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# Contribution of EphA2 to effective transplantation of macrophages/monocytes into the spleen

The spleen is formed by the white pulp and red pulp. The former is subdivided into the T-cell zone and the B-cell follicles, and the latter into the splenic sinus and the cord. The spleen reacts immunologically to blood-borne antigens in the white pulp and filters blood in the red pulp. Recently, it is known that the number of monocytes higher than that in blood circulation lodge in the cords of the subcapsular red pulp, and these monocytes are mobilized into inflamed tissues during tissue injury. Thus, a system of monocyte immigration and emigration likely operates in the spleen. The spleen harbors a unique vascular system that is indispensable for those functions. In rodents, a central artery sheathed by lymphoid tissue runs through the T-cell zone of the white pulp and extends branches that either run into the B-cell follicles of the white pulp or terminate in the marginal sinus of the marginal zone that lies between the white pulp and the red pulp.

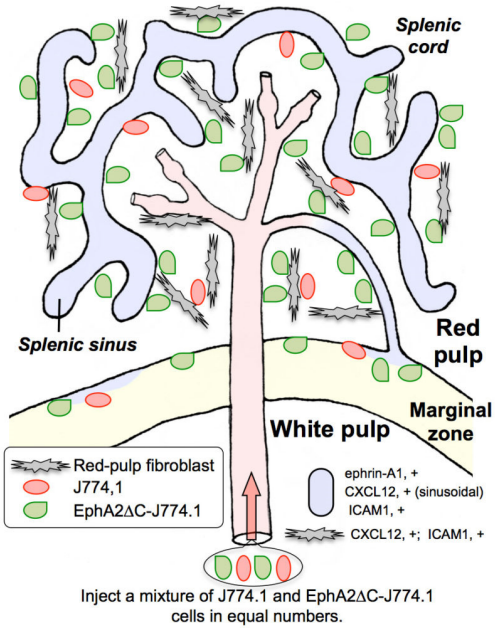


Fig. 1. A schematic drawing showing (1) expressions of ephrin-A1, CXCL12 and ICAM1, and (2) J774.1 (red) and EphA2ΔC-J774.1 (green) cell distribution in the spleen. An equal number of J774.1 cells and EphA2ΔC-J774.1 cells labeled with Far Red and CSFE, respectively, were intravenously injected into BALB/c mice that were intraperitoneally injected with clodronate-encapsulating anionic liposomes one day before the cell injection.

Subsequently, the artery runs over the marginal zone into the splenic cord and extends branches of terminal arterioles, whose terminal ends open into the reticular meshwork of the cord as open circulation. The organization of the white pulp is involved in the migration and lodgment of lymphocytes, and chemokines and chemokine receptors are essential molecules implicated in the formation of the T-cell zone and B-cell follicles, whereas the organization of the red pulp remains unclear, especially with regard to the migration and/or lodgment of monocytes and macrophages.

We previously established a J774.1 monocyte/macrophage subline expressing a truncated EphA2 construct lacking the kinase domain (EphA2ΔC-J774.1), and demonstrated that following ephrin-A1 stimulation, endogenous EphA2 promotes cell adhesion through interaction with integrins and integrin ligands such as ICAM1, and that truncated EphA2 potentiates the adhesion and becomes associated with the integrin/integrin-ligand complex. Based on these findings, we hypothesized that the EphA/ephrin-A system, particularly EphA2/ephrin-A1, regulates transendothelial migration/tissue infiltration of monocytes/macrophages, because ephrin-A1 is widely recognized to be upregulated in inflammatory vasculatures.

To evaluate whether this hypothesis is applicable in the spleen, we screened for ephrin-A1, ICAM1, and CXCL12 expression and reexamined the cellular properties of the J774.1 subline. We found that (1) ephrin-A1 was expressed in the vasculature of the marginal zone and the red pulp, and that its expression was upregulated in response to phagocyte depletion, (2) ICAM1 was expressed in sinusoidal and capillary endothelial cells in the red pulp, the microvascular endothelial cells of the marginal zone, and in red-pulp fibroblasts, and (3) CXCL12 was expressed in sinusoidal endothelial cells and red-pulp fibroblasts (Fig. 1). We further found that CD115, F4/80, and CXCR4 were expressed in J774.1 cells, which serve as a usable substitute for monocytes/macrophages. Moreover, following ephrin-A1 stimulation, truncated EphA2 did not detectably interfere with the phosphorylation of endogenous EphA2, and it potentiated cell adhesion possibly through modulation of integrin avidity (Saeki et al., Cell Adh Migr, 2014). Accordingly, by intravenously injecting mice with equal numbers of J774.1 and the subline cells labeled with distinct fluorochromes, we determined that truncated EphA2 markedly potentiated preferential cell infiltration into the red pulp and the marginal zone (Fig. 1).

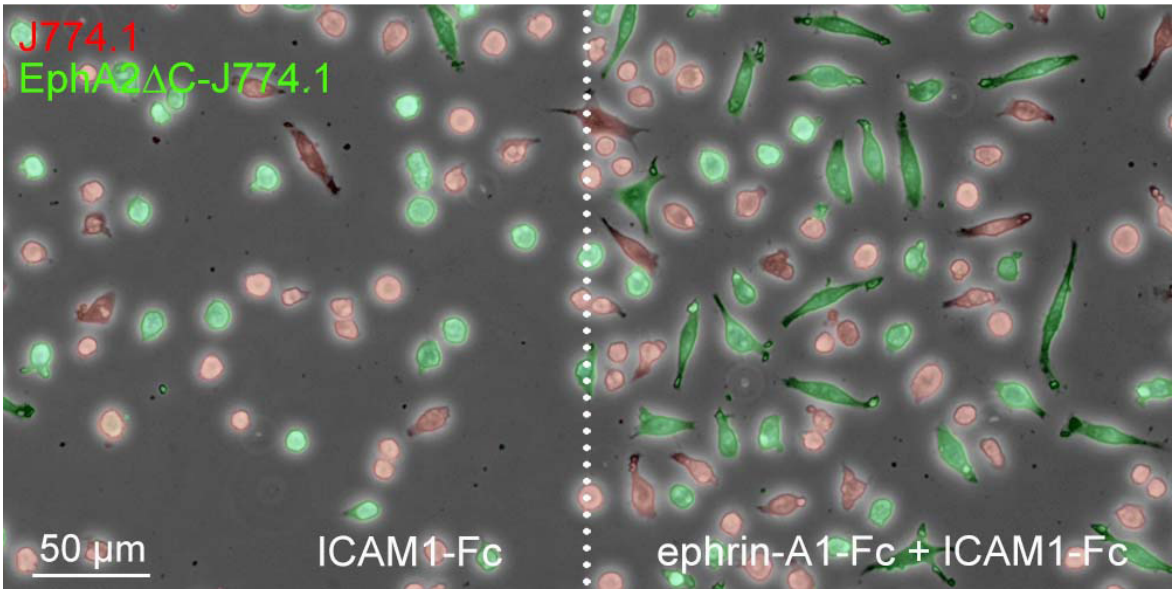


Fig. 2. Phase-contrast image merged with fluorescence images showing Far Red-labeled J774.1 cells (pink or deep red) and CSFE-labeled EphA2ΔC-J774.1 cells (green) cultured on a coverslip surface coated with stripes of ephrin-A1-Fc plus ICAM1-Fc/ICAM1-Fc.

This finding suggests that the additional expression of truncated EphA2 in the subline cells causes effective infiltration and/or lodgment, possibly because of its potentiation of cell adhesion, similar to what was observed in a cell-adhesion stripe assay (Fig. 2). Thus, modulation of EphA2 signaling might contribute to effective transplantation of tissue-specific resident macrophages and/or monocytes.

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## Publication

Truncated EphA2 likely potentiates cell adhesion via integrins as well as infiltration and/or lodgment of a monocyte/macrophage cell line in the red pulp and marginal zone of the mouse spleen, where ephrin-A1 is prominently expressed in the vasculature.
Konda N, Saeki N, Nishino S, Ogawa K
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