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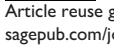


Perspectives on the Biological Role of Chemokine:Glycosaminoglycan Interactions

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Introduction

Chemokines (chemotactic cytokines) are a family of proteins that mediate recruitment and precise positioning of leukocytes in different anatomical locations, and as such are central to the immune response. Despite their importance, there are no Food and Drug Administration–approved therapies targeting chemokines or their receptors to treat inflammatory diseases.[1,2] It has become clear that one of the primary reasons for this failure is the lack of a comprehensive understanding of basic chemokine biology.

Chemokines function by binding to G protein–coupled chemokine receptors on the surface of leukocytes and activating the cell motility machinery that promotes cell movement. However, and of relevance to this perspective, chemokines, with few exceptions, bind to a second type of receptor—glycosaminoglycans (GAGs). A number of papers have demonstrated that this interaction is critical for the ability of chemokines to mediate leukocyte recruitment.[3–7] In fact, it has been suggested that some chemokines, such as CCL18, do not function through chemokine receptors, but rather via GAG binding.[8] The importance of this interaction to chemokine function is demonstrated by the evolution of proteins that inhibit chemokine:GAG interactions. These chemokine-binding proteins are produced by mammals, arachnids, and viruses to stop excessive inflammation and facilitate immune evasion.[9–16] Despite this knowledge, the importance of chemokine:GAG interactions is not fully understood. In this perspective, we will outline a number of emerging concepts and unanswered questions that are being explored to further understand the role of chemokine:GAG interactions in regulating leukocyte recruitment.

How Are Endothelial Immobilized Chemokines Presented to Chemokine Receptors on Leukocytes?

Unlike the fibroblast growth factor system,[17] the chemokine:GAG interaction does not seem to be important for the direct interaction of chemokines with their heptahelical receptors. Instead, interactions with GAGs are thought to facilitate the local retention of chemokines that provide directional signals for migrating cells.[18–20] Chemokine:receptor interactions play an important role in mediating leukocyte firm adhesion to the vascular endothelium via integrin activation, but because of vascular blood flow it seems likely that chemokines would be flushed away in the absence of interactions with endothelial GAGs. This concept has given rise to multiple models for how endothelium-localized chemokines are presented to receptors on leukocytes. One long-standing model, recently coined

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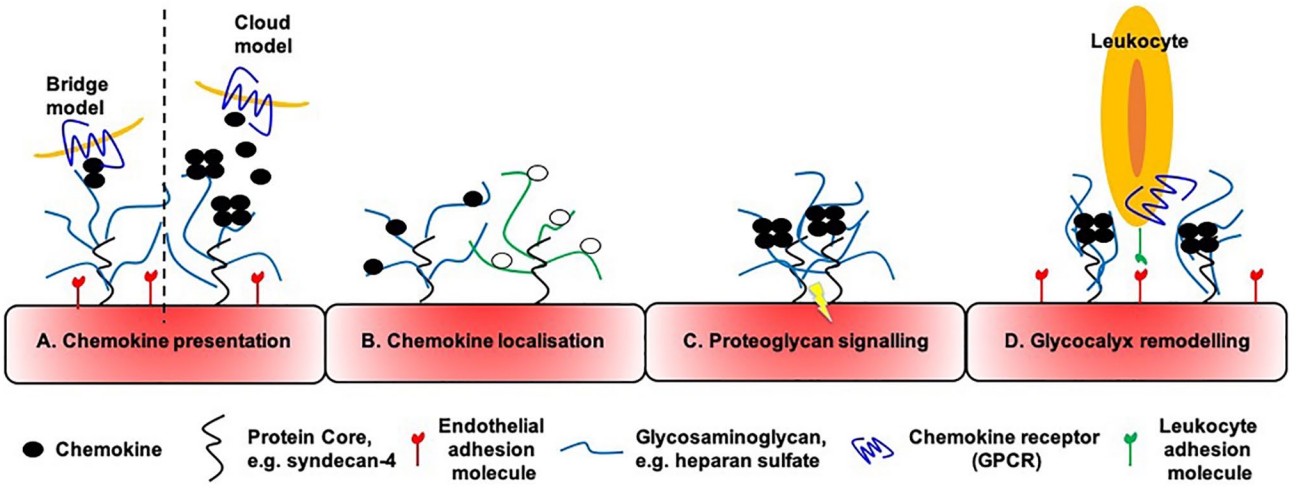

Figure 1. The biological role of chemokine:glycosaminoglycan (GAG) interactions. Chemokines are thought to be presented by GAGs on the endothelial surface to circulating leukocytes to facilitate firm adhesion and leukocyte migration. (A) Two models for this have been proposed: the "bridge" model where chemokines can simultaneously bind to GAGs and their receptors, primarily G protein-coupled receptors (GPCRs), and the "cloud" model where dynamic GAG binding is thought to create localized pools of soluble chemokines that can bind to their receptors. (B) Specific GAG sulphation may tune chemokine binding and allow specific localization. Chemokine-mediated crosslinking of GAG chains may facilitate (C) proteoglycan clustering and signaling and (D) glycocalyx remodeling, leading to increased permeability and adhesion molecule accessibility.

the "bridge model," developed from the observations that (1) many chemokines oligomerize yet they bind their receptors as monomers, and that (2) the GAG and receptor-binding epitopes on chemokines overlap. To reconcile these data, the bridge model suggests that chemokines bind simultaneously to GAGs on endothelial cells and receptors on leukocytes through separate subunits of the chemokine oligomer.[3]

More recently, however, steric considerations stemming from structures of chemokines in complex with full-length receptors vs structures of chemokine oligomers and their GAG-binding epitopes suggest that CC chemokine oligomers cannot bind receptors, which precludes the bridge model.[21] Accordingly, an alternative "cloud model" developed. This model suggests that GAG interactions promote a localized cloud of chemokine at the endothelial cell surface that effectively maintains a dynamic equilibrium of GAG-bound vs soluble chemokine, with only the latter capable of binding chemokine receptors. However, it is possible that CXC chemokine oligomers and chemokines with long flexible GAG-binding tails are not structurally excluded from the bridge model and could in fact simultaneously bind to chemokines and GAGs. Indeed, one study suggests that CXCL12 can simultaneously bind to heparan sulphate and CXCR4 to activate cell adhesion in vitro.[22] This issue, therefore, remains an open question, but if different models apply to different types of chemokines, it will reflect the amazing versatility of these small proteins to alter their functional mechanisms with minor sequence changes.

Do Chemokine:GAG Interactions Encode Functional Specificity?

One of the fundamental challenges in studying and therapeutically targeting the chemokine system has been the issue of redundancy, where multiple chemokine ligands bind a given receptor and a given chemokine binds multiple receptors.[23] However, more recent work using advanced technologies and experiments to address this question suggests that in fact there may be more specificity than currently appreciated.[24–30] Although some specificity seems to be driven by subtly coordinated temporal and spatial patterns of chemokine expression, there are a number of examples where this is not the case.[31] An emerging concept is the ability of GAGs to confer specificity to the chemokine system by differentially localizing ligands to different sites according to their interaction affinity.[31] A number of groups, including our own, have analyzed the nature of the interactions between specific chemokines and GAGs (extensively reviewed in Crijns et al.[4]). These studies have shown that chemokine:GAG complexes have a wide range of affinities (e.g., CCL3 and CCL4 are weak binders, whereas CCL5 and CXCL4 have very high affinities[32,33]) and that there is a strong correlation

between affinity and the degree of oligomerization and the isoelectric point (PI) of the chemokine.[33,34] We also showed that a subset of chemokines that are all associated with monocyte recruitment (CCL2, CCL3, CCL5, CCL7, and CXCL4) have very different abilities to interact with GAGs.[33] Thus, in scenarios where ligands with overlapping receptor-binding patterns are all expressed in the same location, those with low-affinity GAG interactions should be able to diffuse further and encounter receptors at different locations than those that have a high affinity for GAGs. For example, CCL7 has a relatively low-affinity GAG interaction[33] and while being produced at inflammatory sites is thought to travel to the bone marrow to mediate monocyte egress into the circulation.[35] Further insight into the functional consequences of the diverse range of chemokine binding:GAG affinities is needed.

New technologies, for example, GAG sequencing and CRISPR-Cas9 generated cells with specific knockout of GAG synthetic enzymes, have been used to demonstrate that beyond overall affinity, specific sulphation patterns may be important in chemokine binding.[36,37] For example, we demonstrated that 2-O sulphation is important for binding to CCL2 but not to CXCL4.[33] Moreover, even relatively subtle changes in sulphation have been shown to have dramatic effects on the binding of short GAG sequences to CCL2 and CXCL8.[38,39] These results suggest the exciting idea that specific vascular beds may tune their sulphation patterns to facilitate binding and presentation of certain chemokines over others. This is one explanation for recent findings that monocytes use different chemokine ligands to migrate into the lung or skin of mice.[29]

Do Chemokine:GAG Interactions Enable Immune Cell Recruitment via Endothelial Glycocalyx Remodeling and/or Proteoglycan Signaling?

We and others have used assays developed in the context of materials science to determine the effect of chemokine binding on the hydrated layers formed by GAGs.[40,41] This work has demonstrated that certain chemokines, particularly those that oligomerize, effectively crosslink GAG chains, presumably through epitopes on different subunits, thereby reducing the thickness of the hydrated layers they form.[40,41] Although the biological importance of these structural effects remains to be elucidated, doing so may fill gaps in our knowledge of chemokine function.

CXCL12- and CCL5-mediated GAG crosslinking may explain the ability of these chemokines to cause proteoglycan clustering,[42,43] a phenomenon that in other contexts results in endothelial signaling.[44] CCL5 has been shown to signal via the mitogen-activated protein kinase in a GAG-dependent fashion[45] and to induce syndecan shedding alongside CXCL12.[46,47] Thus, crosslinking of GAGs by certain chemokines may promote signaling, either partially or directly, through proteoglycans independent of chemokine receptors,[41] a possibility that we are currently investigating.

The glycocalyx barrier lines blood vessels and controls vascular permeability and leukocyte recruitment.[48] This structure is largely formed by proteoglycans, for example, syndecans, and the non-sulphated GAG hyaluronan. The glycocalyx projects significantly further from the endothelium than adhesion molecules and masks them from circulating leukocytes (Fig. 1), preventing rolling.[48] The health (thickness and permeability) of the glycocalyx barrier is therefore critical to a range of diseases, for example, acute respiratory distress syndrome and COVID-19.[49,50] The glycocalyx can be remodeled by heparinase during inflammation; however, how the underlying endothelial adhesion molecules become accessible to leukocytes is not yet understood.[48] We are now investigating whether certain chemokines, for example, CXCL4, can physically remodel the glycocalyx structure to increase permeability and enable leukocyte rolling.[41]

Further work is needed to determine whether these mechanisms can explain the promigratory effect of chemokines such as CXCL4 and CCL18 that have high GAG-binding propensity but do not signal through chemokine receptors in a classical fashion.

In conclusion, after more than two decades where the importance of chemokine:GAG interactions in leukocyte recruitment and inflammation has been recognized, we are now starting to explore the underlying mechanisms. Recent developments, particularly in the GAG field, are facilitating innovative approaches that will help advance the field (Fig. 1). Such mechanistic breakthroughs will be key in developing new therapeutics to target the chemokine system in an array of inflammatory diseases.

Competing Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author Contributions

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