The roles of chemokines during hematopoiesis of the amphibian *Xenopus laevis*

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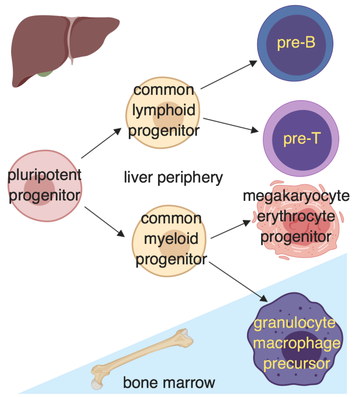
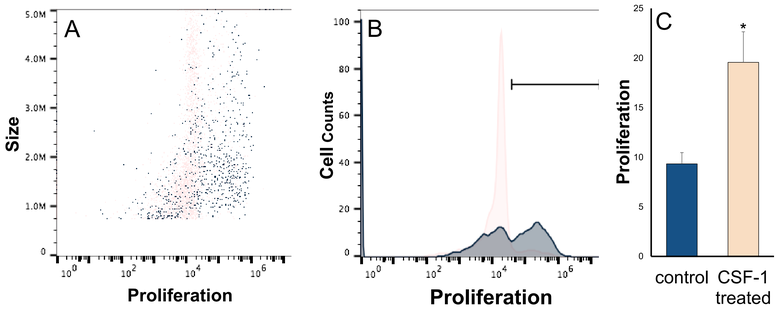
Abstract

Across all vertebrates, hematopoiesis (blood cell development) occurs in designated sites. In the *Xenopus laevis* frog, the main site for hematopoiesis is thought to be the liver periphery (LP). However, macrophage precursors are not found in the LP and instead they reside in the bone marrow (BM). Because of this unique strategy of dividing hematopoiesis across multiple tissues, *X. laevis* is a useful model to study blood cell development, enabling us to better understand the evolution of hematopoiesis across vertebrates. We hypothesized that macrophage precursor cells are produced in the LP and migrate to the bone marrow in response to chemokines produced by the bone marrow stroma (BMS).



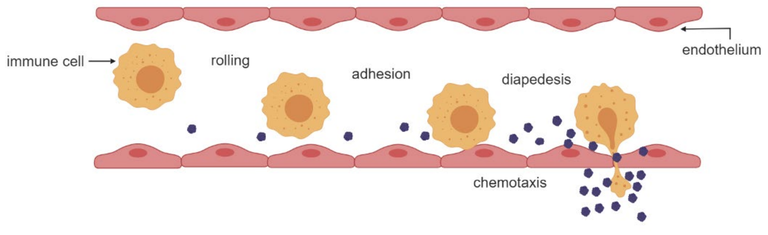
Background

X. laevis bone marrow cells are responsive to CSF-1



Flow cytometry of *X. laevis* bone marrow cells from CSF-1 treated animals, compared to controls. Representative **(A)** scatter plot comparing size (forward scatter) with proliferation and **(B)** histogram plot showing cell count versus proliferation using EdU, a marker for new DNA synthesis. **(C)** Graphical representation of flow cytometry data derived from five animals (*N*=5), $p < 0.05$ significance level. (*) indicates statistical significance relative to the respective control. (*) above a line spanning two columns or bars indicates statistical significance between the respective groups.

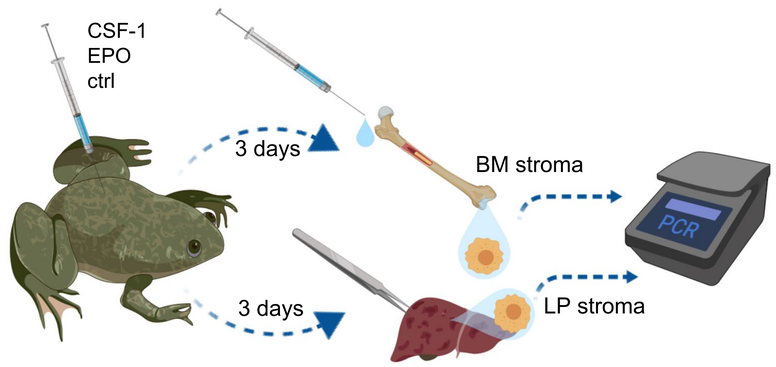
Chemotaxis



During chemotaxis, cells move in response to chemokine (chemotactic cytokine) gradients. Distinct chemokine gradients dictate the homing and localization of different blood cells to immune sites and disparate tissues.

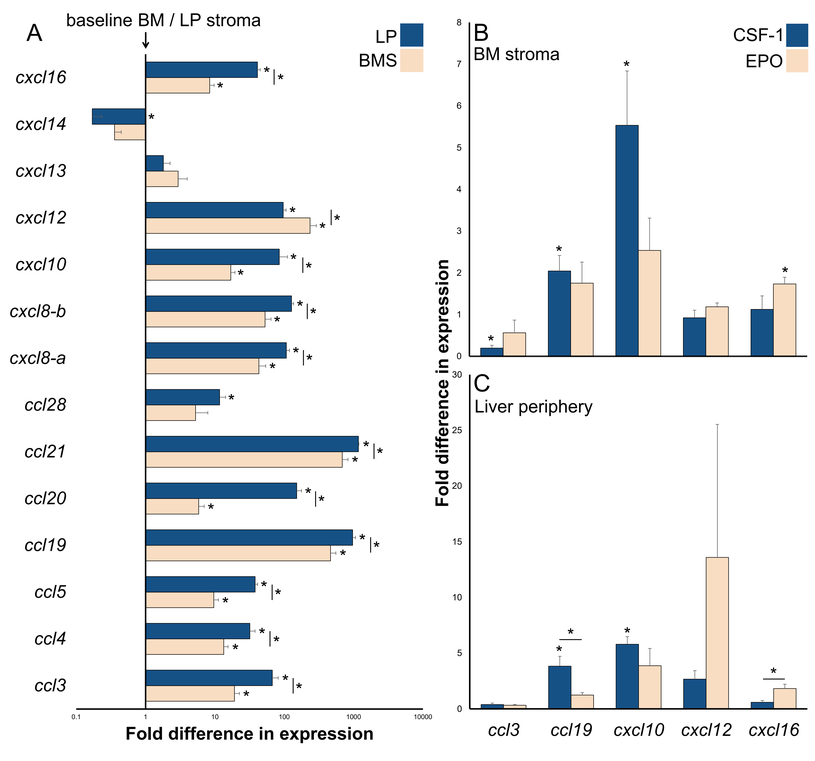
Results

Isolation and analyses of hematopoietic stromal cells



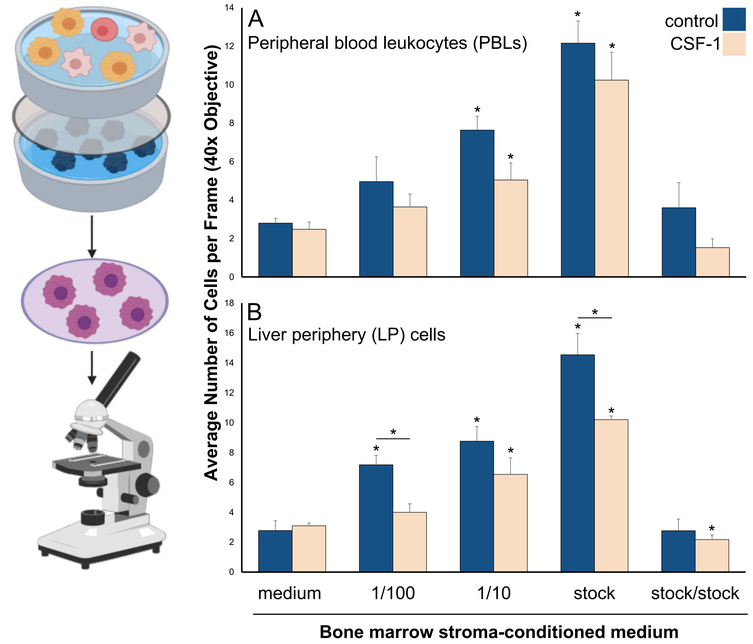
Frogs were injected with macrophage (colony stimulating factor 1; CSF-1) or erythrocyte (erythropoietin; EPO) growth factors, After 3 days, bone marrow (BM) and liver periphery (LP) stromal cells were isolated and analyzed.

Growth factor-induced changes in bone marrow and liver periphery stroma expression of chemokines



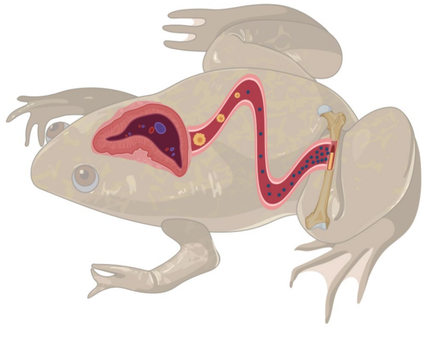
Chemokine expression in bone marrow stroma and liver periphery of *X. laevis*. **(A)** LP displayed greater expression of select chemokines compared to BMS. Animals injected with CSF-1 and EPO exhibited differing transcript levels of *ccl3, ccl19, cxcl10, cxcl12,* and *cxcl16* chemokines relative to each other and controls in **(B)** BMS and **(C)** LP. Data derived from five animals (*N*=5), $p < 0.05$.

PBLs and LP cells are chemoattracted to BMS sup



Chemoattraction of *X. laevis* PBLs and LP cells to BMS supernatant. **(A)** PBLs and **(B)** LP cells exhibit significant attraction to BMS supernatant compared to media. Data derived from five animals (*N*=5), $p < 0.05$.

Conclusions



Liver periphery and bone marrow stroma showcase differential chemokine expression, suggesting that these distinct chemokines facilitate homing of different cell lineages to these respective frog tissues. Additionally, PBL and LP cell attraction to supernatant cultured from BMS suggest that cells migrate from the LP to the bone marrow through the bloodstream.

Research to determine the specific cellular targets of these disparate chemokines will offer new insights into the mechanisms by which blood cell precursors are trafficked during distinct stages of hematopoiesis.

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