Chemokines and their receptors

The Euroconference entitled *Chemokines and their receptors* (11–13 March 1999) was a well-attended and well-organized meeting devoted to important conceptual themes and breaking news in the field. The members of this family of small (8–10 Kd) proteins and their seven transmembrane domains continue to grow, with 16 well-characterized receptors and an unknown number of related receptors whose ligands remain to be identified. There are 40–50 chemokine ligands, making this family of receptors and ligands perhaps the largest single classifiable G protein-coupled receptor (GPCR) family outside of the odourant receptors. Appropriately, therefore, it is not surprising that the initial concept of leukocyte recruitment and activation in inflammation, although still relevant, is beginning to broaden and deepen in both related and surprising areas.

Chemokine structure

Evidence shows that the biochemistry in this field is more complex than originally thought, and this is reflected by the fairly low (15–50%) amino acid sequence identity between chemokines, although the conserved Cys-, Pro- and Gly-residues are key exceptions. However, Ian Clark-Lewis (University of British Columbia, Vancouver) reminded us that the α-carbon backbone structure is surprisingly similar in each of the 12 chemokines whose X-ray or solution structures have been determined. The ability of this family of chemokines to execute such a large set of successful receptor–ligand pairs resides in this dominant structural theme that can have many subtle, but crucial, variations. These variations appear to be in the N-terminal domain, up to 11 residues prior to the first Cys. This is the region of the molecule that is seen by nuclear magnetic resonance to have several different conformations and is, therefore, the most disordered in X-ray or solution structures. For the first time for any chemokine, in this case the truncated peptide comprising the first nine amino acid residues of stromal cell-derived factor (SDF), it was shown that specific signalling responses can be obtained with a very small fragment (Clark-Lewis). Although the universality of this observation remains to be demonstrated, it suggests that the rest of the molecule exists in its highly ordered structure primarily to provide specific binding to the receptor and perhaps to proteoglycans. This observation supports the two-site binding models of chemokines to their receptors, which have been in favour for some time now[1].

Another important feature of chemokine structure was emphasized by Timothy Williams (Imperial College, London, UK), whose elegant re-inventions of chemotaxis assays in the guinea pig mesentery *in vivo* showed that native glycosylated eotaxin was active whereas synthetic material was inactive. Although the interpretation of these results is still in some doubt, it did serve as a reminder that chemokines produced in mammalian cells are glycosylated, sometimes heavily, and that a full understanding of their role *in vivo* will have to take this into account.

Lymphocyte migration patterns

Important advances in our understanding of lymphocyte migration patterns were presented by a number of investigators. An extension of the role of chemokines in inflammation to basal trafficking of leukocytes was presented by Martin Lipp (Max Delbrueck Centre, Berlin, Germany). His focus on the chemokine receptor CCR7 is interesting in this context, as work by others has shown an important role for this receptor in mediating the chemotaxis of naïve T cells to the CCR7 ligand, the secondary lymphoid tissue chemokine (SLC). Mice, in which this receptor had been removed from birth, showed a reduction in the number of T cells in mesenteric and peripheral lymph nodes, an increase in their numbers in the spleen and bone marrow, and an increase in T cells with the naïve $CD62L^{hi}$ phenotype in the peripheral circulation. Thus far, this phenotype is consistent with that of plt mice (paucity of lymph node T cells), which has been recently suggested to lack SLC (M. Gunn, Duke University, NC, USA).

Walter Newman (LeukoSite, MA, USA) expanded on the theme of tissue-homing propensities of lymphocyte subsets defined by chemokine receptors by presenting data that, for the first time, identified the CC chemokine, thymus-expressed chemokine (TECK), as a ligand for the orphan receptor gpr-9-6. By using antibodies to block this receptor, its expression was demonstrated on both thymocytes and a small subset of peripheral blood CD4 cells, both of which responded to TECK in chemotaxis assays. Of particular interest was the selective expression of gpr-9-6 on the mucosal homing population of CD4 cells as characterized by the high levels of expression of the integrin, α4β7.

Inflammation and drug discovery

The conference also included interesting presentations on asthma and transplantation. In the asthma arena,

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Professor Williams showed that the role of eotaxin is more complex than simple recruitment of eosinophils into the lung. Noting that atopic individuals have an increased number of CD34-positive cells in their circulation, Williams has now shown that, in guinea pigs, eotaxin stimulates the release of eosinophil– and granulocyte–macrophage-colony forming precursors from the bone marrow, as well as from more mature marginated eosinophils. This pathway provides another reservoir of cells that presumably express the CCR3 receptor and could move to, and perhaps mature at, sites where eosinophils localize, such as the gut and the lung.

It might be predicted that mice that lack expression of the eotaxin receptor, CCR3, traffick eosinophils in an unusual way, data to this effect being presented by Craig Gerard (Children's Hospital, MA, USA). Work in progress indicated that, in otherwise-untreated mice, the basal trafficking of eosinophils to the gut was greatly reduced and that many of these cells could, in fact, be found in the spleen. Although the responsiveness of these mice in an asthma model is not complete, there does seem to be a reduction in eosinophil recruitment to the lung in ovalbumin-sensitized and -challenged animals.

In the transplant arena, Wayne Hancock (LeukoSite, MA, USA) showed the first *in vivo* evidence that a specific chemokine receptor plays a crucial role in transplant rejection and arteriosclerosis. Mice with a targeted deletion of the CCR1 gene showed indefinite cardiac graft survival across a class 2 mismatch barrier, whereas those that were also given low-dose cyclosporin had an indefinite cardiac graft survival across both a class 1 and a class 2 mismatch barrier. While animals rendered tolerant to cardiac allografts by CD4 monoclonal-antibody treatment show indefinite graft survival, they also show profound arteriosclerosis. This feature is essentially absent from tolerized and engrafted CCR1-knockout mice.

The most dramatic results in an asthma model were presented by Jose-Carlos Gutierrez-Ramos (Millennium, MA, USA). Using a somewhat unusual model, he was nevertheless able to show an effective reduction of the inflammation in the lung interstitium and a concurrent reduction in bronchial hyperreactivity, interleukin-4 (IL-4) production and T-helper 2 (Th2) recruitment by pre-administration of a blocking antibody to the CCR4 ligand, macrophage-derived chemokine (MDC). A therapeutic effect did not correlate with a reduction in recruitment of leukocytes solely to the lavageable airways. Although this might be expected, it highlights the need, in both preclinical and clinical studies, to go beyond counting cells harvested from lavages.

On the continuously evolving subject of chemokines and viral infections, Susan Michaelson (Institut Pasteur, Paris, France) showed interesting data on yet another possible mechanism of viral subversion of chemokine function. In this case, cytomegalovirus (CMV) encoded chemokine-like receptors, US27 and US28. Her data implicated these putative receptors as instrumental in the reduction in binding and internalization of MCP-1 (monocyte chemoattractant protein-1) and RANTES (regulated on activation, normal T-expressed and secreted) produced by CMV-infected fibroblasts.

Lastly, Maria Webb (Pharmacopeia, NJ, USA) presented her group's efforts to produce small-molecule antagonists of the IL-8RA and IL-8RB receptors (also known as CXCR1 and CXCR2 receptors, respectively). Although a potent lead at 37 nM has been developed for the CCR2 receptor, the limited species cross-reactivity (no potency even in primates) and lack of effect on the CXCR1 receptor might limit its development.

The most important points in the meeting were fortunately a reflection of the major issues confronting the field, namely the *in vivo* role of this family. The sophistication apparent in the use of knockouts, conditional knockouts, transgenics, and plain inventive preclinical studies, has increased our understanding of leukocyte traffic patterns in unexpected ways. The next challenge will be to translate this information from small-animal-studies into the clinic.

A second EuroConference on chemokines is planned for Spring 2001.

REFERENCE

1 Montecarlo, F.S. and Charo, I.F. (1996) *J. Biol. Chem.* 271, 19084–19092

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In short…

MDS Inc. (Toronto, Canada) acquired the clinical and bioanalytical operations of **LAB Pharmacological Research** (Montreal, Canada) on 20 April 1999. MDS intend to integrate the LAB facilities and business units with two of its existing operations, thereby expanding specifically the clinical pharmacology and bioanalytical capabilities of the MDS Drug Discovery and Development Sector. By acquiring LAB's 130-bed clinical pharmacology unit, MDS Harris will increase its total bed capacity to 452, broadening its ability to conduct Phase I and II clinical trials. Furthermore, the recruiting capabilities of MDS Harris will be expanded in several population areas including liver, renal, postmenopausal and surgically sterile women, and active asthmatics.