

The study further demonstrates that the purification quality of CLP is important, especially for analyses of T-cell development. The original phenotypic CLP has recently been refined by addition of positivity for Flk2⁵ and now a smaller population of cells including CD27 are shown to contain the T-cell potential. A specific criticism of the CLP as the thymus-seeding cell has been the lack of evidence that CLP can be detected in thymus. The study further challenged the MPP-seeding hypothesis by identifying the presence of a low level of circulating Flk2+CD27+CLP, thus formally demonstrating that CLPs can be detected in transit, presumably to the thymus. Since CLPs have not yet been detected in thymus, likely due to rapid adoption of DN1 phenotype, this is an important step forward by identifying a missing link. Overall, the study provides important information about the inner potential of the originally defined CLP

and offers additional markers to help all labs standardize their CLP isolations. Let's hope that these advances will help to resolve the thymic progenitor debate.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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Comment on Landsman et al, page 963

Fractalkine: one chemokine, many functions

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CX₃CL1 (Fractalkine) is an atypical chemokine with a documented role in the development of numerous inflammatory diseases including atherosclerosis. In this issue of *Blood*, Landsman and colleagues demonstrate that interactions between CX₃CL1 and its receptor CX₃CR1 are involved in monocyte survival via antiapoptotic mechanisms and thus promote atherogenesis.

Chemokines (chemotactic cytokines) are a family of low molecular weight proteins which function to coordinate leukocyte trafficking in health and disease. Divided on the basis of structure into 4 families (C, CC, CXC, and CX₃C), chemokines signal through G protein-coupled 7-transmembrane receptors to induce the directed migration of cells along a chemoattractant gradient.

CX₃CL1 is the only member of the CX₃C family of chemokines and signals through a single G α i-linked receptor: CX₃CR1.^{1,2} Cell surface expression of CX₃CR1 defines 2 circulating monocyte subsets: CX₃CR1^{low} cells correspond to the “inflammatory” Gr1^{hi} (Ly6C^{hi}) subset in mice and the CD14⁺CD16⁻ subset in humans.³ The murine Gr1^{low} (Ly6C^{low}) subset expresses signifi-

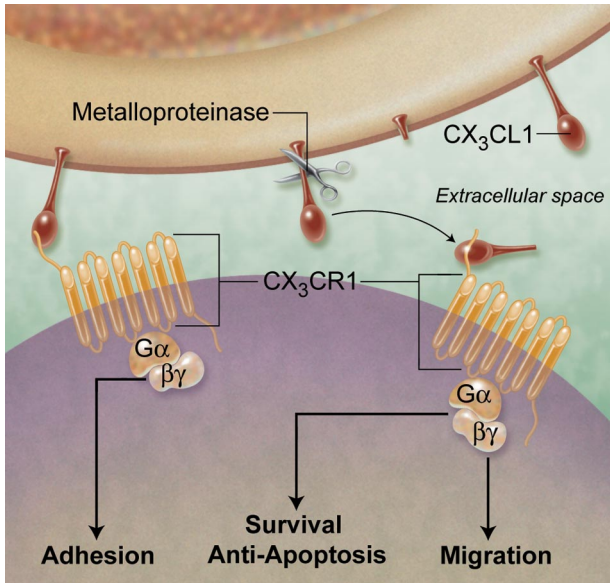
cantly higher levels of CX₃CR1 and corresponds to the CD14⁺CD16⁺ monocyte population in humans. CX₃CL1 is synthesized as a membrane-bound molecule with the chemokine domain presented on a mucin-like stalk which mediates the direct capture of circulating leukocytes, as shown in the figure.² Cleavage at the base of this stalk by metalloproteinases generates a soluble chemokine, which functions as a classical chemoattractant.⁴ It is becoming increasingly clear that CX₃CL1, in common with other chemokines, has a wide range of functional roles that contribute to pathology.

Landsman et al demonstrate that under steady state conditions, mice lacking either CX₃CL1 or CX₃CR1 (Cx₃cr1^{gfp/gfp}) have sig-

nificantly fewer circulating monocytes, specifically those of the Gr1^{low} subset. Furthermore, cotransfer of Cx₃cr1^{gfp/gfp} and wild-type bone marrow into wild-type mice results in a selective reduction of circulating Cx₃cr1^{gfp/gfp} Gr1^{low} monocytes. The forced transgenic expression of the antiapoptotic factor Bcl2 in monocytes rescues this phenotype, indicating that the CX₃CL1–CX₃CR1 interaction confers a survival signal under homeostatic conditions. In human monocytes, soluble CX₃CL1 rescues cells from apoptosis induced by serum-deprivation or cytotoxic stress, though this is not confined to a particular subset.

In the apolipoprotein E (ApoE^{-/-}) deficient mouse model of atherosclerosis, the absence of either CX₃CL1 or CX₃CR1 reduces total lesion area and plaque macrophage content.^{5,6} Landsman et al demonstrate that transfer of Cx₃cr1^{gfp/gfp} bone marrow into ApoE^{-/-} recipients results in reduced atherosclerotic plaque burden (compared with transfer of Cx₃cr1^{+/+} bone marrow), and these mice have reduced levels of circulating Gr1^{low} monocytes. In addition, plaques from these animals contain increased levels of TUNEL⁺ apoptotic cells, consistent with a role for CX₃CR1 in preventing apoptosis. However, enforced survival of monocytes and plaque foam cells in these mice restore atherosclerosis to levels seen in CX₃CR1-proficient animals. These data suggest that plaque resident cells depend on the CX₃CL1–CX₃CR1 interaction for their survival, providing a plausible mechanism for the proatherogenic function of CX₃CL1.

However, the role of CX₃CL1 in controlling levels of circulating monocytes remains controversial. A recently published study failed to show an effect of CX₃CL1 deficiency on levels of total blood monocytes or individual subsets in ApoE^{-/-} mice.⁷ Instead, CCR2 deficiency reduced circulating monocyte numbers, and this was not further diminished in Ccr2^{-/-} Cx₃cl1^{-/-} animals. A second study found significantly reduced levels of circulating Gr1^{low} monocytes in Cx₃cr1^{-/-} mice in both homeostatic and inflammatory (ApoE^{-/-}) conditions.⁸ However, CCL2 deficiency caused a more marked reduction in blood monocytes while Ccl2^{-/-} Cx₃cr1^{-/-} ApoE^{-/-} mice showed an additive reduction in total and individual monocyte subsets. Landsman et al provide a mechanistic explanation for the effect of CX₃CL1 on circulating levels of specific monocyte subsets, but this



Functions of CX₃CL1 (Fractalkine) contributing to atherosclerosis. Membrane-bound CX₃CL1 enables the integrin-independent capture and firm adhesion of leukocytes via the Gα_i coupled receptor CX₃CR1. Under inflammatory conditions, cleavage of cell-surface CX₃CL1 by metalloproteinases generates a soluble chemokine, which binds CX₃CR1 on nearby cells and, via signaling intermediates, can induce cell chemotaxis and survival. Thus, CX₃CL1 can have multiple roles in all stages of atherosclerosis, including the recruitment, retention, and survival of monocytes in early atherosclerotic lesions as well as foam cell persistence in more advanced plaques. Professional illustration by A. Y. Chen.

may prove to be a more general function of chemokines.

Does this study have any implications for targeting chemokines in cardiovascular dis-

reminds us that chemokines, originally discovered and named for their role in cell migration, have a plethora of functions that are only now being revealed.

ease? One key question is whether long-term inhibition of a single chemokine/receptor would be an effective therapeutic strategy. Combined inhibition of both CCL2 and CX₃CL1 may be required to overcome redundancy in the chemokine network, but the resultant chronic monocyte suppression could have significant undesirable effects. Although targeting CX₃CL1 in early atherosclerosis might reduce monocyte accumulation and survival in fatty streak lesions, macrophage survival may be crucial in late disease stages to ensure clearance of necrotic and potentially thrombogenic material. The study presented here adds to our appreciation of CX₃CL1 as an unusual chemokine and

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