

Chemokine interaction with synergy-inducing molecules: fine tuning modulation of cell trafficking

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ABSTRACT

Directed migration and arrest of leukocytes during homeostasis, inflammation, and tumor development is mediated by the chemokine system, which governs leukocyte migration and activities. Although we understand well the effects of different chemokines one by one, much less was known about the potential consequences of the concomitant expression of multiple chemokines or of their interaction with inflammatory molecules on leukocyte migration and functions. In the past 10 yr, several studies revealed the existence of additional features of chemokines: they can antagonize chemokine receptors or synergize with other chemokines, also by forming heterocomplexes. Moreover, recent data show that not only chemokines but also the alarmin high-mobility group box 1 can form a complex with CXCL12, enhancing its potency on CXCR4. The molecular mechanism underlying the effect of the heterocomplex has been partially elucidated, whereas its structure is a matter of current investigations. The present review discusses the current knowledge and relevance of the functions of heterocomplexes formed between chemokines or between the chemokine CXCL12 and the alarmin high-mobility group box 1. These studies highlight the importance of taking into account, when approaching innovative therapies targeting the chemokine system, also the fact that some chemokines and molecules released in inflammation, can considerably affect the activity of chemokine receptor agonists. *J. Leukoc. Biol.* 99: 851–855; 2016.

Introduction

Chemokines are key regulators of leukocyte migration and have fundamental roles in both physiologic and pathologic immune responses, such as inflammatory diseases [1]. The chemokine system includes a set of about 50 ligands, which engage in a promiscuous fashion a panel of >20 chemokine receptors, which are differentially expressed by all leukocytes and many nonhematopoietic cells, including cancer cells [2]. The resulting combinatorial diversity in

responsiveness to chemokines guarantees the proper tissue distribution of distinct leukocyte subsets under normal and pathologic conditions. Chemokines bind to chemokine receptors, which constitute the largest branch of the γ subfamily of rhodopsin-like GPCRs, a receptor superfamily, which, in modern pharmacology, is the most successful target of small molecule inhibitors for treating diseases affecting various organs, including the central nervous, cardiovascular, pulmonary, or gastrointestinal system.

A vast range of in situ experiments, aimed at understanding which chemokines are produced in specific circumstances, has revealed that a variety of chemokines can be concomitantly produced at the target sites of leukocyte trafficking and homing [3–7]. This renders the chemokine system a good target for therapy and has promoted the search, by pharmaceutical companies, for small-molecule chemokine antagonists [8–10]. Although we understand well the effects of different chemokines one by one, much less was known about the potential consequences of the concomitant expression of multiple chemokines or of their interaction with inflammatory molecules on leukocyte migration and function [11, 12].

Pioneer studies on the interactions among different chemokines have revealed that they can antagonize chemokine receptors [13–17] or synergize with other chemokines. The synergism can occur at different levels, involving either 2 chemokine receptors triggered simultaneously or sequentially exposed to their agonists [18–22] or the activation of one type of chemokine receptor triggered by chemokine heterocomplexes [23].

Chemokine biology is more complex than simple ligand–receptor interaction, as several studies have demonstrated that chemokines can dimerize [24] after binding to GAGs on endothelial cells and that their receptors are found as dimers and/or as oligomers at the cell surface [25–27]. Because of the complexity of the chemokine binding and signaling system [28], several mechanisms have been proposed to provide an explanation for the synergy between chemokines in leukocyte migration.

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Abbreviations: DAMPs = damage-associated molecular patterns, GAG = glycosaminoglycan, GPCR = G protein-coupled receptor, HMGB1 = high mobility group box 1, NMR = nuclear magnetic resonance, PTX = *Bordetella pertussis* toxin

In addition to the several chemokines that, by forming a heterocomplex with chemokine receptor agonists, act as enhancers of molecules of the same family, we have recently identified HMGB1, an endogenous DAMP molecule, as an enhancer of the activity of CXCL12 [29–31].

It is now evident that the synergism between chemokines is crucial at the very early stage of inflammation [32–36], whereas the study of the role of synergy-inducing chemokines in the tumor microenvironment is at its infancy [37]. Moreover, the mechanisms underlying the involvement of endogenous DAMPs in chronic diseases are still largely unexplored, and their interaction with other molecules might be a possible approach to understanding their targets and functions.

CHEMOKINE HETEROCOMPLEXES

We and others have revealed, almost 10 yr ago, a regulatory mechanism governing chemokine-induced migration: the heterodimerization occurring between 2 chemokines [33, 38]. Chemokines heterocomplexes induce activation of chemokine receptors in the presence of low concentrations of chemokine-selective agonists that, in the absence of the synergy-inducing chemokine, would, per se, be inactive [23, 36]. This type of synergism implies the activation of one type of chemokine receptor, which is selective for the chemokine that is present in the environment at low concentration.

Our first evidence was the demonstration that CCL21 or CCL19 can form heterocomplexes with CXCL13, leading to binding and activation of CCR7 at lower agonist concentrations [33]. Importantly, this type of synergism is transmitted by 1 chemokine receptor (e.g., CCR7), which is triggered by the agonist in complex with the synergy-inducing chemokine. The synergy-inducing chemokine is able to bind to the receptor only in the presence of its selective agonist [33]. These initial data were supported by the observation that chemokines, coexpressed with CCL22 at specific sites in the inflamed skin, can synergize with CCR4 ligands via CCR4 activation [32]. Indeed, we found that the migration of CCR4⁺ lymphocytes was strongly enhanced upon concomitant application of CCL22 and CXCL10 and that this synergistic increase was independent from binding to CXCR3 or GAGs. More important, using several hybrids synthesized based on either synergistic or nonsynergistic activity with CCL22, we were able to show that the first β -strand of the synergy-inducing chemokine is necessary for synergizing with CCL22 [32]. In the same year, von Hundelshausen et al. [38] demonstrated that CCL5-mediated arrest of monocytes was enhanced by heterophilic interaction with CXCL4, requiring the presence of GAGs on the cell surface. These authors suggested that the heterocomplex formed by CCL5 and CXCL4 could promote or stabilize heterodimerization of chemokine receptors, resulting in different signaling events. GAGs can, therefore, influence homodimerization or heterodimerization, depending on the chemokines or the cell type involved [32, 38, 39]. Later on, we showed that the homeostatic chemokines CCL19 and CCL21 can form heterocomplexes with CCL7, enhancing monocyte migration and preventing CCL7 degradation mediated by the atypical chemokine receptor 2 [34]. Subsequent studies demonstrated that the coexpression and heterocomplex formation

between CXCL9 and CXCL12, in the tumor vasculature of primary central nervous system lymphomas, might be responsible for the enhanced recruitment of CD8⁺ T cells and for the angiocentric positioning of malignant B cells in the perivascular cuff [37].

CXCL12/HMGB1 HETEROCOMPLEXES

Degryse et al. [40] showed >10 yr ago, that the migration of cells toward HMGB1 can be blocked by PTX, suggesting the involvement of a GPCR associated to G_{i/o} proteins; this observation was, later on, independently confirmed [41]. We and others have shown that the migration of mouse embryonic fibroblasts toward HMGB1 requires the CXCR4 receptor [29, 42] and is, indeed, blocked in vitro by anti-CXCR4 antibodies, by the CXCR4 antagonist AMD3100, and by the neutralizing anti-CXCL12 antibody K15C [43], indicating that HMGB1-induced migration not only requires CXCR4 but also requires the concomitant presence of CXCL12. The influx of leukocytes to the muscle damaged by cardiotoxin, which involves the release of HMGB1, can also be blocked by AMD3100 and by glycyrrhizin, a known inhibitor of HMGB1-induced cell migration [29]. In this model, the HMGB1 receptor for advanced glycation end-products has no critical role because leukocyte influx into the damaged muscle is comparable in *Rage*^{-/-} and wild-type mice [29].

We found both biochemical and functional synergy between HMGB1 and CXCL12 [29]. NMR chemical-shift mapping indicated contacts between CXCL12 and the full-length HMGB1 or its individual HMG boxes. The HMGB1/CXCL12 heterocomplex is more efficient than is CXCL12 alone in inducing human monocyte migration, suggesting that the heterocomplex may present 2 CXCL12 ligand molecules in the correct spatial arrangement to dimers of the CXCR4 receptor. Taking into account that the monomeric form of CXCL12 has been shown to efficiently promote cell migration [44], it is tempting to speculate that HMGB1 would bind 2 CXCL12 monomers. Alternatively, HMGB1 may promote CXCL12 binding by fixing its N-terminal domain in the best conformation for triggering CXCR4. Clearly, more structural studies are required to solve the issue, but fluorescence resonance energy transfer studies with tagged CXCR4 molecules indicate that the conformation of CXCR4 dimers interacting with the HMGB1/CXCL12 heterocomplex is different from the conformation of those interacting with CXCL12 alone.

The interaction between HMGB1 and CXCL12 is specific: HMGB1 does not randomly bind any chemokine. We have evidenced that HMGB1 does not affect migration induced in vitro by other inflammatory or homeostatic chemokines, such as CXCL8, CCL2, and CCL7, and only marginally enhances CCL19- and CCL21-induced migration, even if studies on the possible heterocomplex formation between HMGB1 and the CCR7 agonists have still to be performed [29].

Additional roles of HMGB1 in modulating CXCL12 activities have been described. HMGB1 induces CXCL12 transcription [45] and protects the conformational integrity of the chemokine under reducing conditions [46]. The characterization of the CXCL12 epitopes, which are important in the interaction with HMGB1, can also be pursued using different anti-CXCL12 antibodies, such as K15C [43] and 30D8, as recently described by Zhong et al. [47]. No data are so far available on the HMGB1

role in protecting CXCL12 from degradation via the scavenging receptor atypical chemokine receptor 3.

Intracellular HMGB1 is largely in the reduced state [30, 48], whereas the extracellular milieu oxidizes much more in normal conditions, so that the formation of the C23–C45 disulfide bond in HMGB1 might be expected. Recently, the disulfide-containing form of HMGB1 was shown to induce TNF release from macrophages, in contrast to the completely reduced form of HMGB1, in which all 3 cysteine residues are in the thiol state (all-thiol-HMGB1) [49]. Only all-thiol-HMGB1 has chemotactic activity [30], and correspondingly, all-thiol-HMGB1 forms a heterocomplex with CXCL12, whereas disulfide-HMGB1 does not. Disulfide-HMGB1 does not compete with all-thiol-HMGB1 for cell migration, and all-thiol-HMGB1 does not compete with disulfide-HMGB1 in cytokine stimulation. Indeed, the 2 functions of HMGB1, as chemoattractant and as proinflammatory cytokine, require different receptors, CXCR4 [29, 31] and TLR4 [50], respectively. No data are so far available regarding the heterocomplex formation between HMGB1 and CXCL12 intracellularly nor its activities once released. In the tissue microenvironment, the balance between reducing factors, such as thioredoxins [51–53], which are able to maintain HMGB1 in the reduced form, and the reactive oxygen species, modulates cell recruitment and activation [54, 55].

RELEVANCE OF THE HETEROCOMPLEXES FORMED WITH CHEMOKINES

The importance of heterocomplex formation and synergistic interaction between CXCL4 and CCL5, was demonstrated by Koenen et al. [35] in 2009 in an *in vivo* model of atherosclerosis, where the arrest of monocytes on inflamed endothelium, which results from the CXCL4–CCL5 interaction, can be blocked by peptide inhibitors that specifically disrupt the heterocomplex formation, reducing atherosclerosis progression.

In the model of sterile inflammation, mouse tibialis anterior muscle, damaged by cardiotoxin injection, releases the CXCL12/HMGB1 heterocomplex within 30 min [29]. Monocyte influx into the damaged muscle is abrogated by anti-HMGB1 antibodies, by glycyrrhizin, or by AMD3100, showing that both HMGB1 heterocomplex formation and CXCR4 signaling are required for the recruitment of leukocytes to the damaged tissue.

Recruitment of immune cells at the site of inflammation, enhanced by the interaction between chemokines or inflammatory molecules, could be beneficial for its resolution [56]. When resolution of inflammation fails, the presence of these heterocomplexes could be detrimental and eventually leading to chronic inflammation.

Over the years, the importance of inhibiting HMGB1 activities in several disease models have been widely assessed [57–62], and blockade of the CXCL12/HMGB1 interaction could represent an additional therapeutic strategy in chronic diseases and cancer.

CONCLUDING REMARKS

Several review articles point out the importance of the chemokine network in cell trafficking and activation. A number of components, in addition to the direct activation of the receptor via a selective agonist, can regulate chemokine functions via a direct interaction with chemokines or chemokine receptors. The studies discussed in this review support the concept that multiple chemokines within inflamed tissues selectively enhance each other's migratory functions, depending on their concentrations, proximity, and simultaneous exposure to leukocytes.

We have proposed the heterocomplex formation between chemokines or with inflammatory molecules, such as HMGB1, as an additional mechanism for positive regulation of leukocyte traffic in inflammation. This mechanism involves a single type of chemokine receptor, triggered by its agonist bound to a synergy-inducing chemokine or HMGB1 (Fig. 1). Even if structures

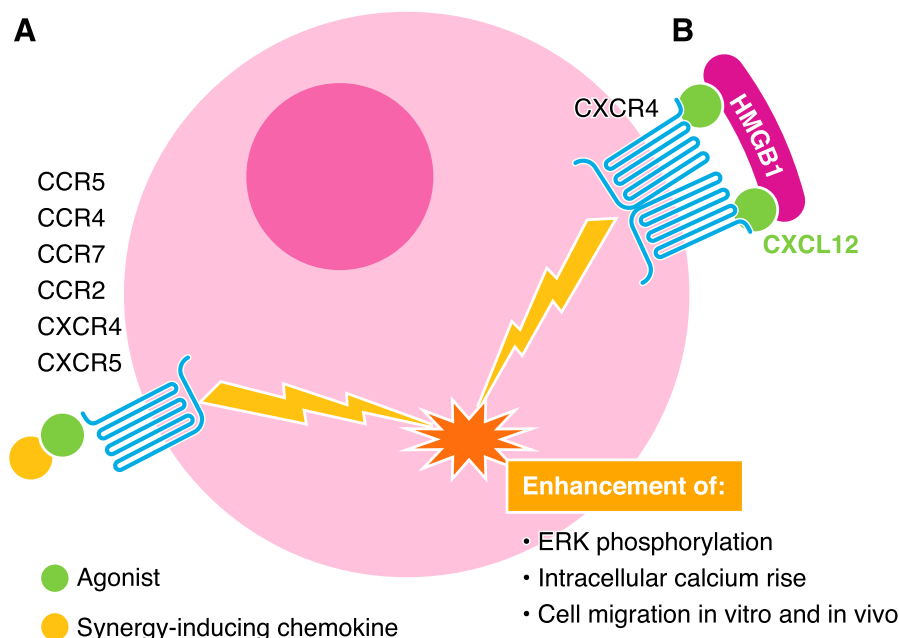


Figure 1. Synergism induced by the formation of heterocomplexes. (A) Heterocomplex formed between 2 chemokines renders the agonist more potent on the selective receptor. (B) HMGB1 forms a heterocomplex with CXCL12 enhancing CXCL12 potency on CXCR4.

of the heterocomplexes are not yet resolved, NMR data indicate that binding of the synergy-inducing molecule induces a modification in the N-terminal domain of the agonist, suggesting that it might fix it in the best conformation for triggering its selective receptor. A thorough study is now necessary to define the structure of the heterocomplex to better define its activity and in designing small-molecule antagonists able to disrupt its formation.

When assessing chemokine activities in inflammation, several aspects need to be taken into account: proteolytic cleavage [63], antagonism, and decoy activities [64], which regulate chemokine signaling, impairing chemokine-mediated effects and, equally important, the protective effect of the heterocomplexes formation. Although, many have studied chemokine synergism, it seems there is still much to uncover about the synergy between chemokines and the molecular mechanisms involved in this cooperation.

The chemokine system remains a promising biologic target for the development of new therapeutic tools for treatment of immunologic disorders. Nevertheless, both well-known and emerging, intrinsic molecular properties represent a limitation, impeding the successful development of conventional, competitive inhibitors. This calls for innovative approaches that will account for some chemokines and molecules being released in inflammation, by forming heterocomplexes, which can affect the activity of chemokine receptor agonists considerably.

AUTHORSHIP

V.C., G.D.A., L.R., and M.U. wrote the review.

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DISCLOSURES

The authors declare no conflicts of interest.

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