NEWS AND COMMENTARY

Starfish MIFs expand chemokine mechanisms

Discovery of a startling star: chemotaxis and chemotactic inhibition by starfish MIFs

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Immune cell recruitment is a crucial process in cell homing and vertebrate immunity, initiating powerful host innate and adaptive defense mechanisms. However, immune cell recruitment also critically contributes to the pathologic sequelae of events driving various inflammatory diseases in humans such as atherosclerosis. The recruitment process is executed by chemokines, which promote the directed migration and arrest of a variety of immune and inflammatory cells in a receptor-specific manner. Specificity and diversity is guaranteed by an impressive repertoire of chemokine ligands interacting with their G protein-coupled receptors (GPCRs). Several organisms including invertebrates do not express classical CC-, CXC-, C-, or CXXC-chemokines or their prototypical GPCR receptors, suggesting that in those organisms, yet additional mechanisms controlling chemotactic immune cell migration are in place. In this issue of Immunology and Cell Biology, Furukawa et al. identify two orthodoxes of macrophage migration inhibitory factor (MIF; i.e., ApMIF1 and ApMIF2) in the starfish Patiria (Asterina)pectinifera (Ap).

Using starfish larvae in vivo, the authors demonstrate that the ApMIFs control the chemotactic recruitment of mesenchyme cells, that is, innate immune-like cells in starfish, in response to foreign substance challenge (Figure 1). The authors show that the ApMIFs phylogenetically resemble with human MIF and its homolog d-dopachrome tautomerase (D-DT or MIF-2). MIF is an evolutionarily conserved inflammatory cytokine, and is a prototypical ‘chemokine-like function’ or ‘atypical chemokine’. MIF and other atypical chemokines are structurally distinct from classical chemokines, yet interact with classical chemokine receptors in a surprisingly specific manner. Therefore, chemokine-like function chemokines enhance the complexity and redundancy in chemokine-driven host immune responses to assure rapid, fine-tuned migration and inflammatory responses of numerous cell types. An elucidation of the responsible residues that underlie binding between atypical chemokines and classical chemokine GPCRs is under intensive investigation.

Intriguingly, the functional knockdown experiments in starfish larvae performed by Furukawa et al. indicate that ApMIF1 and ApMIF2 behave antagonistically. Knockdown of ApMIF1 led to a strong elevation in mesenchymal cell recruitment, whereas interference with ApMIF2 ablated mesenchyme cell movement, suggesting that ApMIF2 has chemokine-like properties, whereas ApMIF1 serves as an inhibitor of cell migration. This opposing behavior is in contrast to what has been observed so far for the human MIF homologs. Although chemotaxis has not directly been studied, signaling studies in several cell models imply that the mammalian MIFs function in an accordant manner, amplifying each other. The current paper thus uncovers a novel immune mechanism in invertebrates that will enhance our understanding of the ligand–receptor mechanisms underlying human MIF and D-DT responses. Their findings may have important implications in developing novel molecular strategies to specifically interfere with the pathologic effects of MIF versus D-DT in human immune and inflammatory diseases.

The chemotaxis-inhibitory effect of ApMIF1 is intriguing as human MIF was initially discovered as ‘macrophage migration inhibitory factor’ half a century ago. Although this ancient eponymous activity refers to an effect on the random movement of macrophages out of capillary devices, some recent studies have reported on chemotaxis-inhibitory effects of MIF. An explanation offered has been desensitization of a MIF GPCR receptor. In fact, in addition to CD74/ invariant chain (Ii), which can engage both MIF and D-DT to trigger inflammatory and pro-survival responses in monocytes/macrophages, activated endothelium, and various tumor cells, MIF is a high-affinity, non-cognate ligand of the classical chemokine receptors CXCR2 and CXCR4. CXCR2 ligation by MIF elicits the recruitment of monocytes/macrophages into atherosclerotic lesions, whereas the MIF/CXCR4 axis supports T lymphocyte and progenitor cell recruitment as well as tumor cell metastasis. The role of MIF-2/D-DT in immune cell recruitment is unknown. Understanding the structural determinants underlying the promiscuous binding of MIF to classical chemokine receptors is an important research task and a prerequisite to design receptor-specific MIF-based therapeutic interference strategies in inflammatory disease. However, although a pseudo-ELR (glutamate, leucine, arginine) motif and an N-like loop have been identified in MIF to contribute to CXCR2 binding, the details of MIF/CXCR4 and MIF-2/CD74 interactions are not well understood. The study by Furukawa et al. provides important insight in this respect. According to their data, ApMIF1 clusters with human MIF but not with ApMIF2 or MIF-2/D-DT. Furthermore, the N-like loop sequence of ApMIF1 is 80% identical to that of human MIF, whereas that in ApMIF2 only shares 40% identity with human MIF or D-DT. A pseudo-ELR motif was only found in ApMIF2, and apparently is
not present in D-DT. Yet, the in vivo knockdown in oil droplet-injected starfish larvae and in vitro chemotaxis experiments with mesenchyme cells performed by the authors clearly suggest that ApMIF1 exerts strong chemotaxis-blocking effects whereas ApMIF2 promotes invertebrate immune cell recruitment. Thus, with respect to receptor usage, and the involved molecular determinants, the starfish MIF data offer the interesting concept that both MIF homologs—across species—are able to either promote or inhibit immune cell chemotaxis. The outcome would depend on the specific receptor expression profile and the associated downstream signaling components. As human D-DT does not feature a pseudo-ELR motif and lacks most of the N-like loop, it will be important to learn whether this homolog promotes or blocks cell recruitment of CXCR2/4-expressing immune cells.

Overall, the study by Furukawa et al. confirms the importance of chemokine-like mediators in controlling immune cell recruitment, and significantly expands the current concept of promiscuous CXCR chemokine receptor usage by MIF proteins and possibly other atypical chemokines. Their data imply that the mere presence of critical receptor binding motifs such as ELR or N-loop mediates receptor interactions but does not qualitatively determine the outcome of the response. A major limitation of this attractive conclusion is that prior studies have failed to identify putative classical chemokine receptors in invertebrates. Starfish larvae hence likely do not express MIF chemokine receptor orthologs. It is also questionable whether P. pectinifera mesenchyme cells express an ortholog of CD74. Nevertheless, the recent discovery of a starfish DOCK2 ortholog, a signaling protein, which is also involved in CXCR2/4-mediated chemotactic processes in human immune cells, argues that in principal, the molecular machinery is in place. An important future task will therefore be to identify the receptor(s) in starfish mesenchyme cells or other invertebrate innate immune cells that mediate recruitment responses. It will be intriguing to find out whether these are GPCR-like receptors or belong to yet unanticipated classes of membrane proteins.

**CONFLICT OF INTEREST**
The authors declare no conflict of interest.

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