokines was found to abolish leukocyte recruitment *in vivo*, when injected intraperitoneally, although *in vitro* chemotaxis was not altered.

The importance of these interactions has been further highlighted by showing that GAG-binding mutants of chemokines can block the action of wild type chemokines in normal animals as well as in murine models of diseases (Ali et al., 2005; Braunersreuther et al., 2008; Johnson et al., 2004). Pathogens also target GAG-binding domains of chemokines to prevent their effects. As mentioned above (§2.1, avoidance strategies), the poorly selective M-T1 and M-T7 proteins from *myxoma* virus, the M3 protein from *herpes* virus or glycoprotein E163 from *ectromelia* virus neutralize chemokines upon binding to their GAG binding domain. The likely consequence of this is that high local concentrations of chemokines giving rise to a "Velcro effect" are reduced, and gradients are disrupted. Therefore, leukocyte attraction no longer takes place. Although no small chemical molecule targeting the GAG binding domain of chemokines has been described up to now, this portion of the protein is validated for chemical biology approaches. Noteworthy are the disaccharides that were used to solve the structure of CCL5 forming complexes with heparin-derived sugars (Shaw et al., 2004) that could be used as starting blocks for drug design. Interestingly too is the small molecule surfen, that was first used as an excipient in drug formulas before being also identified as a heparin neutralizing molecule (Hunter & Hill, 1961)

with antibacterial and trypanocidal activity, which could be used to lower excessive heparan sulfate-involving interactions (Schuksz et al., 2008).

6. Concluding remarks and perspectives

Many articles now report that either genetic manipulations of receptors and chemokines, or development of pharmacological agents leads to selective alterations of a subset of responses out of a series of possible responses. One consequence is that, depending on the desired properties of the molecule to be developed for research or disease treatment, preference for a ligand of receptor or a ligand of ligand will need to be validated experimentally. Indeed, chemokines activate members of the family of G protein-coupled receptors (GPCRs) known to exhibit a significant level (around 10%) of spontaneous isomerization towards active conformations (Alewijse et al., 2000; Claeysen et al., 2000; Lecat et al., 2002; Lefkowitz, Cotecchia, Kjelsberg et al., 1993; Lefkowitz, Cotecchia, Samama et al., 1993; Leurs et al., 2000; Palanche et al., 2001). Spontaneous activity of GPCRs has well established physiological roles (Adan, 2006; Arrang et al., 2007) like control of neurotransmitter (Threlfell et al., 2008) and hormone (Ben-Shlomo et al., 2009) release in relation with higher order behaviors (Fioravanti et al., 2008), or regulation of apoptosis (Lau et al., 2009). Spontaneous activity of chemokine receptors also exists. For herpesvirus-encoded receptors this activity is linked to transforming effects (M. Burger et al., 1999; Holst et al., 2001). Ligands of GPCRs almost never behave as neutral molecules, and most antagonists are either weak partial agonists (that increase the level of receptor activity) or are so called "inverse agonists" capable of diminishing the level of spontaneous activity of the receptor. Small molecules neutralizing ligands may complement the tool palette of pharmacologists since they are expected to leave the spontaneous activity of the receptors unchanged.

There are four preferred ways to identify small molecules modulating protein-protein interactions, ELISA assays, fluorescence resonance energy transfer (FRET) assays, fluorescence anisotropy (Berg, 2003) and surface plasmon resonance. We have designed a general strategy in order to find fluorescent probes that bind to a soluble protein. This fluorescence anisotropy strategy involves four steps:

- 1) Synthesis of a library of fluorescent compounds using known chemical scaffolds that exhibit low specificity. The molecules are organized around generic GPCR-preferring chemical scaffolds, are derivatized with charged, hydrophilic and hydrophobic moieties and bear a lissamine fluorophore at the end of a spacing arm,
- 2) Screening of the library of fluorescent molecules by fluorescence anisotropy measurements in order to successfully fish one fluorescent probe at least. This technique allows to set up a “mix and read” assay that readily pinpoints the probe that interacts with the protein,
- 3) Characterization of the binding properties of the probe in order to fulfil the desired requirements, i.e neutralize binding of the ligand to its receptor, or inhibit oligomerization, or inhibit binding to GAGs, and select the desired and optimized screening assay,
- 4) Screening of libraries of unlabelled and drug-like molecules to identify high affinity molecules to use for chemical biology purposes.

The generalization of such kinds of drug discovery approaches in particular in university laboratories, is highly desirable. The increasing amount of screening data will then lead to large scale chemoinformatics and bioinformatics including data from transcriptional analyses and proteomics (Schadt et al., 2009; Weill & Rognan, 2009), development of complex network modelling as is the case in systems biology in order to establish training sets for interaction networks prediction, and to use these models to predict molecule toxicity and metabolism, bioavailability and patterns of biological activity.

Acknowledgements: the authors wish to thank the Centre National de la Recherche Scientifique (CNRS), the Institut National de la Santé et de la Recherche Médicale (INSERM), the Université de Strasbourg, the Agence Nationale de la Recherche sur le SIDA (ANRS), Sidaction, The Agence Nationale de la Recherche (ANR).

Table 1

Chemokine/ chemokine receptor	biological tool	effect	reference
CCL1 (I-309)	CCR8	Anti CCL1	Post operational peritoneal adhesions (Hoshino et al., 2007)
CCL2 (MCP-1)	CCR2	Anti-CCL2	- prostate cancer growth inhibition - infectious keratitis - atherosclerosis (X. Li et al., 2009; Loberg et al., 2007) (Xue et al., 2007) (Lutgens et al., 2005)
		CCR2 Knock out	- atherosclerosis / Multiple sclerosis (Boring et al., 1998; Izikson et al., 2000)
		CCL2 knock out	- age-related macular degeneration / neuroinflammation - sepsis - atherosclerosis (Belmadani et al., 2006; Ross et al., 2008) (Lu et al., 1998) (Gu et al., 1998)
CCL3 (MIP-1a)	CCR1/CCR3/CCR5	Anti-CCL3	- infectious keratitis - fever - sepsis - inflammation in MS (Xue et al., 2007) (Soares et al., 2009) (Takahashi et al., 2002) (Man et al., 2007)
		CCL3 -/-	- sepsis (Cook et al., 1995)
CCL4		Anti-CCL4	- lung inflammatory response (Bless et al., 2000)
CCL5 (RANTES)	CCR5/CCR1/CCR3	Anti-CCL5	- autocrine proliferation of Hodgkin lymphoma cell lines (Aldinucci et al., 2008; Boring et al., 1998; Izikson et al., 2000; Levina et al., 2008)
		CCL5-/-	- demyelination in MS - glial activation (Glass et al., 2004) (El-Hage et al., 2008)
CCL6 (C10)	CCR1	Anti-CCL6	- lung inflammation and remodeling - airway allergy and hyperresponsiveness - phagocytic activity of macrophages (Ma et al., 2004) (Hogaboam et al., 1999)

				(Steinhauser et al., 2000)
CCL7 (MCP-3)	CCR2	Anti-CCL7	- airway allergy and hypereosinophilia	(Stafford et al., 1997)
CCL8 (MCP-2)	CCR2 / CCR5			
CCL9 (MIP-1g)	CCR1	Anti-CCL9	- osteoclast differentiation	(M. Yang et al., 2006)
CCL11 (Eotaxin)	CCR3	Anti-CCL11 Eotaxin -/-	- airway allergy/ Asthma - bronchiolitis - acute inflammatory response	(Ding et al., 2004; Niimi et al., 2007) (Matthews et al., 2005) (Rothenberg et al., 1997)
CCL12 (MCP-5)	CCR2/CCR5			
CCL13 (MCP-4)	CCR2			
CCL14 (HCC-1)	CCR1			
CCL15 (HCC-2)	CCR1/CCR3			
CCL16 (HCC-4)	CCR1/CCR3			
CCL17 (TARC)	CCR4	Anti-TARC	- Hypereosinophilia / allergic asthma - pulmonary infections/ fibrosis - lung cancer (?) - hepatic failure - skin inflammation	(de Lavareille et al., 2001; Schnyder-Candrian et al., 2006) (Belperio et al., 2004; Carpenter & Hogaboam, 2005) (Qin et al., 2009) (Yoneyama et al., 1998) (Campbell et al., 1999)
CCL18 (PARC)	CCR3 (?)	Anti-CCL18	- rheumatoid arthritis	(van der Voort et al., 2005)
CCL19 (ELC)	CCR7			
CCL20 (MIP-3 alpha)	CCR6	Anti-CCL20 / Anti CCR6 Anti-CCL20	- multiple myeloma - HPV infection / Langerhans cells migration - brain inflammation (MS / EAE)	(Giuliani et al., 2008) (Caberg et al., 2009) (Ambrosini et al., 2003)
CCL21 (SLC)	CCR7	Anti-CCL21 CCL21 -/- mice	- kidney fibrosis - corneal immunity - thymus development	(Sakai et al., 2006; Wada et al., 2007) (Y. Jin et al., 2007) (C. Liu et al., 2005)
CCL22 (MDC)	CCR4	Anti-CCL22	- leukemia cell survival and proliferation - eosinophil activation in lung inflammation - lung cancer	(Ghia et al., 2002) (Pinho et al., 2003) (Qin et al., 2009)
CCL23 (MPIF-1)	CCR3		- vascular endothelial cell migration	(Son et al., 2006)
CCL24 (Eotaxin-2)	CCR3		- HIV pathogenicity	(Fiorucci et al., 2007)

CCL25 (TECK)	CCR9	Anti-CCL25	- intestinal immunity	(N. Feng et al., 2006; Hieshima et al., 2008)
CCL26 (Eotaxin-3)	CCR3			(Cuvelier & Patel, 2001)
CCL27 (CTACK)	CCR10	Anti-CCL27	Dermatitis / skin disease	(L. Chen et al., 2006; Morales et al., 1999; Reiss et al., 2001)
CCL28 (MEC)	CCR10	Anti CCL28	- intestine and colon immunity	(N. Feng et al., 2006; Hieshima et al., 2008)
CXCL1 (Gro alpha)	CXCR2	Anti-CXCL1	- arthritis - kidney sepsis - airway inflammation	(Grespan et al., 2008; Lemos et al., 2009) (H. J. Brown et al., 2007) (Issa et al., 2006)
CXCL2 (Gro-beta)	CXCR2	Anti-CXCL2	- kidney sepsis	(H. J. Brown et al., 2007)
CXCL3 (Gro gamma)	CXCR2			
CXCL4 (PF4)	CXCR3b			
CXCL5 (ENA-78)	CXCR2	Anti-CXCL5	- arthritis - diabetes - NSCLC growth/ angiogenesis	(Grespan et al., 2008; Lemos et al., 2009; Smith et al., 2008) (Chavey et al., 2009) (Pold et al., 2004)
CXCL6 (GCP-2)		Anti-GCP-2	- growth SCLC - arthritis	(Zhu et al., 2006) (Kelchtermans et al., 2007)
CXCL7 (NAP-2)		Anti-NAP-2	- thrombosis	(Amiral et al., 1996; Piccardoni et al., 1996)
CXCL8(IL8)	CXCR1 / CXCR2	anti CXCL8 anti CXCR1 anti CXCL8	- inhibition of NSCLC growth/ angiogenesis - inhibition of NSCLC proliferation - clearance of apoptotic cells	(Pold et al., 2004) (Zhu et al., 2004) (Iyoda et al., 2005)
CXCL9 (Mig)	CXCR3	Anti-CXCL9	- brain immunity and MS - transplant rejection	(M. T. Liu, Armstrong et al., 2001; Salmaggi et al., 2002) (Belperio et al., 2003; Colvin et al., 2005; Whiting et al., 2004)
CXCL10 (IP-10)	CXCR3	Anti CXCL10	- axon sprouting and vasculature remodelling following injury	(Glaser et al., 2004; Glaser et al., 2006)

			<ul style="list-style-type: none"> - inflammatory demyelination in MS - coronavirus-induced neurological and liver damage - transplant rejection 	<p>(M. T. Liu, Keirstead et al., 2001; Narumi et al., 2002) (Walsh et al., 2007)</p> <p>(Belperio et al., 2002)</p>
CXCL11 (I-TAC)	CXCR3	Anti-CXCL11	- brain immunity	(Rupprecht et al., 2005)
CXCL12 (SDF-1 α)	CXCR4	Anti CXCL12	<ul style="list-style-type: none"> - autoimmune disease / Lupus erythematosus - metastases/ tumor proliferation - pulmonary hypertension / airway inflammation 	<p>(Balabanian et al., 2003; Matin et al., 2002; A. Wang et al., 2009) (Cardones et al., 2003; Muller et al., 2001; Orimo et al., 2005; Otsuka & Bebb, 2008; Pan et al., 2006; Phillips et al., 2003) (Gonzalo et al., 2000; Hachet-Haas et al., 2008; Lukacs et al., 2002; Young et al., 2009) (Bertolini et al., 2002; Hinton et al., 2008; H. Li et al., 2009) (Otsuka & Bebb, 2008)</p>
		Anti CXCR4	<ul style="list-style-type: none"> - tumor invasion - NSCLC proliferation - airway inflammation 	(Otsuka & Bebb, 2008)
		CXCL12 -/-	- development	(Nagasawa et al., 1996)
CXCL13 (BCA-1)	CXCR5	Anti-CXCL13	<ul style="list-style-type: none"> - autoimmunity / myasthenia gravis - arthritis - graft rejection 	<p>(Meraouna et al., 2006) (Zheng et al., 2005) (Lee et al., 2006)</p>
CXCL14 (BRAK, BMAC)				
CXCL15 (Lungkine)		CXCL15 knock out	- sepsis	(S. C. Chen et al., 2001)
CXCL16	CXCR6	Anti CXCL16	<ul style="list-style-type: none"> - kidney inflammation - sepsis - arthritis - graft tolerance 	<p>(S. H. Yang et al., 2008) (Shimaoka et al., 2003; Xu et al., 2005) (Nanki et al., 2005) (Jiang et al., 2005)</p>
		CXCL16 -/-	- atherosclerosis	(Aslanian & Charo, 2006)

CX3CL1 (fractalkine) CX3CR1	Anti-CX3CL1 XC3CL1 knock out	- graft tolerance - autoimmune disease - atherosclerosis - no phenotype	(Ueha et al., 2007) (Suzuki et al., 2005) (Schulz et al., 2007) (Cook et al., 2001)
XCL1 (lymphotactin) XCR1	Overexpression	- cancer immunotherapy - anti infection immunotherapy	(Q. Wang et al., 2002) (Yue et al., 2009)

NSCLC: non small cell lung cancer; SCLC: small cell lung cancer; MS: Multiple sclerosis; EAE: Experimental autoimmune encephalomyelitis

FIGURE LEGENDS

Figure 1: Examples of different possible routes that can be followed by chemokines / cytokines in the presence of neutralizing macromolecules. Route 1 leads to signalling in the target cell expressing the chemokine/cytokine receptor. Route 2 is used either endogenously or by pathogens. Binding of chemokines takes place without signalling. This event may either lead to degradation of the chemokine or to its transcytosis. Route 3 is used by pathogens that express soluble proteins capable of binding chemokines, generally with moderate affinity and selectivity, and prevents them from normal signalling to the immune system. In Route 4, the neutralizing molecule prevents chemokine binding to glycosaminoglycans. The resulting effect is a collapse of the chemotactic gradient that abolishes leukocyte attraction in the inflamed tissue.⁷

Figure 2: Folding of chemokines: chemokine adopts a typical structure with 3 anti-parallel beta strands and one carboxy terminal helix. C-C denotes disulfide bridges

Figure 3: Superimposition of peptide backbones from CC, CXC and CX3C chemokine groups shows that they have a canonical three dimensional structure.

Figure 4: Illustration of the different modes of action of neutralizing molecules.

A) The small molecule competitively binds to the same site as the receptor; B) The small chemical molecule alters the quaternary structure of the protein ligand; C) The protein ligand undergoes structural changes that regulate its activity: the small molecule alters tertiary structure of the protein ligand; D) The small molecule interferes with ligand bioavailability.

Figure 5: Proposed model for the interaction between CXCL12 and neutralizing chalcone molecule 4. Redrawn from (Hachet-Haas et al., 2008).

References

- Adan, R. A. (2006). Constitutive receptor activity series: endogenous inverse agonists and constitutive receptor activity in the melanocortin system. *Trends Pharmacol Sci*, 27(4), 183-186.
- Addison, C. L., Daniel, T. O., Burdick, M. D., Liu, H., Ehlert, J. E., Xue, Y. Y., et al. (2000). The CXC chemokine receptor 2, CXCR2, is the putative receptor for ELR+ CXC chemokine-induced angiogenic activity. *J Immunol*, 165(9), 5269-5277.
- Alcami, A. (2003). Viral mimicry of cytokines, chemokines and their receptors. *Nat Rev Immunol*, 3(1), 36-50.
- Alcami, A., & Smith, G. L. (1992). A soluble receptor for interleukin-1 beta encoded by vaccinia virus: a novel mechanism of virus modulation of the host response to infection. *Cell*, 71(1), 153-167.
- Aldinucci, D., Lorenzon, D., Cattaruzza, L., Pinto, A., Gloghini, A., Carbone, A., et al. (2008). Expression of CCR5 receptors on Reed-Sternberg cells and Hodgkin lymphoma cell lines: involvement of CCL5/Rantes in tumor cell growth and microenvironmental interactions. *Int J Cancer*, 122(4), 769-776.
- Alewijnse, A. E., Timmerman, H., Jacobs, E. H., Smit, M. J., Roovers, E., Cotecchia, S., et al. (2000). The effect of mutations in the DRY motif on the constitutive activity and structural instability of the histamine H(2) receptor. *Mol Pharmacol*, 57(5), 890-898.
- Alexander, J. M., Nelson, C. A., van Berkel, V., Lau, E. K., Studts, J. M., Brett, T. J., et al. (2002). Structural basis of chemokine sequestration by a herpesvirus decoy receptor. *Cell*, 111(3), 343-356.
- Ali, S., Robertson, H., Wain, J. H., Isaacs, J. D., Malik, G., & Kirby, J. A. (2005). A non-glycosaminoglycan-binding variant of CC chemokine ligand 7 (monocyte chemoattractant protein-3) antagonizes chemokine-mediated inflammation. *J Immunol*, 175(2), 1257-1266.
- Amara, A., Lorthioir, O., Valenzuela, A., Magerus, A., Thelen, M., Montes, M., et al. (1999). Stromal cell-derived factor-1alpha associates with heparan sulfates through the first beta-strand of the chemokine. *J Biol Chem*, 274(34), 23916-23925.
- Ambrosini, E., Columba-Cabezas, S., Serafini, B., Muscella, A., & Aloisi, F. (2003). Astrocytes are the major intracerebral source of macrophage inflammatory protein-3alpha/CCL20 in relapsing experimental autoimmune encephalomyelitis and in vitro. *Glia*, 41(3), 290-300.
- Amiral, J., Marfaing-Koka, A., Wolf, M., Alessi, M. C., Tardy, B., Boyer-Neumann, C., et al. (1996). Presence of autoantibodies to interleukin-8 or neutrophil-activating peptide-2 in patients with heparin-associated thrombocytopenia. *Blood*, 88(2), 410-416.
- Andreasen, C., & Carbonetti, N. H. (2008). Pertussis toxin inhibits early chemokine production to delay neutrophil recruitment in response to Bordetella pertussis respiratory tract infection in mice. *Infect Immun*, 76(11), 5139-5148.
- Ariel, A., Fredman, G., Sun, Y. P., Kantarci, A., Van Dyke, T. E., Luster, A. D., et al. (2006). Apoptotic neutrophils and T cells sequester chemokines during immune response resolution through modulation of CCR5 expression. *Nat Immunol*, 7(11), 1209-1216.
- Arkin, M. (2005). Protein-protein interactions and cancer: small molecules going in for the kill. *Curr Opin Chem Biol*, 9(3), 317-324.
- Arkin, M., & Moasser, M. M. (2008). HER-2-directed, small-molecule antagonists. *Curr Opin Investig Drugs*, 9(12), 1264-1276.

- Arkin, M. R., Randal, M., DeLano, W. L., Hyde, J., Luong, T. N., Oslob, J. D., et al. (2003). Binding of small molecules to an adaptive protein-protein interface. *Proc Natl Acad Sci U S A*, *100*(4), 1603-1608.
- Arkin, M. R., & Wells, J. A. (2004). Small-molecule inhibitors of protein-protein interactions: progressing towards the dream. *Nat Rev Drug Discov*, *3*(4), 301-317.
- Arrang, J. M., Morisset, S., & Gbahou, F. (2007). Constitutive activity of the histamine H3 receptor. *Trends Pharmacol Sci*, *28*(7), 350-357.
- Aslanian, A. M., & Charo, I. F. (2006). Targeted disruption of the scavenger receptor and chemokine CXCL16 accelerates atherosclerosis. *Circulation*, *114*(6), 583-590.
- Auwerx, J., Avner, P., Baldock, R., Ballabio, A., Balling, R., Barbacid, M., et al. (2004). The European dimension for the mouse genome mutagenesis program. *Nat Genet*, *36*(9), 925-927.
- Balabanian, K., Couderc, J., Bouchet-Delbos, L., Amara, A., Berrebi, D., Foussat, A., et al. (2003). Role of the chemokine stromal cell-derived factor 1 in autoantibody production and nephritis in murine lupus. *J Immunol*, *170*(6), 3392-3400.
- Balabanian, K., Lagane, B., Infantino, S., Chow, K. Y., Harriague, J., Moepps, B., et al. (2005). The chemokine SDF-1/CXCL12 binds to and signals through the orphan receptor RDC1 in T lymphocytes. *J Biol Chem*, *280*(42), 35760-35766.
- Balkwill, F. (2009). Tumour necrosis factor and cancer. *Nat Rev Cancer*, *9*(5), 361-371.
- Bamias, G., Siakavellas, S. I., Stamatelopoulos, K. S., Chrysoschoou, E., Papamichael, C., & Sfikakis, P. P. (2008). Circulating levels of TNF-like cytokine 1A (TL1A) and its decoy receptor 3 (DcR3) in rheumatoid arthritis. *Clin Immunol*, *129*(2), 249-255.
- Bannert, N., Craig, S., Farzan, M., Sogah, D., Santo, N. V., Choe, H., et al. (2001). Sialylated O-glycans and sulfated tyrosines in the NH2-terminal domain of CC chemokine receptor 5 contribute to high affinity binding of chemokines. *J Exp Med*, *194*(11), 1661-1673.
- Bedard, E. L., Kim, P., Jiang, J., Parry, N., Liu, L., Wang, H., et al. (2003). Chemokine-binding viral protein M-T7 prevents chronic rejection in rat renal allografts. *Transplantation*, *76*(1), 249-252.
- Behera, A. K., Hildebrand, E., Bronson, R. T., Perides, G., Uematsu, S., Akira, S., et al. (2006). MyD88 deficiency results in tissue-specific changes in cytokine induction and inflammation in interleukin-18-independent mice infected with *Borrelia burgdorferi*. *Infect Immun*, *74*(3), 1462-1470.
- Bellail, A. C., Tse, M. C., Song, J. H., Phuphanich, S., Olson, J. J., Sun, S. Y., et al. (2009). DR5-mediated DISC controls caspase-8 cleavage and initiation of apoptosis in human glioblastomas. *J Cell Mol Med*.
- Belmadani, A., Tran, P. B., Ren, D., & Miller, R. J. (2006). Chemokines regulate the migration of neural progenitors to sites of neuroinflammation. *J Neurosci*, *26*(12), 3182-3191.
- Belperio, J. A., Dy, M., Murray, L., Burdick, M. D., Xue, Y. Y., Strieter, R. M., et al. (2004). The role of the Th2 CC chemokine ligand CCL17 in pulmonary fibrosis. *J Immunol*, *173*(7), 4692-4698.
- Belperio, J. A., Keane, M. P., Burdick, M. D., Lynch, J. P., 3rd, Xue, Y. Y., Li, K., et al. (2002). Critical role for CXCR3 chemokine biology in the pathogenesis of bronchiolitis obliterans syndrome. *J Immunol*, *169*(2), 1037-1049.
- Belperio, J. A., Keane, M. P., Burdick, M. D., Lynch, J. P., 3rd, Zisman, D. A., Xue, Y. Y., et al. (2003). Role of CXCL9/CXCR3 chemokine biology during pathogenesis of acute lung allograft rejection. *J Immunol*, *171*(9), 4844-4852.

- Ben-Shlomo, A., Zhou, C., Pichurin, O., Chesnokova, V., Liu, N. A., Culler, M. D., et al. (2009). Constitutive somatostatin receptor activity determines tonic pituitary cell response. *Mol Endocrinol*, *23*(3), 337-348.
- Berard, J. L., Velez, R. L., Freeman, R. B., & Tsunoda, S. M. (1999). A review of interleukin-2 receptor antagonists in solid organ transplantation. *Pharmacotherapy*, *19*(10), 1127-1137.
- Berg, T. (2003). Modulation of protein-protein interactions with small organic molecules. *Angew Chem Int Ed Engl*, *42*(22), 2462-2481.
- Berg, T. (2006). Inhibition of TNF-alpha signaling: divide and conquer. *ChemMedChem*, *1*(7), 687-688.
- Bertolini, F., Dell'Agnola, C., Mancuso, P., Rabascio, C., Burlini, A., Monestiroli, S., et al. (2002). CXCR4 neutralization, a novel therapeutic approach for non-Hodgkin's lymphoma. *Cancer Res*, *62*(11), 3106-3112.
- Bezerra, M. C., Carvalho, J. F., Prokopowitsch, A. S., & Pereira, R. M. (2005). RANK, RANKL and osteoprotegerin in arthritic bone loss. *Braz J Med Biol Res*, *38*(2), 161-170.
- Blain, K. Y., Kwiatkowski, W., Zhao, Q., La Fleur, D., Naik, C., Chun, T. W., et al. (2007). Structural and functional characterization of CC chemokine CCL14. *Biochemistry*, *46*(35), 10008-10015.
- Blanpain, C., Doranz, B. J., Vakili, J., Rucker, J., Govaerts, C., Baik, S. S., et al. (1999). Multiple charged and aromatic residues in CCR5 amino-terminal domain are involved in high affinity binding of both chemokines and HIV-1 Env protein. *J Biol Chem*, *274*(49), 34719-34727.
- Blazer, L. L., & Neubig, R. R. (2009). Small molecule protein-protein interaction inhibitors as CNS therapeutic agents: current progress and future hurdles. *Neuropsychopharmacology*, *34*(1), 126-141.
- Bless, N. M., Huber-Lang, M., Guo, R. F., Warner, R. L., Schmal, H., Czermak, B. J., et al. (2000). Role of CC chemokines (macrophage inflammatory protein-1 beta, monocyte chemoattractant protein-1, RANTES) in acute lung injury in rats. *J Immunol*, *164*(5), 2650-2659.
- Boeglin, D., Bonnet, D., & Hibert, M. (2007). Solid-phase preparation of a pilot library derived from the 2,3,4,5-tetrahydro-1H-benzo[b]azepin-5-amine scaffold. *J Comb Chem*, *9*(3), 487-500.
- Boismenu, R., Feng, L., Xia, Y. Y., Chang, J. C., & Havran, W. L. (1996). Chemokine expression by intraepithelial gamma delta T cells. Implications for the recruitment of inflammatory cells to damaged epithelia. *J Immunol*, *157*(3), 985-992.
- Boldajipour, B., Mahabaleshwar, H., Kardash, E., Reichman-Fried, M., Blaser, H., Minina, S., et al. (2008). Control of chemokine-guided cell migration by ligand sequestration. *Cell*, *132*(3), 463-473.
- Bonecchi, R., Borroni, E. M., Anselmo, A., Doni, A., Savino, B., Mirolo, M., et al. (2008). Regulation of D6 chemokine scavenging activity by ligand- and Rab11-dependent surface up-regulation. *Blood*, *112*(3), 493-503.
- Bonecchi, R., Borroni, E. M., Savino, B., Buracchi, C., Mantovani, A., & Locati, M. (2008). Non-signaling chemokine receptors: mechanism of action and role in vivo. *J Neuroimmunol*, *198*(1-2), 14-19.
- Boring, L., Gosling, J., Cleary, M., & Charo, I. F. (1998). Decreased lesion formation in CCR2^{-/-} mice reveals a role for chemokines in the initiation of atherosclerosis. *Nature*, *394*(6696), 894-897.

- Braunersreuther, V., Steffens, S., Arnaud, C., Pelli, G., Burger, F., Proudfoot, A., et al. (2008). A novel RANTES antagonist prevents progression of established atherosclerotic lesions in mice. *Arterioscler Thromb Vasc Biol*, 28(6), 1090-1096.
- Brown, H. J., Lock, H. R., Wolfs, T. G., Buurman, W. A., Sacks, S. H., & Robson, M. G. (2007). Toll-like receptor 4 ligation on intrinsic renal cells contributes to the induction of antibody-mediated glomerulonephritis via CXCL1 and CXCL2. *J Am Soc Nephrol*, 18(6), 1732-1739.
- Brown, S. D., Chambon, P., & de Angelis, M. H. (2005). EMPReSS: standardized phenotype screens for functional annotation of the mouse genome. *Nat Genet*, 37(11), 1155.
- Burger, J. A., & Peled, A. (2009). CXCR4 antagonists: targeting the microenvironment in leukemia and other cancers. *Leukemia*, 23(1), 43-52.
- Burger, M., Burger, J. A., Hoch, R. C., Oades, Z., Takamori, H., & Schraufstatter, I. U. (1999). Point mutation causing constitutive signaling of CXCR2 leads to transforming activity similar to Kaposi's sarcoma herpesvirus-G protein-coupled receptor. *J Immunol*, 163(4), 2017-2022.
- Burns, J. M., Summers, B. C., Wang, Y., Melikian, A., Berahovich, R., Miao, Z., et al. (2006). A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development. *J Exp Med*, 203(9), 2201-2213.
- Caberg, J. H., Hubert, P., Herman, L., Herfs, M., Roncarati, P., Boniver, J., et al. (2009). Increased migration of Langerhans cells in response to HPV16 E6 and E7 oncogene silencing: role of CCL20. *Cancer Immunol Immunother*, 58(1), 39-47.
- Cain, S. A., & Monk, P. N. (2002). The orphan receptor C5L2 has high affinity binding sites for complement fragments C5a and C5a des-Arg(74). *J Biol Chem*, 277(9), 7165-7169.
- Campbell, J. J., Haraldsen, G., Pan, J., Rottman, J., Qin, S., Ponath, P., et al. (1999). The chemokine receptor CCR4 in vascular recognition by cutaneous but not intestinal memory T cells. *Nature*, 400(6746), 776-780.
- Caput, D., Laurent, P., Kaghad, M., Lelias, J. M., Lefort, S., Vita, N., et al. (1996). Cloning and characterization of a specific interleukin (IL)-13 binding protein structurally related to the IL-5 receptor alpha chain. *J Biol Chem*, 271(28), 16921-16926.
- Cardones, A. R., Murakami, T., & Hwang, S. T. (2003). CXCR4 enhances adhesion of B16 tumor cells to endothelial cells in vitro and in vivo via beta(1) integrin. *Cancer Res*, 63(20), 6751-6757.
- Carpenter, K. J., & Hogaboam, C. M. (2005). Immunosuppressive effects of CCL17 on pulmonary antifungal responses during pulmonary invasive aspergillosis. *Infect Immun*, 73(11), 7198-7207.
- Cartier, L., Hartley, O., Dubois-Dauphin, M., & Krause, K. H. (2005). Chemokine receptors in the central nervous system: role in brain inflammation and neurodegenerative diseases. *Brain Res Brain Res Rev*, 48(1), 16-42.
- Catusse, J., Clark, D. J., & Gompels, U. A. (2009). CCR5 signalling, but not DARC or D6 regulatory, chemokine receptors are targeted by herpesvirus U83A chemokine which delays receptor internalisation via diversion to a caveolin-linked pathway. *J Inflamm (Lond)*, 6, 22.
- Catusse, J., Parry, C. M., Dewin, D. R., & Gompels, U. A. (2007). Inhibition of HIV-1 infection by viral chemokine U83A via high-affinity CCR5 interactions that block human chemokine-induced leukocyte chemotaxis and receptor internalization. *Blood*, 109(9), 3633-3639.
- Chames, P., Van Regenmortel, M., Weiss, E., & Baty, D. (2009). Therapeutic antibodies: successes, limitations and hopes for the future. *Br J Pharmacol*, 157(2), 220-233.

- Chanel, C., Staropoli, I., Baleux, F., Amara, A., Valenzuela-Fernandez, A., Virelizier, J. L., et al. (2002). Low levels of co-receptor CCR5 are sufficient to permit HIV envelope-mediated fusion with resting CD4 T cells. *Aids*, *16*(17), 2337-2340.
- Chavey, C., Lazennec, G., Lagarrigue, S., Clape, C., Iankova, I., Teyssier, J., et al. (2009). CXC ligand 5 is an adipose-tissue derived factor that links obesity to insulin resistance. *Cell Metab*, *9*(4), 339-349.
- Chen, L., Lin, S. X., Agha-Majzoub, R., Overbergh, L., Mathieu, C., & Chan, L. S. (2006). CCL27 is a critical factor for the development of atopic dermatitis in the keratin-14 IL-4 transgenic mouse model. *Int Immunol*, *18*(8), 1233-1242.
- Chen, S. C., Mehrad, B., Deng, J. C., Vassileva, G., Manfra, D. J., Cook, D. N., et al. (2001). Impaired pulmonary host defense in mice lacking expression of the CXC chemokine lungkine. *J Immunol*, *166*(5), 3362-3368.
- Chensue, S. W., Lukacs, N. W., Yang, T. Y., Shang, X., Frait, K. A., Kunkel, S. L., et al. (2001). Aberrant in vivo T helper type 2 cell response and impaired eosinophil recruitment in CC chemokine receptor 8 knockout mice. *J Exp Med*, *193*(5), 573-584.
- Chitnis, C. E., & Sharma, A. (2008). Targeting the Plasmodium vivax Duffy-binding protein. *Trends Parasitol*, *24*(1), 29-34.
- Claeysen, S., Sebben, M., Becamel, C., Eglen, R. M., Clark, R. D., Bockaert, J., et al. (2000). Pharmacological properties of 5-Hydroxytryptamine(4) receptor antagonists on constitutively active wild-type and mutated receptors. *Mol Pharmacol*, *58*(1), 136-144.
- Clubb, R. T., Omichinski, J. G., Clore, G. M., & Gronenborn, A. M. (1994). Mapping the binding surface of interleukin-8 complexed with an N-terminal fragment of the type 1 human interleukin-8 receptor. *FEBS Lett*, *338*(1), 93-97.
- Colotta, F., Orlando, S., Fadlon, E. J., Sozzani, S., Matteucci, C., & Mantovani, A. (1995). Chemoattractants induce rapid release of the interleukin 1 type II decoy receptor in human polymorphonuclear cells. *J Exp Med*, *181*(6), 2181-2186.
- Colotta, F., Re, F., Muzio, M., Bertini, R., Polentarutti, N., Sironi, M., et al. (1993). Interleukin-1 type II receptor: a decoy target for IL-1 that is regulated by IL-4. *Science*, *261*(5120), 472-475.
- Colvin, B. L., Wang, Z., Nakano, H., Wu, W., Kakiuchi, T., Fairchild, R. L., et al. (2005). CXCL9 antagonism further extends prolonged cardiac allograft survival in CCL19/CCL21-deficient mice. *Am J Transplant*, *5*(9), 2104-2113.
- Comerford, I., Milasta, S., Morrow, V., Milligan, G., & Nibbs, R. (2006). The chemokine receptor CCX-CKR mediates effective scavenging of CCL19 in vitro. *Eur J Immunol*, *36*(7), 1904-1916.
- Cook, D. N., Beck, M. A., Coffman, T. M., Kirby, S. L., Sheridan, J. F., Pragnell, I. B., et al. (1995). Requirement of MIP-1 alpha for an inflammatory response to viral infection. *Science*, *269*(5230), 1583-1585.
- Cook, D. N., Chen, S. C., Sullivan, L. M., Manfra, D. J., Wiekowski, M. T., Prosser, D. M., et al. (2001). Generation and analysis of mice lacking the chemokine fractalkine. *Mol Cell Biol*, *21*(9), 3159-3165.
- Crown, S. E., Yu, Y., Sweeney, M. D., Leary, J. A., & Handel, T. M. (2006). Heterodimerization of CCR2 chemokines and regulation by glycosaminoglycan binding. *J Biol Chem*, *281*(35), 25438-25446.
- Cuvelier, S. L., & Patel, K. D. (2001). Shear-dependent eosinophil transmigration on interleukin 4-stimulated endothelial cells: a role for endothelium-associated eotaxin-3. *J Exp Med*, *194*(12), 1699-1709.

- Dambly-Chaudiere, C., Cubedo, N., & Ghysen, A. (2007). Control of cell migration in the development of the posterior lateral line: antagonistic interactions between the chemokine receptors CXCR4 and CXCR7/RDC1. *BMC Dev Biol*, 7, 23.
- Damon, I., Murphy, P. M., & Moss, B. (1998). Broad spectrum chemokine antagonistic activity of a human poxvirus chemokine homolog. *Proc Natl Acad Sci U S A*, 95(11), 6403-6407.
- Darbonne, W. C., Rice, G. C., Mohler, M. A., Apple, T., Hebert, C. A., Valente, A. J., et al. (1991). Red blood cells are a sink for interleukin 8, a leukocyte chemotaxin. *J Clin Invest*, 88(4), 1362-1369.
- Dawson, T. C., Lentsch, A. B., Wang, Z., Cowhig, J. E., Rot, A., Maeda, N., et al. (2000). Exaggerated response to endotoxin in mice lacking the Duffy antigen/receptor for chemokines (DARC). *Blood*, 96(5), 1681-1684.
- de Lavarelle, A., Roufousse, F., Schandene, L., Stordeur, P., Cogan, E., & Goldman, M. (2001). Clonal Th2 cells associated with chronic hypereosinophilia: TARC-induced CCR4 down-regulation in vivo. *Eur J Immunol*, 31(4), 1037-1046.
- de Moura, P. R., Watanabe, L., Bleicher, L., Colau, D., Dumoutier, L., Lemaire, M. M., et al. (2009). Crystal structure of a soluble decoy receptor IL-22BP bound to interleukin-22. *FEBS Lett*, 583(7), 1072-1077.
- Deruaz, M., Frauenschuh, A., Alessandri, A. L., Dias, J. M., Coelho, F. M., Russo, R. C., et al. (2008). Ticks produce highly selective chemokine binding proteins with antiinflammatory activity. *J Exp Med*, 205(9), 2019-2031.
- Ding, C., Li, J., & Zhang, X. (2004). Bertilimumab Cambridge Antibody Technology Group. *Curr Opin Investig Drugs*, 5(11), 1213-1218.
- Dobson, C. M. (1999). Protein misfolding, evolution and disease. *Trends Biochem Sci*, 24(9), 329-332.
- Dorf, M. E., Berman, M. A., Tanabe, S., Heesen, M., & Luo, Y. (2000). Astrocytes express functional chemokine receptors. *J Neuroimmunol*, 111(1-2), 109-121.
- Duma, L., Haussinger, D., Rogowski, M., Lusso, P., & Grzesiek, S. (2007). Recognition of RANTES by extracellular parts of the CCR5 receptor. *J Mol Biol*, 365(4), 1063-1075.
- El-Hage, N., Bruce-Keller, A. J., Knapp, P. E., & Hauser, K. F. (2008). CCL5/RANTES gene deletion attenuates opioid-induced increases in glial CCL2/MCP-1 immunoreactivity and activation in HIV-1 Tat-exposed mice. *J Neuroimmune Pharmacol*, 3(4), 275-285.
- Emerson, S. D., Palermo, R., Liu, C. M., Tilley, J. W., Chen, L., Danho, W., et al. (2003). NMR characterization of interleukin-2 in complexes with the IL-2Ralpha receptor component, and with low molecular weight compounds that inhibit the IL-2/IL-Ralpha interaction. *Protein Sci*, 12(4), 811-822.
- Feng, L. Y., Ou, Z. L., Wu, F. Y., Shen, Z. Z., & Shao, Z. M. (2009). Involvement of a novel chemokine decoy receptor CCX-CKR in breast cancer growth, metastasis and patient survival. *Clin Cancer Res*, 15(9), 2962-2970.
- Feng, N., Jaimes, M. C., Lazarus, N. H., Monak, D., Zhang, C., Butcher, E. C., et al. (2006). Redundant role of chemokines CCL25/TECK and CCL28/MEC in IgA+ plasmablast recruitment to the intestinal lamina propria after rotavirus infection. *J Immunol*, 176(10), 5749-5759.
- Fili, S., Karalaki, M., & Schaller, B. (2009). Mechanism of bone metastasis: The role of osteoprotegerin and of the host-tissue microenvironment-related survival factors. *Cancer Lett*.
- Fioravanti, B., De Felice, M., Stucky, C. L., Medler, K. A., Luo, M. C., Gardell, L. R., et al. (2008). Constitutive activity at the cannabinoid CB1 receptor is required for behavioral response to noxious chemical stimulation of TRPV1: antinociceptive actions of CB1 inverse agonists. *J Neurosci*, 28(45), 11593-11602.

- Fiorucci, G., Olivetta, E., Chiantore, M. V., & Federico, M. (2007). Microarray analysis reveals CCL24/eotaxin-2 as an effector of the pathogenetic effects induced by HIV-1 Nef. *Curr Drug Discov Technol*, 4(1), 12-23.
- Fong, A. M., Alam, S. M., Imai, T., Haribabu, B., & Patel, D. D. (2002). CX3CR1 tyrosine sulfation enhances fractalkine-induced cell adhesion. *J Biol Chem*, 277(22), 19418-19423.
- Foxwell, B., Andreakos, E., Brennan, F., Feldmann, M., Smith, C., & Conron, M. (2003). Prospects for the development of small molecular weight compounds to replace anti-tumour necrosis factor biological agents. *Ann Rheum Dis*, 62 Suppl 2, ii90-93.
- Fra, A. M., Locati, M., Otero, K., Sironi, M., Signorelli, P., Massardi, M. L., et al. (2003). Cutting edge: scavenging of inflammatory CC chemokines by the promiscuous putatively silent chemokine receptor D6. *J Immunol*, 170(5), 2279-2282.
- Frauenschuh, A., Power, C. A., Deruaz, M., Ferreira, B. R., Silva, J. S., Teixeira, M. M., et al. (2007). Molecular cloning and characterization of a highly selective chemokine-binding protein from the tick *Rhipicephalus sanguineus*. *J Biol Chem*, 282(37), 27250-27258.
- Funke, B., Autschbach, F., Kim, S., Lasitschka, F., Strauch, U., Rogler, G., et al. (2009). Functional characterisation of decoy receptor 3 in Crohn's disease. *Gut*, 58(4), 483-491.
- Galliera, E., Jala, V. R., Trent, J. O., Bonecchi, R., Signorelli, P., Lefkowitz, R. J., et al. (2004). beta-Arrestin-dependent constitutive internalization of the human chemokine decoy receptor D6. *J Biol Chem*, 279(24), 25590-25597.
- Gardner, L., Wilson, C., Patterson, A. M., Bresnihan, B., FitzGerald, O., Stone, M. A., et al. (2006). Temporal expression pattern of Duffy antigen in rheumatoid arthritis: up-regulation in early disease. *Arthritis Rheum*, 54(6), 2022-2026.
- Gayle, R. B., 3rd, Sleath, P. R., Srinivason, S., Birks, C. W., Weerawarna, K. S., Cerretti, D. P., et al. (1993). Importance of the amino terminus of the interleukin-8 receptor in ligand interactions. *J Biol Chem*, 268(10), 7283-7289.
- Gerard, C., Frossard, J. L., Bhatia, M., Saluja, A., Gerard, N. P., Lu, B., et al. (1997). Targeted disruption of the beta-chemokine receptor CCR1 protects against pancreatitis-associated lung injury. *J Clin Invest*, 100(8), 2022-2027.
- Ghia, P., Strola, G., Granziero, L., Geuna, M., Guida, G., Sallusto, F., et al. (2002). Chronic lymphocytic leukemia B cells are endowed with the capacity to attract CD4+, CD40L+ T cells by producing CCL22. *Eur J Immunol*, 32(5), 1403-1413.
- Giuliani, N., Lisignoli, G., Colla, S., Lazzaretti, M., Storti, P., Mancini, C., et al. (2008). CC-Chemokine ligand 20/macrophage inflammatory protein-3alpha and CC-chemokine receptor 6 are overexpressed in myeloma microenvironment related to osteolytic bone lesions. *Cancer Res*, 68(16), 6840-6850.
- Glaser, J., Gonzalez, R., Perreau, V. M., Cotman, C. W., & Keirstead, H. S. (2004). Neutralization of the chemokine CXCL10 enhances tissue sparing and angiogenesis following spinal cord injury. *J Neurosci Res*, 77(5), 701-708.
- Glaser, J., Gonzalez, R., Sadr, E., & Keirstead, H. S. (2006). Neutralization of the chemokine CXCL10 reduces apoptosis and increases axon sprouting after spinal cord injury. *J Neurosci Res*, 84(4), 724-734.
- Glass, W. G., Hickey, M. J., Hardison, J. L., Liu, M. T., Manning, J. E., & Lane, T. E. (2004). Antibody targeting of the CC chemokine ligand 5 results in diminished leukocyte infiltration into the central nervous system and reduced neurologic disease in a viral model of multiple sclerosis. *J Immunol*, 172(7), 4018-4025.

- Glass, W. G., Lim, J. K., Cholera, R., Pletnev, A. G., Gao, J. L., & Murphy, P. M. (2005). Chemokine receptor CCR5 promotes leukocyte trafficking to the brain and survival in West Nile virus infection. *J Exp Med*, *202*(8), 1087-1098.
- Gonzalo, J. A., Lloyd, C. M., Peled, A., Delaney, T., Coyle, A. J., & Gutierrez-Ramos, J. C. (2000). Critical involvement of the chemotactic axis CXCR4/stromal cell-derived factor-1 alpha in the inflammatory component of allergic airway disease. *J Immunol*, *165*(1), 499-508.
- Gosling, J., Dairaghi, D. J., Wang, Y., Hanley, M., Talbot, D., Miao, Z., et al. (2000). Cutting edge: identification of a novel chemokine receptor that binds dendritic cell- and T cell-active chemokines including ELC, SLC, and TECK. *J Immunol*, *164*(6), 2851-2856.
- Gosling, J., Slaymaker, S., Gu, L., Tseng, S., Zlot, C. H., Young, S. G., et al. (1999). MCP-1 deficiency reduces susceptibility to atherosclerosis in mice that overexpress human apolipoprotein B. *J Clin Invest*, *103*(6), 773-778.
- Graham, G. J. (2009). D6 and the atypical chemokine receptor family: novel regulators of immune and inflammatory processes. *Eur J Immunol*, *39*(2), 342-351.
- Graham, G. J., & McKimmie, C. S. (2006). Chemokine scavenging by D6: a movable feast? *Trends Immunol*, *27*(8), 381-386.
- Grespan, R., Fukada, S. Y., Lemos, H. P., Vieira, S. M., Napimoga, M. H., Teixeira, M. M., et al. (2008). CXCR2-specific chemokines mediate leukotriene B4-dependent recruitment of neutrophils to inflamed joints in mice with antigen-induced arthritis. *Arthritis Rheum*, *58*(7), 2030-2040.
- Gu, L., Okada, Y., Clinton, S. K., Gerard, C., Sukhova, G. K., Libby, P., et al. (1998). Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. *Mol Cell*, *2*(2), 275-281.
- Guerau-de-Arellano, M., & Huber, B. T. (2005). Chemokines and Toll-like receptors in Lyme disease pathogenesis. *Trends Mol Med*, *11*(3), 114-120.
- Hachet-Haas, M., Balabanian, K., Rohmer, F., Pons, F., Franchet, C., Lecat, S., et al. (2008). Small neutralizing molecules to inhibit actions of the chemokine CXCL12. *J Biol Chem*, *283*(34), 23189-23199.
- Hajnicka, V., Kocakova, P., Slavikova, M., Slovak, M., Gasperik, J., Fuchsberger, N., et al. (2001). Anti-interleukin-8 activity of tick salivary gland extracts. *Parasite Immunol*, *23*(9), 483-489.
- Handel, T. M., Johnson, Z., Rodrigues, D. H., Dos Santos, A. C., Cirillo, R., Muzio, V., et al. (2008). An engineered monomer of CCL2 has anti-inflammatory properties emphasizing the importance of oligomerization for chemokine activity in vivo. *J Leukoc Biol*, *84*(4), 1101-1108.
- He, M. M., Smith, A. S., Oslob, J. D., Flanagan, W. M., Braisted, A. C., Whitty, A., et al. (2005). Small-molecule inhibition of TNF-alpha. *Science*, *310*(5750), 1022-1025.
- Hibert, M. F. (2009). French/European academic compound library initiative. *Drug Discov Today*.
- Hieshima, K., Nagakubo, D., Nakayama, T., Shirakawa, A. K., Jin, Z., & Yoshie, O. (2008). Tax-inducible production of CC chemokine ligand 22 by human T cell leukemia virus type 1 (HTLV-1)-infected T cells promotes preferential transmission of HTLV-1 to CCR4-expressing CD4+ T cells. *J Immunol*, *180*(2), 931-939.
- Hinton, C. V., Avraham, S., & Avraham, H. K. (2008). Role of the CXCR4/CXCL12 signaling axis in breast cancer metastasis to the brain. *Clin Exp Metastasis*.
- Hirschfeld, M., Kirschning, C. J., Schwandner, R., Wesche, H., Weis, J. H., Wooten, R. M., et al. (1999). Cutting edge: inflammatory signaling by *Borrelia burgdorferi* lipoproteins is mediated by toll-like receptor 2. *J Immunol*, *163*(5), 2382-2386.

- Hogaboam, C. M., Gallinat, C. S., Taub, D. D., Strieter, R. M., Kunkel, S. L., & Lukacs, N. W. (1999). Immunomodulatory role of C10 chemokine in a murine model of allergic bronchopulmonary aspergillosis. *J Immunol*, *162*(10), 6071-6079.
- Holst, P. J., Rosenkilde, M. M., Manfra, D., Chen, S. C., Wiekowski, M. T., Holst, B., et al. (2001). Tumorigenesis induced by the HHV8-encoded chemokine receptor requires ligand modulation of high constitutive activity. *J Clin Invest*, *108*(12), 1789-1796.
- Horuk, R. (2009). Promiscuous drugs as therapeutics for chemokine receptors. *Expert Rev Mol Med*, *11*, e1.
- Hoshino, A., Kawamura, Y. I., Yasuhara, M., Toyama-Sorimachi, N., Yamamoto, K., Matsukawa, A., et al. (2007). Inhibition of CCL1-CCR8 interaction prevents aggregation of macrophages and development of peritoneal adhesions. *J Immunol*, *178*(8), 5296-5304.
- Hughes, A., & Nelson, M. (2009). HIV entry: new insights and implications for patient management. *Curr Opin Infect Dis*, *22*(1), 35-42.
- Hunter, D. T., Jr., & Hill, J. M. (1961). Surfen: a quinoline with oncogenic and heparin-neutralizing properties. *Nature*, *191*, 1378-1379.
- Issa, R., Xie, S., Lee, K. Y., Stanbridge, R. D., Bhavsar, P., Sukkar, M. B., et al. (2006). GRO-alpha regulation in airway smooth muscle by IL-1beta and TNF-alpha: role of NF-kappaB and MAP kinases. *Am J Physiol Lung Cell Mol Physiol*, *291*(1), L66-74.
- Iyoda, T., Nagata, K., Akashi, M., & Kobayashi, Y. (2005). Neutrophils accelerate macrophage-mediated digestion of apoptotic cells in vivo as well as in vitro. *J Immunol*, *175*(6), 3475-3483.
- Izikson, L., Klein, R. S., Charo, I. F., Weiner, H. L., & Luster, A. D. (2000). Resistance to experimental autoimmune encephalomyelitis in mice lacking the CC chemokine receptor (CCR)2. *J Exp Med*, *192*(7), 1075-1080.
- Jamieson, T., Cook, D. N., Nibbs, R. J., Rot, A., Nixon, C., McLean, P., et al. (2005). The chemokine receptor D6 limits the inflammatory response in vivo. *Nat Immunol*, *6*(4), 403-411.
- Jiang, X., Shimaoka, T., Kojo, S., Harada, M., Watarai, H., Wakao, H., et al. (2005). Cutting edge: critical role of CXCL16/CXCR6 in NKT cell trafficking in allograft tolerance. *J Immunol*, *175*(4), 2051-2055.
- Jin, H., Shen, X., Baggett, B. R., Kong, X., & LiWang, P. J. (2007). The human CC chemokine MIP-1beta dimer is not competent to bind to the CCR5 receptor. *J Biol Chem*, *282*(38), 27976-27983.
- Jin, Y., Shen, L., Chong, E. M., Hamrah, P., Zhang, Q., Chen, L., et al. (2007). The chemokine receptor CCR7 mediates corneal antigen-presenting cell trafficking. *Mol Vis*, *13*, 626-634.
- Johnson, Z., Kosco-Vilbois, M. H., Herren, S., Cirillo, R., Muzio, V., Zaratini, P., et al. (2004). Interference with heparin binding and oligomerization creates a novel anti-inflammatory strategy targeting the chemokine system. *J Immunol*, *173*(9), 5776-5785.
- Kalatskaya, I., Berchiche, Y. A., Gravel, S., Limberg, B. J., Rosenbaum, J. S., & Heveker, N. (2009). AMD3100 is a CXCR7 ligand with allosteric agonist properties. *Mol Pharmacol*, *75*(5), 1240-1247.
- Kelchtermans, H., Struyf, S., De Klerck, B., Mitera, T., Alen, M., Geboes, L., et al. (2007). Protective role of IFN-gamma in collagen-induced arthritis conferred by inhibition of mycobacteria-induced granulocyte chemotactic protein-2 production. *J Leukoc Biol*, *81*(4), 1044-1053.
- Khosla, S. (2001). Minireview: the OPG/RANKL/RANK system. *Endocrinology*, *142*(12), 5050-5055.

- Kindberg, E., Mickiene, A., Ax, C., Akerlind, B., Vene, S., Lindquist, L., et al. (2008). A deletion in the chemokine receptor 5 (CCR5) gene is associated with tickborne encephalitis. *J Infect Dis*, *197*(2), 266-269.
- Kokkoli, E., Kasinskas, R. W., Mardilovich, A., & Garg, A. (2005). Fractalkine targeting with a receptor-mimicking peptide-amphiphile. *Biomacromolecules*, *6*(3), 1272-1279.
- Kong, K. O., Tan, A. W., Thong, B. Y., Lian, T. Y., Cheng, Y. K., Teh, C. L., et al. (2009). Enhanced expression of interferon-inducible protein-10 correlates with disease activity and clinical manifestations in systemic lupus erythematosus. *Clin Exp Immunol*, *156*(1), 134-140.
- Kriegova, E., Tsyrlunyk, A., Arakelyan, A., Mrazek, F., Ordeltova, M., Petzmann, S., et al. (2006). Expression of CCX CKR in pulmonary sarcoidosis. *Inflamm Res*, *55*(10), 441-445.
- Kuloglu, E. S., McCaslin, D. R., Markley, J. L., & Volkman, B. F. (2002). Structural rearrangement of human lymphotactin, a C chemokine, under physiological solution conditions. *J Biol Chem*, *277*(20), 17863-17870.
- Kwong, J., Kulbe, H., Wong, D., Chakravarty, P., & Balkwill, F. (2009). An antagonist of the chemokine receptor CXCR4 induces mitotic catastrophe in ovarian cancer cells. *Mol Cancer Ther*, *8*(7), 1893-1905.
- Laguri, C., Arenzana-Seisdedos, F., & Lortat-Jacob, H. (2008). Relationships between glycosaminoglycan and receptor binding sites in chemokines-the CXCL12 example. *Carbohydr Res*, *343*(12), 2018-2023.
- Lalani, A. S., Graham, K., Mossman, K., Rajarathnam, K., Clark-Lewis, I., Kelvin, D., et al. (1997). The purified myxoma virus gamma interferon receptor homolog M-T7 interacts with the heparin-binding domains of chemokines. *J Virol*, *71*(6), 4356-4363.
- Lau, P. N., Chow, K. B., Chan, C. B., Cheng, C. H., & Wise, H. (2009). The constitutive activity of the ghrelin receptor attenuates apoptosis via a protein kinase C-dependent pathway. *Mol Cell Endocrinol*, *299*(2), 232-239.
- Lecat, S., Bucher, B., Mely, Y., & Galzi, J. L. (2002). Mutations in the extracellular amino-terminal domain of the NK2 neurokinin receptor abolish cAMP signaling but preserve intracellular calcium responses. *J Biol Chem*, *277*(44), 42034-42048.
- Lee, B. P., Chen, W., Shi, H., Der, S. D., Forster, R., & Zhang, L. (2006). CXCR5/CXCL13 interaction is important for double-negative regulatory T cell homing to cardiac allografts. *J Immunol*, *176*(9), 5276-5283.
- Lefkowitz, R. J., Cotecchia, S., Kjelsberg, M. A., Pitcher, J., Koch, W. J., Inglese, J., et al. (1993). Adrenergic receptors: recent insights into their mechanism of activation and desensitization. *Adv Second Messenger Phosphoprotein Res*, *28*, 1-9.
- Lefkowitz, R. J., Cotecchia, S., Samama, P., & Costa, T. (1993). Constitutive activity of receptors coupled to guanine nucleotide regulatory proteins. *Trends Pharmacol Sci*, *14*(8), 303-307.
- Lemos, H. P., Grespan, R., Vieira, S. M., Cunha, T. M., Verri, W. A., Jr., Fernandes, K. S., et al. (2009). Prostaglandin mediates IL-23/IL-17-induced neutrophil migration in inflammation by inhibiting IL-12 and IFN γ production. *Proc Natl Acad Sci U S A*, *106*(14), 5954-5959.
- Leurs, R., Rodriguez Pena, M. S., Bakker, R. A., Alewijnse, A. E., & Timmerman, H. (2000). Constitutive activity of G protein coupled receptors and drug action. *Pharm Acta Helv*, *74*(2-3), 327-331.
- Levina, V., Nolen, B. M., Marrangoni, A. M., Cheng, P., Marks, J. R., Szczepanski, M. J., et al. (2009). Role of eotaxin-1 signaling in ovarian cancer. *Clin Cancer Res*, *15*(8), 2647-2656.

- Levina, V., Su, Y., Nolen, B., Liu, X., Gordin, Y., Lee, M., et al. (2008). Chemotherapeutic drugs and human tumor cells cytokine network. *Int J Cancer*, *123*(9), 2031-2040.
- Levoye, A., Balabanian, K., Baleux, F., Bachelier, F., & Lagane, B. (2009). CXCR7 heterodimerizes with CXCR4 and regulates CXCL12-mediated G protein signaling. *Blood*, *113*(24), 6085-6093.
- Li, B. Q., Fu, T., Gong, W. H., Dunlop, N., Kung, H., Yan, Y., et al. (2000). The flavonoid baicalin exhibits anti-inflammatory activity by binding to chemokines. *Immunopharmacology*, *49*(3), 295-306.
- Li, H., Yang, W., Chen, P. W., Alizadeh, H., & Niederkorn, J. Y. (2009). Inhibition of Chemokine Receptor Expression on Uveal Melanomas by CXCR4 siRNA Blocks Tumor Cell Invasion and Liver Metastasis of Uveal Melanoma Cells. *Invest Ophthalmol Vis Sci*.
- Li, X., Loberg, R., Liao, J., Ying, C., Snyder, L. A., Pienta, K. J., et al. (2009). A destructive cascade mediated by CCL2 facilitates prostate cancer growth in bone. *Cancer Res*, *69*(4), 1685-1692.
- Lim, J. K., Louie, C. Y., Glaser, C., Jean, C., Johnson, B., Johnson, H., et al. (2008). Genetic deficiency of chemokine receptor CCR5 is a strong risk factor for symptomatic West Nile virus infection: a meta-analysis of 4 cohorts in the US epidemic. *J Infect Dis*, *197*(2), 262-265.
- Liu, C., Ueno, T., Kuse, S., Saito, F., Nitta, T., Piali, L., et al. (2005). The role of CCL21 in recruitment of T-precursor cells to fetal thymus. *Blood*, *105*(1), 31-39.
- Liu, L., Dai, E., Miller, L., Seet, B., Lalani, A., Macauley, C., et al. (2004). Viral chemokine-binding proteins inhibit inflammatory responses and aortic allograft transplant vasculopathy in rat models. *Transplantation*, *77*(11), 1652-1660.
- Liu, L., Lalani, A., Dai, E., Seet, B., Macauley, C., Singh, R., et al. (2000). The viral anti-inflammatory chemokine-binding protein M-T7 reduces intimal hyperplasia after vascular injury. *J Clin Invest*, *105*(11), 1613-1621.
- Liu, M. T., Armstrong, D., Hamilton, T. A., & Lane, T. E. (2001). Expression of Mig (monokine induced by interferon-gamma) is important in T lymphocyte recruitment and host defense following viral infection of the central nervous system. *J Immunol*, *166*(3), 1790-1795.
- Liu, M. T., Keirstead, H. S., & Lane, T. E. (2001). Neutralization of the chemokine CXCL10 reduces inflammatory cell invasion and demyelination and improves neurological function in a viral model of multiple sclerosis. *J Immunol*, *167*(7), 4091-4097.
- Loberg, R. D., Ying, C., Craig, M., Day, L. L., Sargent, E., Neeley, C., et al. (2007). Targeting CCL2 with systemic delivery of neutralizing antibodies induces prostate cancer tumor regression in vivo. *Cancer Res*, *67*(19), 9417-9424.
- Lu, B., Rutledge, B. J., Gu, L., Fiorillo, J., Lukacs, N. W., Kunkel, S. L., et al. (1998). Abnormalities in monocyte recruitment and cytokine expression in monocyte chemoattractant protein 1-deficient mice. *J Exp Med*, *187*(4), 601-608.
- Lukacs, N. W., Berlin, A., Schols, D., Skerlj, R. T., & Bridger, G. J. (2002). AMD3100, a CxCR4 antagonist, attenuates allergic lung inflammation and airway hyperreactivity. *Am J Pathol*, *160*(4), 1353-1360.
- Luker, K., Gupta, M., & Luker, G. (2009 b). Bioluminescent CXCL12 fusion protein for cellular studies of CXCR4 and CXCR7. *Biotechniques*, *47*(1), 625-632.
- Luker, K. E., Gupta, M., Steele, J. M., Foerster, B. R., & Luker, G. D. (2009 a). Imaging ligand-dependent activation of CXCR7. *Neoplasia*, *11*(10), 1022-1035.
- Lutgens, E., Faber, B., Schapira, K., Evelo, C. T., van Haaften, R., Heeneman, S., et al. (2005). Gene profiling in atherosclerosis reveals a key role for small inducible

- cytokines: validation using a novel monocyte chemoattractant protein monoclonal antibody. *Circulation*, *111*(25), 3443-3452.
- Ma, B., Zhu, Z., Homer, R. J., Gerard, C., Strieter, R., & Elias, J. A. (2004). The C10/CCL6 chemokine and CCR1 play critical roles in the pathogenesis of IL-13-induced inflammation and remodeling. *J Immunol*, *172*(3), 1872-1881.
- Madrigal, J. L., Leza, J. C., Polak, P., Kalinin, S., & Feinstein, D. L. (2009). Astrocyte-derived MCP-1 mediates neuroprotective effects of noradrenaline. *J Neurosci*, *29*(1), 263-267.
- Magge, S. N., Malik, S. Z., Royo, N. C., Chen, H. I., Yu, L., Snyder, E. Y., et al. (2009). Role of monocyte chemoattractant protein-1 (MCP-1/CCL2) in migration of neural progenitor cells toward glial tumors. *J Neurosci Res*, *87*(7), 1547-1555.
- Mahabaleswar, H., Boldajipour, B., & Raz, E. (2008). Killing the messenger: The role of CXCR7 in regulating primordial germ cell migration. *Cell Adh Migr*, *2*(2), 69-70.
- Maillet, E. L., Pellegrini, N., Valant, C., Bucher, B., Hibert, M., Bourguignon, J. J., et al. (2007). A novel, conformation-specific allosteric inhibitor of the tachykinin NK2 receptor (NK2R) with functionally selective properties. *Faseb J*, *21*(9), 2124-2134.
- Man, S. M., Ma, Y. R., Shang, D. S., Zhao, W. D., Li, B., Guo, D. W., et al. (2007). Peripheral T cells overexpress MIP-1alpha to enhance its transendothelial migration in Alzheimer's disease. *Neurobiol Aging*, *28*(4), 485-496.
- Mantovani, A., Allavena, P., Sica, A., & Balkwill, F. (2008). Cancer-related inflammation. *Nature*, *454*(7203), 436-444.
- Mantovani, A., Bonecchi, R., & Locati, M. (2006). Tuning inflammation and immunity by chemokine sequestration: decoys and more. *Nat Rev Immunol*, *6*(12), 907-918.
- Mantovani, A., Garlanda, C., Locati, M., Rodriguez, T. V., Feo, S. G., Savino, B., et al. (2007). Regulatory pathways in inflammation. *Autoimmun Rev*, *7*(1), 8-11.
- Martin, A. P., Canasto-Chibuque, C., Shang, L., Rollins, B. J., & Lira, S. A. (2006). The chemokine decoy receptor M3 blocks CC chemokine ligand 2 and CXC chemokine ligand 13 function in vivo. *J Immunol*, *177*(10), 7296-7302.
- Martinez de la Torre, Y., Locati, M., Buracchi, C., Dupor, J., Cook, D. N., Bonecchi, R., et al. (2005). Increased inflammation in mice deficient for the chemokine decoy receptor D6. *Eur J Immunol*, *35*(5), 1342-1346.
- Matin, K., Salam, M. A., Akhter, J., Hanada, N., & Senpuku, H. (2002). Role of stromal-cell derived factor-1 in the development of autoimmune diseases in non-obese diabetic mice. *Immunology*, *107*(2), 222-232.
- Matthews, S. P., Tregoning, J. S., Coyle, A. J., Hussell, T., & Openshaw, P. J. (2005). Role of CCL11 in eosinophilic lung disease during respiratory syncytial virus infection. *J Virol*, *79*(4), 2050-2057.
- Mayer, K. L., & Stone, M. J. (2000). NMR solution structure and receptor peptide binding of the CC chemokine eotaxin-2. *Biochemistry*, *39*(29), 8382-8395.
- Mazzinghi, B., Ronconi, E., Lazzeri, E., Sagrinati, C., Ballerini, L., Angelotti, M. L., et al. (2008). Essential but differential role for CXCR4 and CXCR7 in the therapeutic homing of human renal progenitor cells. *J Exp Med*, *205*(2), 479-490.
- McFadden, G., Lalani, A., Everett, H., Nash, P., & Xu, X. (1998). Virus-encoded receptors for cytokines and chemokines. *Semin Cell Dev Biol*, *9*(3), 359-368.
- Meijer, J., Zeelenberg, I. S., Sipos, B., & Roos, E. (2006). The CXCR5 chemokine receptor is expressed by carcinoma cells and promotes growth of colon carcinoma in the liver. *Cancer Res*, *66*(19), 9576-9582.
- Meng, C. Q., Ni, L., Worsencroft, K. J., Ye, Z., Weingarten, M. D., Simpson, J. E., et al. (2007). Carboxylated, heteroaryl-substituted chalcones as inhibitors of vascular cell

- adhesion molecule-1 expression for use in chronic inflammatory diseases. *J Med Chem*, 50(6), 1304-1315.
- Meraouna, A., Cizeron-Clairac, G., Panse, R. L., Bismuth, J., Truffault, F., Tallaksen, C., et al. (2006). The chemokine CXCL13 is a key molecule in autoimmune myasthenia gravis. *Blood*, 108(2), 432-440.
- Middel, P., Thelen, P., Blaschke, S., Polzien, F., Reich, K., Blaschke, V., et al. (2001). Expression of the T-cell chemoattractant chemokine lymphotactin in Crohn's disease. *Am J Pathol*, 159(5), 1751-1761.
- Mizoue, L. S., Bazan, J. F., Johnson, E. C., & Handel, T. M. (1999). Solution structure and dynamics of the CX3C chemokine domain of fractalkine and its interaction with an N-terminal fragment of CX3CR1. *Biochemistry*, 38(5), 1402-1414.
- Monod, J., Wyman, J., & Changeux, J. P. (1965). On the Nature of Allosteric Transitions: a Plausible Model. *J Mol Biol*, 12, 88-118.
- Monteclaro, F. S., & Charo, I. F. (1996). The amino-terminal extracellular domain of the MCP-1 receptor, but not the RANTES/MIP-1 α receptor, confers chemokine selectivity. Evidence for a two-step mechanism for MCP-1 receptor activation. *J Biol Chem*, 271(32), 19084-19092.
- Monteclaro, F. S., & Charo, I. F. (1997). The amino-terminal domain of CCR2 is both necessary and sufficient for high affinity binding of monocyte chemoattractant protein 1. Receptor activation by a pseudo-tethered ligand. *J Biol Chem*, 272(37), 23186-23190.
- Morales, J., Homey, B., Vicari, A. P., Hudak, S., Oldham, E., Hedrick, J., et al. (1999). CTACK, a skin-associated chemokine that preferentially attracts skin-homing memory T cells. *Proc Natl Acad Sci U S A*, 96(25), 14470-14475.
- Mortier, A., Van Damme, J., & Proost, P. (2008). Regulation of chemokine activity by posttranslational modification. *Pharmacol Ther*, 120(2), 197-217.
- Mueller, A. M., Pedre, X., Killian, S., David, M., & Steinbrecher, A. (2009). The Decoy Receptor 3 (DcR3, TNFRSF6B) suppresses Th17 immune responses and is abundant in human cerebrospinal fluid. *J Neuroimmunol*, 209(1-2), 57-64.
- Muller, A., Homey, B., Soto, H., Ge, N., Catron, D., Buchanan, M. E., et al. (2001). Involvement of chemokine receptors in breast cancer metastasis. *Nature*, 410(6824), 50-56.
- Murphy, J. W., Cho, Y., Sachpatzidis, A., Fan, C., Hodsdon, M. E., & Lolis, E. (2007). Structural and functional basis of CXCL12 (stromal cell-derived factor-1 α) binding to heparin. *J Biol Chem*, 282(13), 10018-10027.
- Murphy, P. M. (2000). Viral antichemokines: from pathogenesis to drug discovery. *J Clin Invest*, 105(11), 1515-1517.
- Murphy, P. M. (2002). International Union of Pharmacology. XXX. Update on chemokine receptor nomenclature. *Pharmacol Rev*, 54(2), 227-229.
- Nagasawa, T., Hirota, S., Tachibana, K., Takakura, N., Nishikawa, S., Kitamura, Y., et al. (1996). Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. *Nature*, 382(6592), 635-638.
- Nanki, T., Shimaoka, T., Hayashida, K., Taniguchi, K., Yonehara, S., & Miyasaka, N. (2005). Pathogenic role of the CXCL16-CXCR6 pathway in rheumatoid arthritis. *Arthritis Rheum*, 52(10), 3004-3014.
- Narumi, S., Kaburaki, T., Yoneyama, H., Iwamura, H., Kobayashi, Y., & Matsushima, K. (2002). Neutralization of IFN-inducible protein 10/CXCL10 exacerbates experimental autoimmune encephalomyelitis. *Eur J Immunol*, 32(6), 1784-1791.
- Nelson, A. L., & Reichert, J. M. (2009). Development trends for therapeutic antibody fragments. *Nat Biotechnol*, 27(4), 331-337.

- Nibbs, R. J., Gilchrist, D. S., King, V., Ferra, A., Forrow, S., Hunter, K. D., et al. (2007). The atypical chemokine receptor D6 suppresses the development of chemically induced skin tumors. *J Clin Invest*, *117*(7), 1884-1892.
- Niimi, K., Asano, K., Shiraishi, Y., Nakajima, T., Wakaki, M., Kagyo, J., et al. (2007). TLR3-mediated synthesis and release of eotaxin-1/CCL11 from human bronchial smooth muscle cells stimulated with double-stranded RNA. *J Immunol*, *178*(1), 489-495.
- Omari, K. M., Lutz, S. E., Santambrogio, L., Lira, S. A., & Raine, C. S. (2009). Neuroprotection and remyelination after autoimmune demyelination in mice that inducibly overexpress CXCL1. *Am J Pathol*, *174*(1), 164-176.
- Orimo, A., Gupta, P. B., Sgroi, D. C., Arenzana-Seisdedos, F., Delaunay, T., Naeem, R., et al. (2005). Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell*, *121*(3), 335-348.
- Otsuka, S., & Bebb, G. (2008). The CXCR4/SDF-1 chemokine receptor axis: a new target therapeutic for non-small cell lung cancer. *J Thorac Oncol*, *3*(12), 1379-1383.
- Palanche, T., Ilien, B., Zoffmann, S., Reck, M. P., Bucher, B., Edelstein, S. J., et al. (2001). The neurokinin A receptor activates calcium and cAMP responses through distinct conformational states. *J Biol Chem*, *276*(37), 34853-34861.
- Pan, J., Mestas, J., Burdick, M. D., Phillips, R. J., Thomas, G. V., Reckamp, K., et al. (2006). Stromal derived factor-1 (SDF-1/CXCL12) and CXCR4 in renal cell carcinoma metastasis. *Mol Cancer*, *5*, 56.
- Park, M. H., Lee, Y. K., Lee, Y. H., Kim, Y. B., Yun, Y. W., Nam, S. Y., et al. (2009). Chemokines released from astrocytes promote chemokine receptor 5-mediated neuronal cell differentiation. *Exp Cell Res*.
- Pease, J. E., Wang, J., Ponath, P. D., & Murphy, P. M. (1998). The N-terminal extracellular segments of the chemokine receptors CCR1 and CCR3 are determinants for MIP-1 α and eotaxin binding, respectively, but a second domain is essential for efficient receptor activation. *J Biol Chem*, *273*(32), 19972-19976.
- Peterson, F. C., Elgin, E. S., Nelson, T. J., Zhang, F., Hoeger, T. J., Linhardt, R. J., et al. (2004). Identification and characterization of a glycosaminoglycan recognition element of the C chemokine lymphotactin. *J Biol Chem*, *279*(13), 12598-12604.
- Phillips, R. J., Burdick, M. D., Lutz, M., Belperio, J. A., Keane, M. P., & Strieter, R. M. (2003). The stromal derived factor-1/CXCL12-CXC chemokine receptor 4 biological axis in non-small cell lung cancer metastases. *Am J Respir Crit Care Med*, *167*(12), 1676-1686.
- Piccardoni, P., Evangelista, V., Piccoli, A., de Gaetano, G., Walz, A., & Cerletti, C. (1996). Thrombin-activated human platelets release two NAP-2 variants that stimulate polymorphonuclear leukocytes. *Thromb Haemost*, *76*(5), 780-785.
- Pinho, V., Oliveira, S. H., Souza, D. G., Vasconcelos, D., Alessandri, A. L., Lukacs, N. W., et al. (2003). The role of CCL22 (MDC) for the recruitment of eosinophils during allergic pleurisy in mice. *J Leukoc Biol*, *73*(3), 356-362.
- Pitti, R. M., Marsters, S. A., Lawrence, D. A., Roy, M., Kischkel, F. C., Dowd, P., et al. (1998). Genomic amplification of a decoy receptor for Fas ligand in lung and colon cancer. *Nature*, *396*(6712), 699-703.
- Pold, M., Zhu, L. X., Sharma, S., Burdick, M. D., Lin, Y., Lee, P. P., et al. (2004). Cyclooxygenase-2-dependent expression of angiogenic CXC chemokines ENA-78/CXC Ligand (CXCL) 5 and interleukin-8/CXCL8 in human non-small cell lung cancer. *Cancer Res*, *64*(5), 1853-1860.

- Prado, G. N., Suetomi, K., Shumate, D., Maxwell, C., Ravindran, A., Rajarathnam, K., et al. (2007). Chemokine signaling specificity: essential role for the N-terminal domain of chemokine receptors. *Biochemistry*, *46*(31), 8961-8968.
- Proudfoot, A. E., Fritchley, S., Borlat, F., Shaw, J. P., Vilbois, F., Zwahlen, C., et al. (2001). The BBXB motif of RANTES is the principal site for heparin binding and controls receptor selectivity. *J Biol Chem*, *276*(14), 10620-10626.
- Proudfoot, A. E., Handel, T. M., Johnson, Z., Lau, E. K., LiWang, P., Clark-Lewis, I., et al. (2003). Glycosaminoglycan binding and oligomerization are essential for the in vivo activity of certain chemokines. *Proc Natl Acad Sci U S A*, *100*(4), 1885-1890.
- Pruenster, M., Mudde, L., Bombosi, P., Dimitrova, S., Zsak, M., Middleton, J., et al. (2009). The Duffy antigen receptor for chemokines transports chemokines and supports their promigratory activity. *Nat Immunol*, *10*(1), 101-108.
- Qin, X. J., Shi, H. Z., Deng, J. M., Liang, Q. L., Jiang, J., & Ye, Z. J. (2009). CCL22 recruits CD4-positive CD25-positive regulatory T cells into malignant pleural effusion. *Clin Cancer Res*, *15*(7), 2231-2237.
- Rahaman, S. O., Sharma, P., Harbor, P. C., Aman, M. J., Vogelbaum, M. A., & Haque, S. J. (2002). IL-13R(alpha)2, a decoy receptor for IL-13 acts as an inhibitor of IL-4-dependent signal transduction in glioblastoma cells. *Cancer Res*, *62*(4), 1103-1109.
- Raimundo, B. C., Oslob, J. D., Braisted, A. C., Hyde, J., McDowell, R. S., Randal, M., et al. (2004). Integrating fragment assembly and biophysical methods in the chemical advancement of small-molecule antagonists of IL-2: an approach for inhibiting protein-protein interactions. *J Med Chem*, *47*(12), 3111-3130.
- Rajagopalan, L., & Rajarathnam, K. (2004). Ligand selectivity and affinity of chemokine receptor CXCR1. Role of N-terminal domain. *J Biol Chem*, *279*(29), 30000-30008.
- Raz, E., & Mahabaleshwar, H. (2009). Chemokine signaling in embryonic cell migration: a fish-eye view. *Development*, *136*(8), 1223-1229.
- Reiss, Y., Proudfoot, A. E., Power, C. A., Campbell, J. J., & Butcher, E. C. (2001). CC chemokine receptor (CCR)4 and the CCR10 ligand cutaneous T cell-attracting chemokine (CTACK) in lymphocyte trafficking to inflamed skin. *J Exp Med*, *194*(10), 1541-1547.
- Reutershan, J., Harry, B., Chang, D., Bagby, G. J., & Ley, K. (2009). DARC on RBC limits lung injury by balancing compartmental distribution of CXC chemokines. *Eur J Immunol*, *39*(6), 1597-1607.
- Rosenkilde, M. M. (2005). Virus-encoded chemokine receptors--putative novel antiviral drug targets. *Neuropharmacology*, *48*(1), 1-13.
- Ross, R. J., Zhou, M., Shen, D., Fariss, R. N., Ding, X., Bojanowski, C. M., et al. (2008). Immunological protein expression profile in Ccl2/Cx3cr1 deficient mice with lesions similar to age-related macular degeneration. *Exp Eye Res*, *86*(4), 675-683.
- Rostene, W., Kitabgi, P., & Parsadaniantz, S. M. (2007). Chemokines: a new class of neuromodulator? *Nat Rev Neurosci*, *8*(11), 895-903.
- Rothenberg, M. E., MacLean, J. A., Pearlman, E., Luster, A. D., & Leder, P. (1997). Targeted disruption of the chemokine eotaxin partially reduces antigen-induced tissue eosinophilia. *J Exp Med*, *185*(4), 785-790.
- Roychoudhury, K., Dasgupta, B., Sen, P., Laskay, T., Solbach, W., De, T., et al. (2006). Evidence of direct interactions between the CC-chemokines CCL3, CCL4 and CCL5 and Leishmania promastigotes. *Mol Biochem Parasitol*, *150*(2), 374-377.
- Rupprecht, T. A., Koedel, U., Muhlberger, B., Wilske, B., Fontana, A., & Pfister, H. W. (2005). CXCL11 is involved in leucocyte recruitment to the central nervous system in neuroborreliosis. *J Neurol*, *252*(7), 820-823.

- Sadir, R., Imberly, A., Baleux, F., & Lortat-Jacob, H. (2004). Heparan sulfate/heparin oligosaccharides protect stromal cell-derived factor-1 (SDF-1)/CXCL12 against proteolysis induced by CD26/dipeptidyl peptidase IV. *J Biol Chem*, 279(42), 43854-43860.
- Sakai, N., Wada, T., Yokoyama, H., Lipp, M., Ueha, S., Matsushima, K., et al. (2006). Secondary lymphoid tissue chemokine (SLC/CCL21)/CCR7 signaling regulates fibrocytes in renal fibrosis. *Proc Natl Acad Sci U S A*, 103(38), 14098-14103.
- Salazar-Mather, T. P., Orange, J. S., & Biron, C. A. (1998). Early murine cytomegalovirus (MCMV) infection induces liver natural killer (NK) cell inflammation and protection through macrophage inflammatory protein 1alpha (MIP-1alpha)-dependent pathways. *J Exp Med*, 187(1), 1-14.
- Salmaggi, A., Gelati, M., Dufour, A., Corsini, E., Pagano, S., Bacalini, R., et al. (2002). Expression and modulation of IFN-gamma-inducible chemokines (IP-10, Mig, and I-TAC) in human brain endothelium and astrocytes: possible relevance for the immune invasion of the central nervous system and the pathogenesis of multiple sclerosis. *J Interferon Cytokine Res*, 22(6), 631-640.
- Samson, M., Libert, F., Doranz, B. J., Rucker, J., Liesnard, C., Farber, C. M., et al. (1996). Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. *Nature*, 382(6593), 722-725.
- Sancho, M., Vieira, J. M., Casalou, C., Mesquita, M., Pereira, T., Cavaco, B. M., et al. (2006). Expression and function of the chemokine receptor CCR7 in thyroid carcinomas. *J Endocrinol*, 191(1), 229-238.
- Santiago, B., Baleux, F., Palao, G., Gutierrez-Canas, I., Ramirez, J. C., Arenzana-Seisdedos, F., et al. (2006). CXCL12 is displayed by rheumatoid endothelial cells through its basic amino-terminal motif on heparan sulfate proteoglycans. *Arthritis Res Ther*, 8(2), R43.
- Schadt, E. E., Friend, S. H., & Shaywitz, D. A. (2009). A network view of disease and compound screening. *Nat Rev Drug Discov*, 8(4), 286-295.
- Schippling, D. S., & Martin, R. (2008). Spotlight on anti-CD25: daclizumab in MS. *Int MS J*, 15(3), 94-98.
- Schnyder-Candrian, S., Togbe, D., Couillin, I., Mercier, I., Brombacher, F., Quesniaux, V., et al. (2006). Interleukin-17 is a negative regulator of established allergic asthma. *J Exp Med*, 203(12), 2715-2725.
- Schuksz, M., Fuster, M. M., Brown, J. R., Crawford, B. E., Ditto, D. P., Lawrence, R., et al. (2008). Surfen, a small molecule antagonist of heparan sulfate. *Proc Natl Acad Sci U S A*, 105(35), 13075-13080.
- Schulz, C., Schafer, A., Stolla, M., Kerstan, S., Lorenz, M., von Bruhl, M. L., et al. (2007). Chemokine fractalkine mediates leukocyte recruitment to inflammatory endothelial cells in flowing whole blood: a critical role for P-selectin expressed on activated platelets. *Circulation*, 116(7), 764-773.
- Scola, A. M., Johswich, K. O., Morgan, B. P., Klos, A., & Monk, P. N. (2009). The human complement fragment receptor, C5L2, is a recycling decoy receptor. *Mol Immunol*, 46(6), 1149-1162.
- Scotton, C. J., Wilson, J. L., Scott, K., Stamp, G., Wilbanks, G. D., Fricker, S., et al. (2002). Multiple actions of the chemokine CXCL12 on epithelial tumor cells in human ovarian cancer. *Cancer Res*, 62(20), 5930-5938.
- Segerer, S., Regele, H., Mac, K. M., Kain, R., Cartron, J. P., Colin, Y., et al. (2000). The Duffy antigen receptor for chemokines is up-regulated during acute renal transplant rejection and crescentic glomerulonephritis. *Kidney Int*, 58(4), 1546-1556.

- Shaw, J. P., Johnson, Z., Borlat, F., Zwahlen, C., Kungl, A., Roulin, K., et al. (2004). The X-ray structure of RANTES: heparin-derived disaccharides allows the rational design of chemokine inhibitors. *Structure*, *12*(11), 2081-2093.
- Shimaoka, T., Nakayama, T., Kume, N., Takahashi, S., Yamaguchi, J., Minami, M., et al. (2003). Cutting edge: SR-PSOX/CXC chemokine ligand 16 mediates bacterial phagocytosis by APCs through its chemokine domain. *J Immunol*, *171*(4), 1647-1651.
- Sierro, F., Biben, C., Martinez-Munoz, L., Mellado, M., Ransohoff, R. M., Li, M., et al. (2007). Disrupted cardiac development but normal hematopoiesis in mice deficient in the second CXCL12/SDF-1 receptor, CXCR7. *Proc Natl Acad Sci U S A*, *104*(37), 14759-14764.
- Simonet, W. S., Lacey, D. L., Dunstan, C. R., Kelley, M., Chang, M. S., Luthy, R., et al. (1997). Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell*, *89*(2), 309-319.
- Smith, E., McGettrick, H. M., Stone, M. A., Shaw, J. S., Middleton, J., Nash, G. B., et al. (2008). Duffy antigen receptor for chemokines and CXCL5 are essential for the recruitment of neutrophils in a multicellular model of rheumatoid arthritis synovium. *Arthritis Rheum*, *58*(7), 1968-1973.
- Soares, D. M., Figueiredo, M. J., Martins, J. M., Machado, R. R., Kanashiro, A., Malvar Ddo, C., et al. (2009). CCL3/MIP-1 alpha is not involved in the LPS-induced fever and its pyrogenic activity depends on CRF. *Brain Res*, *1269*, 54-60.
- Son, K. N., Hwang, J., Kwon, B. S., & Kim, J. (2006). Human CC chemokine CCL23 enhances expression of matrix metalloproteinase-2 and invasion of vascular endothelial cells. *Biochem Biophys Res Commun*, *340*(2), 498-504.
- Sozzani, S., Luini, W., Bianchi, G., Allavena, P., Wells, T. N., Napolitano, M., et al. (1998). The viral chemokine macrophage inflammatory protein-II is a selective Th2 chemoattractant. *Blood*, *92*(11), 4036-4039.
- Stafford, S., Li, H., Forsythe, P. A., Ryan, M., Bravo, R., & Alam, R. (1997). Monocyte chemotactic protein-3 (MCP-3)/fibroblast-induced cytokine (FIC) in eosinophilic inflammation of the airways and the inhibitory effects of an anti-MCP-3/FIC antibody. *J Immunol*, *158*(10), 4953-4960.
- Steinhauser, M. L., Hogaboam, C. M., Matsukawa, A., Lukacs, N. W., Strieter, R. M., & Kunkel, S. L. (2000). Chemokine C10 promotes disease resolution and survival in an experimental model of bacterial sepsis. *Infect Immun*, *68*(11), 6108-6114.
- Suzuki, F., Nanki, T., Imai, T., Kikuchi, H., Hirohata, S., Kohsaka, H., et al. (2005). Inhibition of CX3CL1 (fractalkine) improves experimental autoimmune myositis in SJL/J mice. *J Immunol*, *175*(10), 6987-6996.
- Takahashi, H., Tashiro, T., Miyazaki, M., Kobayashi, M., Pollard, R. B., & Suzuki, F. (2002). An essential role of macrophage inflammatory protein 1alpha/CCL3 on the expression of host's innate immunities against infectious complications. *J Leukoc Biol*, *72*(6), 1190-1197.
- Thanos, C. D., DeLano, W. L., & Wells, J. A. (2006). Hot-spot mimicry of a cytokine receptor by a small molecule. *Proc Natl Acad Sci U S A*, *103*(42), 15422-15427.
- Thelen, M., & Thelen, S. (2008). CXCR7, CXCR4 and CXCL12: an eccentric trio? *J Neuroimmunol*, *198*(1-2), 9-13.
- Threlfell, S., Exley, R., Cragg, S. J., & Greenfield, S. A. (2008). Constitutive histamine H2 receptor activity regulates serotonin release in the substantia nigra. *J Neurochem*, *107*(3), 745-755.
- Tilley, J. W., Chen, L., Fry, D. C., Emerson, S. D., Power, G. D., Biondi, D., et al. (1997). Identification of a Small Molecule Inhibitor of the IL-2/IL-2R α Receptor Interaction Which Binds to IL-2. *J. Amer chem soc*, *119*, 7589-7590.

- Townson, J. R., & Nibbs, R. J. (2002). Characterization of mouse CCX-CKR, a receptor for the lymphocyte-attracting chemokines TECK/mCCL25, SLC/mCCL21 and MIP-3beta/mCCL19: comparison to human CCX-CKR. *Eur J Immunol*, *32*(5), 1230-1241.
- Tuinstra, R. L., Peterson, F. C., Kutlesa, S., Elgin, E. S., Kron, M. A., & Volkman, B. F. (2008). Interconversion between two unrelated protein folds in the lymphotactin native state. *Proc Natl Acad Sci U S A*, *105*(13), 5057-5062.
- Ueha, S., Murai, M., Yoneyama, H., Kitabatake, M., Imai, T., Shimaoka, T., et al. (2007). Intervention of MAdCAM-1 or fractalkine alleviates graft-versus-host reaction associated intestinal injury while preserving graft-versus-tumor effects. *J Leukoc Biol*, *81*(1), 176-185.
- Vaday, G. G., Peehl, D. M., Kadam, P. A., & Lawrence, D. M. (2006). Expression of CCL5 (RANTES) and CCR5 in prostate cancer. *Prostate*, *66*(2), 124-134.
- Valenzuela, J. G., Charlab, R., Mather, T. N., & Ribeiro, J. M. (2000). Purification, cloning, and expression of a novel salivary anticomplement protein from the tick, *Ixodes scapularis*. *J Biol Chem*, *275*(25), 18717-18723.
- Valenzuela-Fernandez, A., Palanche, T., Amara, A., Magerus, A., Altmeyer, R., Delaunay, T., et al. (2001). Optimal inhibition of X4 HIV isolates by the CXC chemokine stromal cell-derived factor 1 alpha requires interaction with cell surface heparan sulfate proteoglycans. *J Biol Chem*, *276*(28), 26550-26558.
- van Berkel, V., Barrett, J., Tiffany, H. L., Fremont, D. H., Murphy, P. M., McFadden, G., et al. (2000). Identification of a gammaherpesvirus selective chemokine binding protein that inhibits chemokine action. *J Virol*, *74*(15), 6741-6747.
- van der Voort, R., Kramer, M., Lindhout, E., Torensma, R., Eleveld, D., van Lieshout, A. W., et al. (2005). Novel monoclonal antibodies detect elevated levels of the chemokine CCL18/DC-CK1 in serum and body fluids in pathological conditions. *J Leukoc Biol*, *77*(5), 739-747.
- Vancova, I., Slovak, M., Hajnicka, V., Labuda, M., Simo, L., Peterkova, K., et al. (2007). Differential anti-chemokine activity of *Amblyomma variegatum* adult ticks during blood-feeding. *Parasite Immunol*, *29*(4), 169-177.
- Vandercappellen, J., Van Damme, J., & Struyf, S. (2008). The role of CXC chemokines and their receptors in cancer. *Cancer Lett*, *267*(2), 226-244.
- Veldkamp, C. T., Peterson, F. C., Pelzek, A. J., & Volkman, B. F. (2005). The monomer-dimer equilibrium of stromal cell-derived factor-1 (CXCL 12) is altered by pH, phosphate, sulfate, and heparin. *Protein Sci*, *14*(4), 1071-1081.
- Veldkamp, C. T., Seibert, C., Peterson, F. C., De la Cruz, N. B., Haugner, J. C., 3rd, Basnet, H., et al. (2008). Structural basis of CXCR4 sulfotyrosine recognition by the chemokine SDF-1/CXCL12. *Sci Signal*, *1*(37), ra4.
- Vergote, D., Butler, G. S., Ooms, M., Cox, J. H., Silva, C., Hollenberg, M. D., et al. (2006). Proteolytic processing of SDF-1alpha reveals a change in receptor specificity mediating HIV-associated neurodegeneration. *Proc Natl Acad Sci U S A*, *103*(50), 19182-19187.
- Vollmer, J. Y., Alix, P., Chollet, A., Takeda, K., & Galzi, J. L. (1999). Subcellular compartmentalization of activation and desensitization of responses mediated by NK2 neurokinin receptors. *J Biol Chem*, *274*(53), 37915-37922.
- Wada, T., Sakai, N., Matsushima, K., & Kaneko, S. (2007). Fibrocytes: a new insight into kidney fibrosis. *Kidney Int*, *72*(3), 269-273.
- Waldmann, T. A., & O'Shea, J. (1998). The use of antibodies against the IL-2 receptor in transplantation. *Curr Opin Immunol*, *10*(5), 507-512.
- Walsh, K. B., Edwards, R. A., Romero, K. M., Kotlajich, M. V., Stohlman, S. A., & Lane, T. E. (2007). Expression of CXC chemokine ligand 10 from the mouse hepatitis virus

- genome results in protection from viral-induced neurological and liver disease. *J Immunol*, 179(2), 1155-1165.
- Wang, A., Fairhurst, A. M., Tus, K., Subramanian, S., Liu, Y., Lin, F., et al. (2009). CXCR4/CXCL12 hyperexpression plays a pivotal role in the pathogenesis of lupus. *J Immunol*, 182(7), 4448-4458.
- Wang, B., Hendricks, D. T., Wamunyokoli, F., & Parker, M. I. (2006). A growth-related oncogene/CXC chemokine receptor 2 autocrine loop contributes to cellular proliferation in esophageal cancer. *Cancer Res*, 66(6), 3071-3077.
- Wang, J., Ou, Z. L., Hou, Y. F., Luo, J. M., Shen, Z. Z., Ding, J., et al. (2006). Enhanced expression of Duffy antigen receptor for chemokines by breast cancer cells attenuates growth and metastasis potential. *Oncogene*, 25(54), 7201-7211.
- Wang, J., Shiozawa, Y., Wang, J., Wang, Y., Jung, Y., Pienta, K. J., et al. (2008). The role of CXCR7/RDC1 as a chemokine receptor for CXCL12/SDF-1 in prostate cancer. *J Biol Chem*, 283(7), 4283-4294.
- Wang, J. D., Nonomura, N., Takahara, S., Li, B. S., Azuma, H., Ichimaru, N., et al. (1998). Lymphotactin: a key regulator of lymphocyte trafficking during acute graft rejection. *Immunology*, 95(1), 56-61.
- Wang, Q., Yu, H., Zhang, L., Ju, D., Pan, J., Xia, D., et al. (2002). Adenovirus-mediated intratumoral lymphotactin gene transfer potentiates the antibody-targeted superantigen therapy of cancer. *J Mol Med*, 80(9), 585-594.
- Waxman, L., Smith, D. E., Arcuri, K. E., & Vlasuk, G. P. (1990). Tick anticoagulant peptide (TAP) is a novel inhibitor of blood coagulation factor Xa. *Science*, 248(4955), 593-596.
- Weber, M., Blair, E., Simpson, C. V., O'Hara, M., Blackburn, P. E., Rot, A., et al. (2004). The chemokine receptor D6 constitutively traffics to and from the cell surface to internalize and degrade chemokines. *Mol Biol Cell*, 15(5), 2492-2508.
- Weill, N., & Rognan, D. (2009). Development and validation of a novel protein-ligand fingerprint to mine chemogenomic space: application to G protein-coupled receptors and their ligands. *J Chem Inf Model*, 49(4), 1049-1062.
- Wells, J. A., & McClendon, C. L. (2007). Reaching for high-hanging fruit in drug discovery at protein-protein interfaces. *Nature*, 450(7172), 1001-1009.
- Wells, T. N., Power, C. A., Shaw, J. P., & Proudfoot, A. E. (2006). Chemokine blockers--therapeutics in the making? *Trends Pharmacol Sci*, 27(1), 41-47.
- Wermuth, C. G. (2006). Selective optimization of side activities: the SOSA approach. *Drug Discov Today*, 11(3-4), 160-164.
- Wesolowski, J., Alzogaray, V., Reyelt, J., Unger, M., Juarez, K., Urrutia, M., et al. (2009). Single domain antibodies: promising experimental and therapeutic tools in infection and immunity. *Med Microbiol Immunol*, 198(3), 157-174.
- Whiting, D., Hsieh, G., Yun, J. J., Banerji, A., Yao, W., Fishbein, M. C., et al. (2004). Chemokine monokine induced by IFN-gamma/CXC chemokine ligand 9 stimulates T lymphocyte proliferation and effector cytokine production. *J Immunol*, 172(12), 7417-7424.
- Wu, F. Y., Ou, Z. L., Feng, L. Y., Luo, J. M., Wang, L. P., Shen, Z. Z., et al. (2008). Chemokine decoy receptor d6 plays a negative role in human breast cancer. *Mol Cancer Res*, 6(8), 1276-1288.
- Xu, H., Xu, W., Chu, Y., Gong, Y., Jiang, Z., & Xiong, S. (2005). Involvement of up-regulated CXC chemokine ligand 16/scavenger receptor that binds phosphatidylserine and oxidized lipoprotein in endotoxin-induced lethal liver injury via regulation of T-cell recruitment and adhesion. *Infect Immun*, 73(7), 4007-4016.

- Xue, M. L., Thakur, A., Cole, N., Lloyd, A., Stapleton, F., Wakefield, D., et al. (2007). A critical role for CCL2 and CCL3 chemokines in the regulation of polymorphonuclear neutrophils recruitment during corneal infection in mice. *Immunol Cell Biol*, 85(7), 525-531.
- Yang, M., Mailhot, G., MacKay, C. A., Mason-Savas, A., Aubin, J., & Odgren, P. R. (2006). Chemokine and chemokine receptor expression during colony stimulating factor-1-induced osteoclast differentiation in the toothless osteopetrotic rat: a key role for CCL9 (MIP-1gamma) in osteoclastogenesis in vivo and in vitro. *Blood*, 107(6), 2262-2270.
- Yang, S. H., Kim, S. J., Kim, N., Oh, J. E., Lee, J. G., Chung, N. H., et al. (2008). NKT cells inhibit the development of experimental crescentic glomerulonephritis. *J Am Soc Nephrol*, 19(9), 1663-1671.
- Ye, J., Kohli, L. L., & Stone, M. J. (2000). Characterization of binding between the chemokine eotaxin and peptides derived from the chemokine receptor CCR3. *J Biol Chem*, 275(35), 27250-27257.
- Yoneyama, H., Harada, A., Imai, T., Baba, M., Yoshie, O., Zhang, Y., et al. (1998). Pivotal role of TARC, a CC chemokine, in bacteria-induced fulminant hepatic failure in mice. *J Clin Invest*, 102(11), 1933-1941.
- Young, K. C., Torres, E., Hatzistergos, K. E., Hehre, D., Suguihara, C., & Hare, J. M. (2009). Inhibition of the SDF-1/CXCR4 axis attenuates neonatal hypoxia-induced pulmonary hypertension. *Circ Res*, 104(11), 1293-1301.
- Yue, Y., Xu, W., Hu, L., Jiang, Z., & Xiong, S. (2009). Enhanced resistance to coxsackievirus B3-induced myocarditis by intranasal co-immunization of lymphotactin gene encapsulated in chitosan particle. *Virology*, 386(2), 438-447.
- Zabel, B. A., Wang, Y., Lewen, S., Berahovich, R. D., Penfold, M. E., Zhang, P., et al. (2009). Elucidation of CXCR7-mediated signaling events and inhibition of CXCR4-mediated tumor cell transendothelial migration by CXCR7 ligands. *J Immunol*, 183(5), 3204-3211.
- Zheng, B., Ozen, Z., Zhang, X., De Silva, S., Marinova, E., Guo, L., et al. (2005). CXCL13 neutralization reduces the severity of collagen-induced arthritis. *Arthritis Rheum*, 52(2), 620-626.
- Zhu, Y. M., Bagstaff, S. M., & Woll, P. J. (2006). Production and upregulation of granulocyte chemotactic protein-2/CXCL6 by IL-1beta and hypoxia in small cell lung cancer. *Br J Cancer*, 94(12), 1936-1941.
- Zhu, Y. M., Webster, S. J., Flower, D., & Woll, P. J. (2004). Interleukin-8/CXCL8 is a growth factor for human lung cancer cells. *Br J Cancer*, 91(11), 1970-1976.
- Zoffmann, S., Chollet, A., & Galzi, J. L. (2002). Identification of the extracellular loop 2 as the point of interaction between the N terminus of the chemokine MIP-1alpha and its CCR1 receptor. *Mol Pharmacol*, 62(3), 729-736.
- Zou, Y. R., Kottmann, A. H., Kuroda, M., Taniuchi, I., & Littman, D. R. (1998). Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. *Nature*, 393(6685), 595-599.

Figure 2

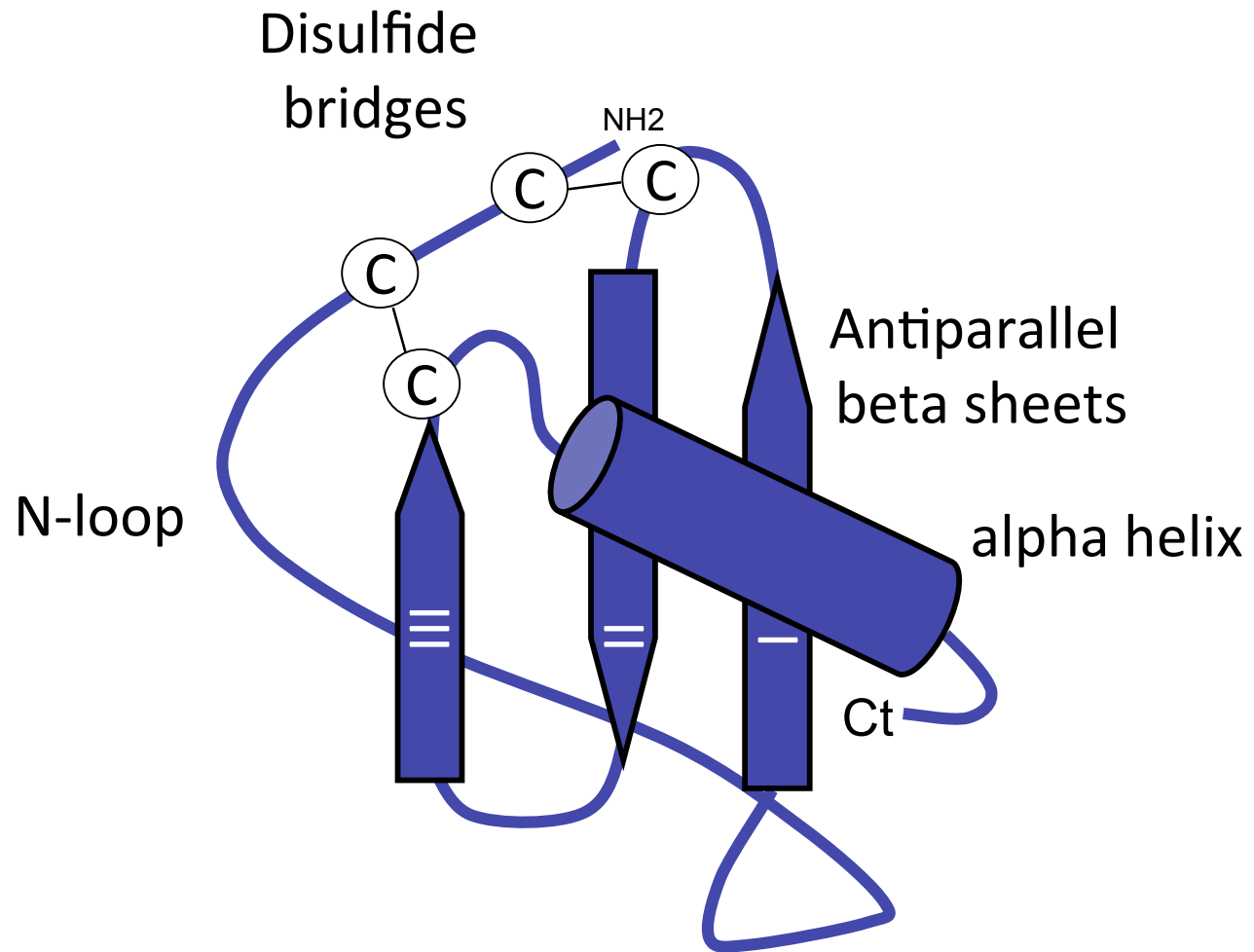


Figure 3

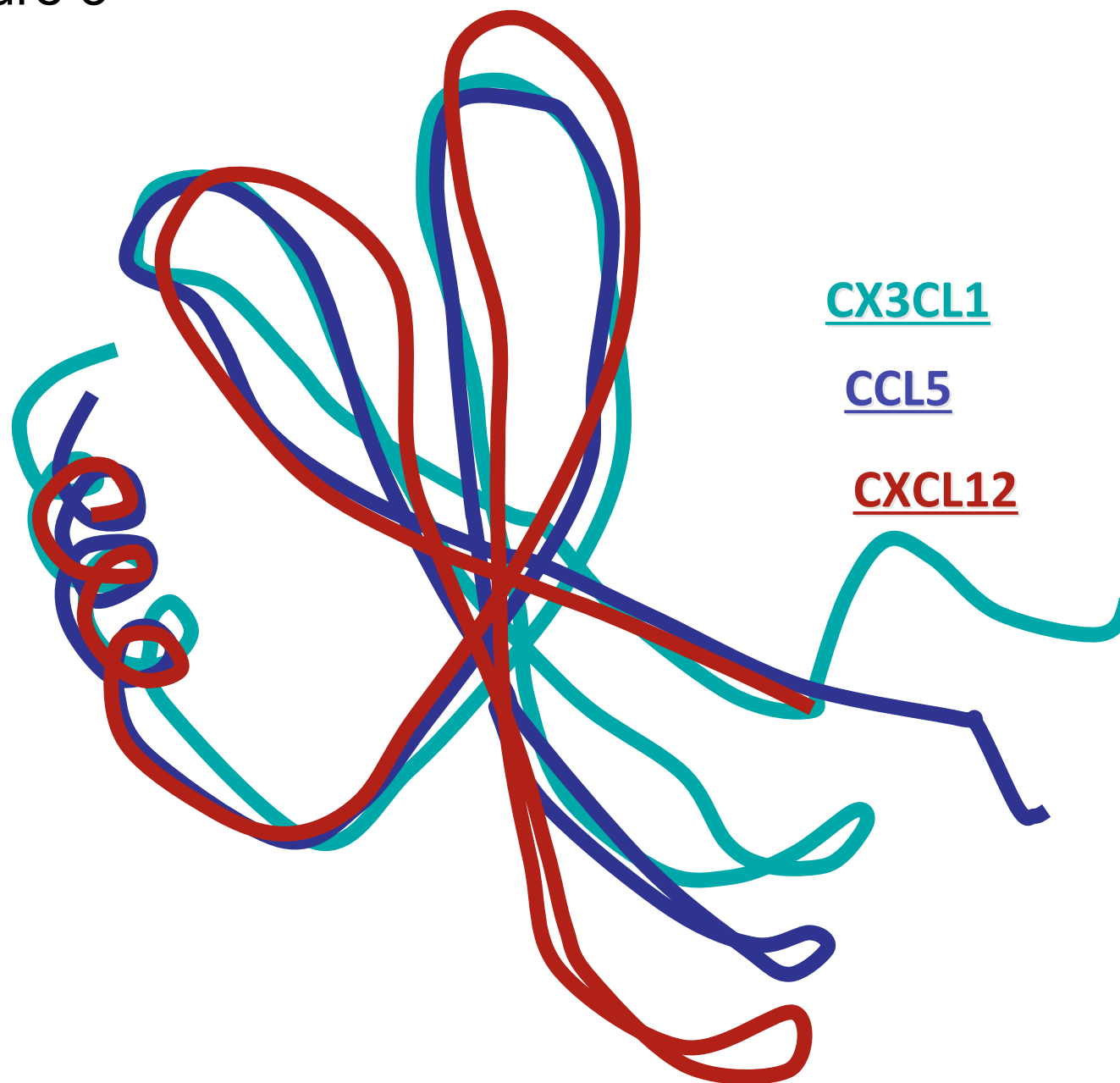
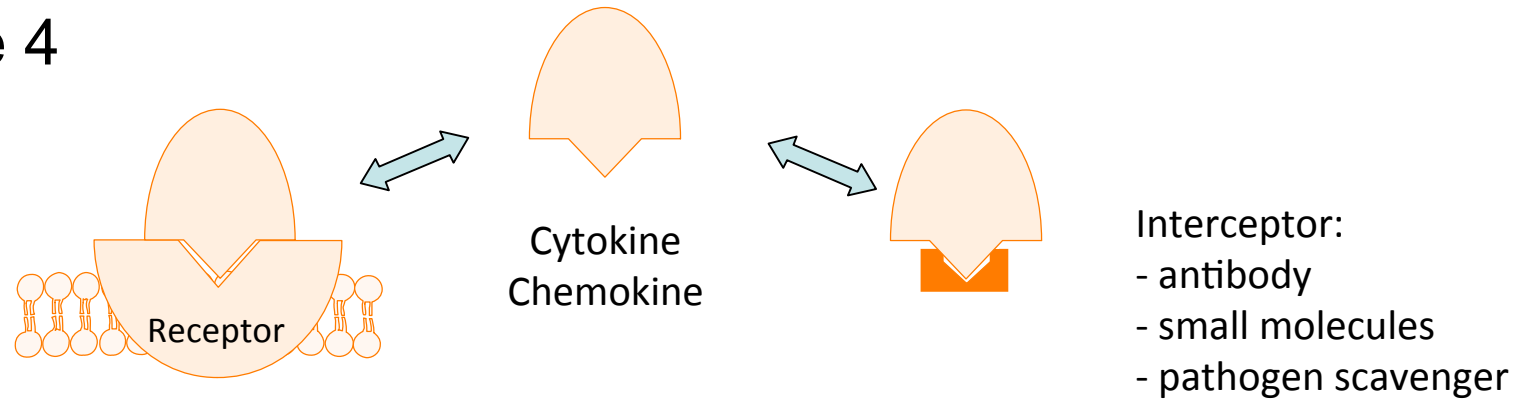
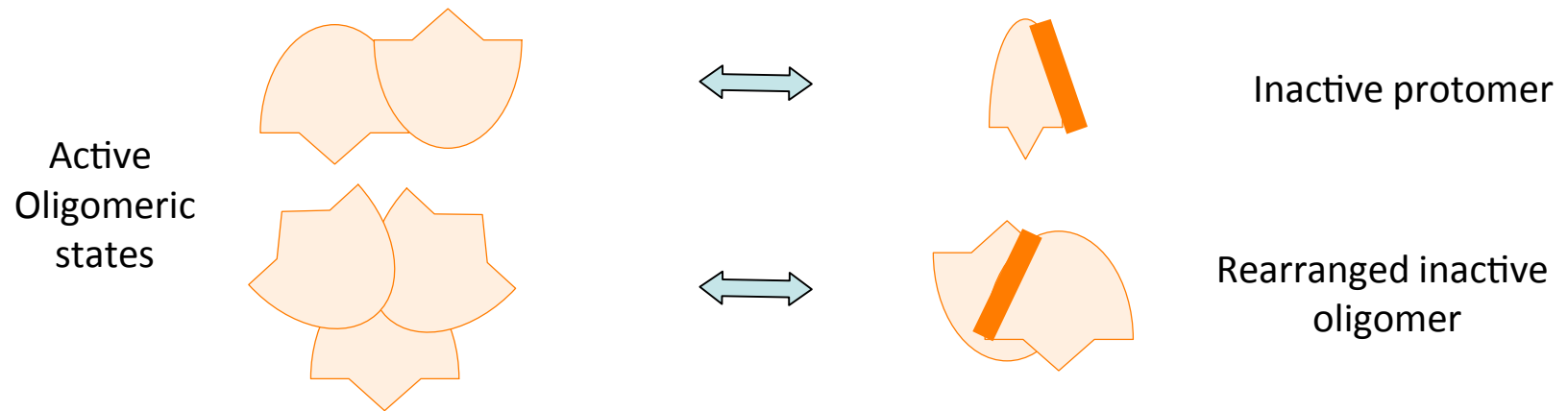


Figure 4

a



b



c

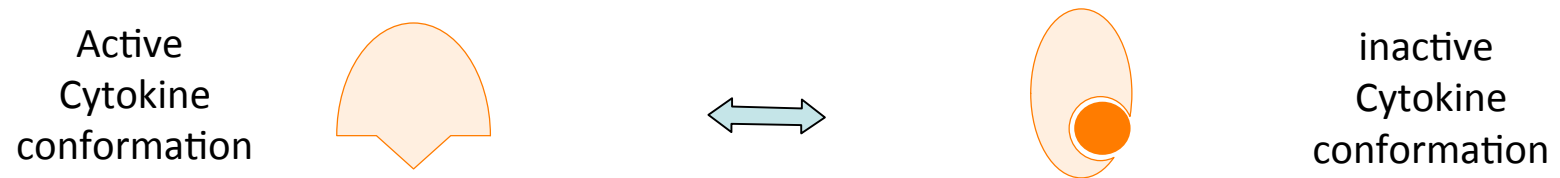


Figure 5

