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Commentary

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Chemokines as relay signals in human dendritic cell migration: Serum amyloid A kicks off chemotaxis

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Cell migration is a response highly conserved in evolution. Chemotactic factors secreted in injured and inflamed tissues generate a concentration-based, chemotactic gradient that directs leukocytes from the blood compartment into tissue. In this issue of the *European Journal of Immunology*, Gouwy et al. [Eur. J. Immunol. 2015. 45: 101–112] show that the SAA1α isoform of serum amyloid A (SAA), which is an acute phase protein upregulated in inflammation and shown to chemoattract some leukocyte subsets, is also able to chemoattract monocyte-derived immature dendritic cells (DCs). The authors also show that the chemotactic activity of SAA1α for monocytes and DCs is indirectly mediated by rapid chemokine induction, providing evidence that proposes a new level of regulation of leukocyte migration.

Keywords: Chemokines · Chemotaxis · Dendritic cells · Monocytes · Migration · Serum amyloid A

See accompanying article by Gouwy et al.

Cell migration is a fundamental process in many diverse physiological and pathological conditions, such as organogenesis, angiogenesis, inflammation, tumor growth and dissemination, and immunity (reviewed in [1]). During immune responses, the appropriate distribution of leukocytes from the circulation to different anatomical compartments is required for the establishment of protective responses. The process of leukocyte migration involves different classes of molecules, such as adhesion molecules, proteases, glycosaminoglycans, and chemotactic factors. A crucial step in leukocyte migration is the formation of a chemotactic gradient that directs the cells from the circulation to the site of inflammation [2]. Chemotactic factors differ in their nature and include bacterial components such as formylated peptides, lipids such as leukotriene B4 (LTB4) and platelet activating factor (PAF), nucleotides such as ATP, and proteins, among which chemokines represent the largest family (reviewed in [3, 4]). Although it is expected that in vivo each chemotactic factor is characterized by specific spatiotemporal kinetics, it is likely that at the site of inflammation cells are exposed to a wide array of chemotactic agonists and that the final outcome will result from the integration of multiple signals. Previous in vitro and in vivo studies have shown that the concomitant activation of multiple chemokine receptors, such as CXCR1 and CXCR4, promotes, in a synergic manner, the chemotactic response of different leukocyte subsets, including dendritic cells (DCs) [5, 6]. This cooperative interaction of chemotactic factors has also been observed between chemokines, such as CCL2, CCL5, and CCL7 and lipid mediators, such as PAF, arachidonic acid, prostaglandin E2 (PGE2), and LTB4 [7–11].

Searching for new synergistic interactions between cytokines and chemokines, Gouwy et al., in a study published in this issue [12], investigated the chemotactic activity of serum amyloid A (SAA) for human DCs. SAA represent a family of acute phase proteins rapidly produced by hepatocytes in response to injury, microbial infection, and inflammation. In reactive conditions, extrahepatic expression of SAA has also been reported in adipose tissue, endothelial cells, and macrophages [13]. SAA is a pleiotropic cytokine involved in cholesterol transport, remodeling

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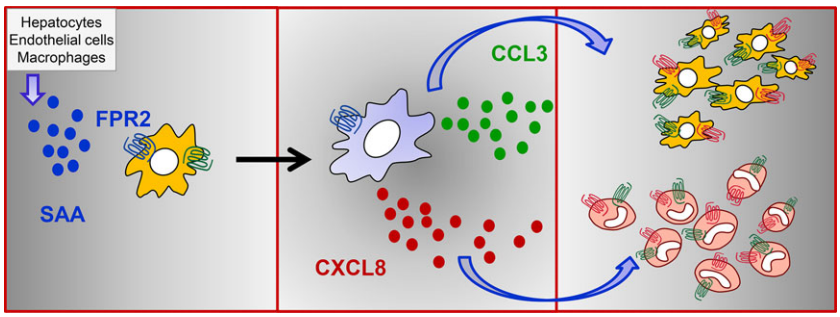

Figure 1. SAA-induced chemokines as relay signals in DC migration. The chemotactic activity of SAA (blue circles, left), produced by hepatocytes, adipose tissue, endothelial cells and macrophages, induces a FPR2-dependent migration of monocytes and DCs through the secondary production of CCL3 (green symbols) and CXCL8 (red symbols) by migrating cells, as shown in Gouwy et al. [12]. These secondarily released chemokines cooperate with SAA to induce DC migration and may promote the recruitment of distant cells (long blue arrows).

of extracellular matrix proteins and inflammation, through the secretion of cytokines, such as CXCL8, IL-12, IL-23, and the IL-1-family member IL-33 [14, 15]. SAA has been shown to induce, in vitro and in vivo, the adhesion and migration of many circulating leukocyte subsets, including mast cells, neutrophils, and monocytes [16, 17]. SAA interacts with multiple chemotactic receptors, including FPR2 (previously known as FPRL1) a promiscuous low affinity chemotactic receptor for formylated peptides [18]. FPR2 is expressed in myeloid cells, including immature DCs (reviewed in [19]). The peculiar aspects of the study by Gouwy et al. [12] are: (i) in addition to monocytes, mast cells, T cells, and granulocytes, which are known targets of SAA-induced migration, DCs are also a target for SAA-induced migration; (ii) in human DCs, SAA-induced chemotactic activity is strongly dependent on the secondary induction of CCL3 in migrating DCs. Consistent with this observation, Gouwy et al. [12] show that SAA-induced migration was significantly reduced either following CCL3 receptor desensitization, or in the presence of a neutralizing anti-CCL3 antibody, or finally, by targeting CCL3 receptors by a combination of anti-CCR1 and CCR5 receptor antagonists. Similar results were also obtained with human monocytes. However, in the case of monocytes, SAA chemotactic activity was sustained by the secondary production of two chemokines, namely CCL3 and CXCL8. Consistent with these results, increased CCL3 and CXCL8 levels were detected in the lower well of the chemotactic chamber, indicating that SAA-stimulated monocytes are responsible for the generation of a chemotactic gradient during the chemotaxis assay.

These results are reminiscent of those previously reported for another cytokine, activin A, a member of the TGF-β cytokine family [20]. Activin A is a homodimeric protein that signals through the activation of heterodimeric transmembrane serine/threonine kinase receptor complexes consisting of type I (ALK 2, 4 or 7) and type II receptors (ActRIIA and ActRIIB) (reviewed in [21]). These receptors, which are a departure from the classic Gαi-protein-coupled seven-transmembrane-domain chemotactic receptors, activate an intracellular Smad-dependent signaling pathway typical of the TGF-β receptor family (reviewed in [22]). In the case of the study with activin A, this cytokine was shown to induce DC migration through the secondary production of two chemokines, CXCL12 and CXCL14 [20]. Both chemokines were rapidly released by migrating DCs through a polarized secretion, allowing the formation of a chemotactic gradient between the lower and the upper wells of the Boyden chamber. Although the study by Gouwy et al. [12] does not directly address the mechanism by which SAA-induced chemokine release is accomplished in order to form a chemotactic gradient, one may speculate that SAA shares comparable secretion mechanisms with activin A in DCs.

It is interesting to note that both SAA and activin A are cytokines with pleiotropic functions that play a role during the early phase of inflammation. For instance, it was shown that blocking activin A production after in vivo injection of LPS results in the inhibition of tumor necrosis factor production [23]. Therefore, an additional role for both SAA and activin A in the amplification of the inflammatory response consists in the promotion of leukocyte recruitment. By showing that CXCL8, a prototypic neutrophil chemotactic factor, may also play a role in monocyte recruitment when cells are exposed to an inflammatory environment, such as that created by SAA stimulation, the work by Gouwy et al. [12] also strengthens the idea that there is a high degree of plasticity within the chemokine system. A similar role for CXCL8 in monocyte adhesion and migration has also been suggested in previous studies [24, 25].

In addition to promoting cell migration, chemokines are also known to activate target cells in terms of lipid secretion and gene transcription. The production of bioactive lipids, such as LTB4, PGE2, and PAF, is the result of membrane phospholipid remodeling by the action of phospholipases (PL), such as PLA2, as was shown for many CC chemokines, like CCL2 and CCL3 [8]. Chemokine-induced gene transcription is usually restricted to a limited set of genes that are functionally relevant for cell extravasation and tissue invasion, such as chemokines and chemokine receptors and molecules involved in extracellular matrix recognition and digestion, such as CCL2, CCR1, urokinase-type plasminogen activator receptor, and matrix metalloproteases-9 and -19 [26].

Studies performed two decades ago highlighted the functional relevance of chemokine-induced PLA2 activation in monocyte migration, showing that PAF and other arachidonic acid-derived products, including 5-oxo-eicosanoids and PGE2, can act in a synergic manner with chemokines to induce cell migration [7, 11, 27]. Similarly, different CC and CXC chemokines can cooperate in a positive manner in inducing leukocyte migration. The molecular basis for this effect is slowly being elucidated and multiple molecular mechanisms are currently proposed. First, synergism may result from the simultaneous or sequential activation of two chemokine receptors by their respective ligands. Alternatively, in

the presence of high concentrations of the ligands, synergism may derive from the activation of a single chemokine receptor by homo- or heteromers of the chemokine ligand (reviewed in [28]). Synergistic activation by chemokines may also derive from the amplification of the receptor signaling cascade. For instance, MAPK phophorylation and increased intracellular calcium levels have been proposed as mechanisms underlying the synergistic activation of monocytes and DCs. Finally, a last proposed mechanism of cooperation between chemokine receptors is based on the formation of homo- or hetero receptor dimers [28]. Independently of the mechanism, synergism between chemokines, usually observed at suboptimal chemokine concentrations, has been clearly documented both in vitro and in vivo and is likely to be one of the mechanisms that finely tunes leukocyte recruitment in inflammation.

Recent work has proposed a new conceptual model that extends the biological meaning of secondary-induced chemokines (reviewed in [29]). In vitro and in vivo experiments have shown that neutrophils, induced to migrate to the site of tissue injury in response to cytokines released from dying cells or bacteria, secrete LTB4 during their migration [30]. In this experimental setting, LTB4 production was shown to function as a signal relay to propagate the information produced at the primary inflammatory site and enhance the radius of directed interstitial neutrophil recruitment [30]. Although this issue is not directly addressed in Gouwy et al. [12], if we were to nevertheless place their findings within this conceptual framework, the secondary and persistent production (up to 24 hours) of CCL3 and CXCL8 by SAA-activated DCs may very well function as a signal relay by migrating cells for upcoming cells. A similar scenario may also apply to other non-professional chemotactic cytokines, such as activin A, which are able to induce chemokine production after stimulation of their target cells [20].

In conclusion, inflammation comprises several amplification mechanisms that sustain the strength, the persistence, and the propagation of the response. Migrating leukocytes are simultaneously exposed to numerous stimuli and their response is the result of the integration of multiple pieces of information. Migrating leukocytes activated by cytokines and chemotactic factors can produce lipids and proteins. These chemotactic agonists can either act in a cooperative manner to potentiate the response of already migrating cells, or alternatively, the release of secondary chemokines may function as a signal relay mechanism. This signal relay would extend, to more distant cells, the information generated by the primary chemotactic signals released at the site of injury, thereby further amplifying the recruitment of leukocytes to the pathological tissue (Fig. 1).

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References

1 **Rot, A. and von Andrian, U. H.**, Chemokines in innate and adaptive host defense: basic chemokinese grammar for immune cells. *Annu. Rev. Immunol.* 2004. **22**: 891–928.

2 **Weber, M., Hauschild, R., Schwarz, J., Moussion, C., de Vries, I., Legler, D. F., Luther, S. A. et al.,** Interstitial dendritic cell guidance by haptotactic chemokine gradients. *Science* 2013. **339**: 328–332.

3 **Del Prete, A., Locati, M., Otero, K., Riboldi, E., Mantovani, A., Vecchi, A. and Sozzani, S.**, Migration of dendritic cells across blood and lymphatic endothelial barriers. *Thromb. Haemost.* 2006. **95**: 22–28.

4 **Sozzani, S.**, Dendritic cell trafficking: more than just chemokines. *Cytokine Growth Factor Rev.* 2005. **16**: 581–592.

5 **Gouwy, M., Struyf, S., Leutenez, L., Portner, N., Sozzani, S. and Van Damme, J.**, Chemokines and other GPCR ligands synergize in receptor-mediated migration of monocyte-derived immature and mature dendritic cells. *Immunobiology* 2014. **219**: 218–229.

6 **Gouwy, M., Struyf, S., Proost, P. and Van Damme, J.**, Synergy in cytokine and chemokine networks amplifies the inflammatory response. *Cytokine Growth Factor Rev.* 2005. **16**: 561–580.

7 **Sozzani, S., Rieppi, M., Locati, M., Zhou, D., Bussolino, F., Proost, P., Van Damme, J. et al.,** Synergism between platelet activating factor and C-C chemokines for arachidonate release in human monocytes. *Biochem. Biophys. Res. Commun.* 1994. **199**: 761–766.

8 **Locati, M., Zhou, D., Luini, W., Evangelista, V., Mantovani, A. and Sozzani, S.**, Rapid induction of arachidonic acid release by monocyte chemotactic protein-1 and related chemokines. Role of Ca2+ influx, synergism with platelet-activating factor and significance for chemotaxis. *J. Biol. Chem.* 1994. **269**: 4746–4753.

9 **Del Prete, A., Shao, W. H., Mitola, S., Santoro, G., Sozzani, S. and Haribabu, B.**, Regulation of dendritic cell migration and adaptive immune response by leukotriene B4 receptors: a role for LTB4 in up-regulation of CCR7 expression and function. *Blood* 2007. **109**: 626–631.

10 **Sadik, C. D. and Luster, A. D.**, Lipid-cytokine-chemokine cascades orchestrate leukocyte recruitment in inflammation. *J. Leukoc. Biol.* 2012. **91**: 207–215.

11 **Panzer, U. and Uguccioni, M.**, Prostaglandin E2 modulates the functional responsiveness of human monocytes to chemokines. *Eur. J. Immunol.* 2004. **34**: 3682–3689.

12 **Gouwy, M., De Buck, M., Portner, N., Opdenakker, G., Proost, P., Struyf, S. and Van Damme, J.**, Serum amyloid A chemoattracts immature dendritic cells and indirectly provokes monocyte chemotaxis by induction of cooperating CC and CXC chemokines. *Eur. J. Immunol.* 2015. **45**: 101–112.

13 **Hardardottir, I., Sipe, J., Moser, A. H., Fielding, C. J., Feingold, K. R. and Grunfeld, C.**, LPS and cytokines regulate extra hepatic mRNA levels of apolipoproteins during the acute phase response in Syrian hamsters. *Biochim. Biophys. Acta* 1997. **1344**: 210–220.

14 **King, V. L., Thompson, J. and Tannock, L. R.**, Serum amyloid A in atherosclerosis. *Curr. Opin. Lipidol.* 2011. **22**: 302–307.

15 **Sun, L., Zhu, Z., Cheng, N., Yan, Q. and Ye, R. D.**, Serum amyloid A induces interleukin-33 expression through an IRF7-dependent pathway. *Eur. J. Immunol.* 2014. **44**: 2153–2164.

16 **Badolato, R., Wang, J. M., Murphy, W. J., Lloyd, A. R., Michiel, D. F., Bausserman, L. L., Kelvin, D. J. et al.,** Serum amyloid A is a chemoattractant: induction of migration, adhesion, and tissue infiltration of monocytes and polymorphonuclear leukocytes. *J. Exp. Med.* 1994. **180**: 203–209.

17 **Olsson, N., Siegbahn, A. and Nilsson, G.**, Serum amyloid A induces chemotaxis of human mast cells by activating a pertussis toxin-sensitive signal transduction pathway. *Biochem. Biophys. Res. Commun.* 1999. **254**: 143–146.

18 **Su, S. B., Gong, W., Gao, J. L., Shen, W., Murphy, P. M., Oppenheim, J. J. and Wang, J. M.**, A seven-transmembrane, G protein-coupled receptor, FPRL1, mediates the chemotactic activity of serum amyloid A for human phagocytic cells. *J. Exp. Med.* 1999. **189**: 395–402.

19 **Ye, R. D., Boulay, F., Wang, J. M., Dahlgren, C., Gerard, C., Parmentier, M., Serhan, C. N. and Murphy, P. M.**, International Union of Basic and Clinical Pharmacology. LXXIII. Nomenclature for the formyl peptide receptor (FPR) family. *Pharmacol. Rev.* 2009. **61**: 119–161.

20 **Salogni, L., Musso, T., Bosisio, D., Mirolo, M., Jala, V. R., Haribabu, B., Locati, M. et al.,** Activin A induces dendritic cell migration through the polarized release of CXC chemokine ligands 12 and 14. *Blood* 2009. **113**: 5848–5856.

21 **Werner, S. and Alzheimer, C.**, Roles of activin in tissue repair, fibrosis, and inflammatory disease. *Cytokine Growth Factor Rev.* 2006. **17**: 157–171.

22 **Sozzani, S. and Musso, T.**, The yin and yang of Activin A. *Blood* 2011. **117**: 5013–5015.

23 **Jones, K. L., Mansell, A., Patella, S., Scott, B. J., Hedger, M. P., de Kretser, D. M. and Phillips, D. J.**, Activin A is a critical component of the inflammatory response, and its binding protein, follistatin, reduces mortality in endotoxemia. *Proc. Natl. Acad. Sci. U S A* 2007. **104**: 16239–16244.

24 **Bonecchi, R., Facchetti, F., Dusi, S., Luini, W., Lissandrini, D., Simmelink, M., Locati, M., et al.,** Induction of functional IL-8 receptors by IL-4 and IL-13 in human monocytes. *J. Immunol.* 2000. **164**: 3862–3869.

25 **Gerszten, R. E., Garcia-Zepeda, E. A., Lim, Y. C., Yoshida, M., Ding, H. A., Gimbrone, M. A., Jr., Luster, A. D. et al.,** MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. *Nature* 1999. **398**: 718–723.

26 **Locati, M., Deuschle, U., Massardi, M. L., Martinez, F. O., Sironi, M., Sozzani, S., Bartfai, T. et al.,** Analysis of the gene expression profile activated by the CC chemokine ligand 5/RANTES and by lipopolysaccharide in human monocytes. *J. Immunol.* 2002. **168**: 3557–3562.

27 **Sozzani, S., Zhou, D., Locati, M., Bernasconi, S., Luini, W., Mantovani, A. and O'Flaherty, J. T.**, Stimulating properties of 5-oxo-eicosanoids for human monocytes: synergism with monocyte chemotactic protein-1 and -3. *J. Immunol.* 1996. **157**: 4664–4671.

28 **Gouwy, M., Schiraldi, M., Struyf, S., Van Damme, J. and Uguccioni, M.**, Possible mechanisms involved in chemokine synergy fine tuning the inflammatory response. *Immunol. Lett.* 2012. **145**: 10–14.

29 **Majumdar, R., Sixt, M. and Parent, C. A.**, New paradigms in the establishment and maintenance of gradients during directed cell migration. *Curr. Opin. Cell Biol.* 2014. **30**: 33–40.

30 **Lammermann, T., Afonso, P. V., Angermann, B. R., Wang, J. M., Kastenmuller, W., Parent, C. A. and Germain, R. N.**, Neutrophil swarms require LTB4 and integrins at sites of cell death in vivo. *Nature* 2013. **498**: 371–375.

Abbreviations: **LTB4**: leukotriene B4 · **PAF**: platelet activating factor · **PGE2**: prostaglandin E2 · **SAA**: serum amyloid A

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