Elucidation of the mechanisms of hairy-cell localization in tissues and the process of the bone marrow fibrosis in hairy-cell leukemia

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ABSTRACT

Hairy cell leukemia is a chronic B cell leukemia with a number of distinctive features including the unusual tissue distribution of the leukemic cells, hairy cells, and the bone marrow fibrosis. We have been working, for a number of years, on the potential mechanisms behind hairy-cell localization in tissues. In this review, it is summarized how our work has shed very important information regarding these mechanisms and led, eventually, to the full elucidation of the process of the bone marrow fibrosis in hairy cell leukemia.

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Hairy cell leukemia (HCL) is a chronic lymphoproliferative disease of B cell phenotype.[1] The leukemic cells show features of activated B cells, prominent among these, is the presence of numerous microvilli projecting from the cell surface and which, gives the cells their name, hairy cells (HCs).[1-3] A number of unusual features unique to HCs include the expression of the monocytic adhesion receptor CD11c and the in-vivo localization to the splenic red pulp, hepatic sinusoids, hepatic portal tracts and the bone marrow. Infiltration of the latter organ by HCs is accompanied by fibrosis.[1,4] The origin of HCs has been a subject of debate in the past. Due to a number of features including the morphology of the cells, the surface expression of CD11c and the ability to phagocytose, HCs were originally thought to have a monocytic-histocytic origin and the disease was labeled leukemic retculoendotheliosis.[5,6] However, subsequently, it was conclusively proven that HCs were of B-cell origin, expressing B-cell specific receptors including CD21, CD24, CD25, CD27 and CD40 ligand in addition to the expression of the monocytic CD11c surface receptor.[1,5,7] Lymphocytes, including B-cells, are known to migrate to lymphoid organs including the splenic white pulp and lymph nodes. In contrast, HCs fail to migrate to such sites, but rather, they migrate to the splenic red pulp, the liver and the bone marrow.[1] It was not clear whey HCs fail to migrate to the lymphoid organs. Moreover, it was not known how the cells migrate and localize in the splenic red pulp and the bone marrow, or the cell surface receptors, which control such localization.

For cells to localize in tissues, they must first migrate from the circulation across the blood vessel wall and then transmigrate across tissues to reach the sites of localization. Movement of lymphocytes from blood vessels to tissues involves a number of distinct and sequential stages. Each stage is mediated by specific adhesion receptors on lymphocyte surfaces that interact

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with specific ligands on the surface of the endothelium and the extracellular tissues.[8,9] The first stage involves rolling of lymphocytes on an inflamed endothelium, or the high endothelial venule (HEV) and such stage is mediated by a receptor on lymphocyte surfaces, belonging to the selectin family of adhesion molecules; L-selectin, interacting with CD34 or GlyCAM on the surface of the endothelium. Such interactions lead to activation of lymphocytes and to the second stage of adhesion. The latter stage is mediated by adhesion molecules on the surface of lymphocytes, belonging to the integrin family; leukocyte functional antigen-1 (LFA-1) and α4β1 interacting with adhesion molecule-ligands belonging to the immunoglobulin family; vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), on the surface of the endothelium.[9] The third stage which involves migration of lymphocytes across the endothelium and subsequent localization in tissues is also mediated by the α4β1 receptor interacting, not only with VCAM-1, but also with the extracellular matrix proteins including fibronectin and collagen.[9-11] **Table 1** gives a summary of the adhesion receptors on lymphocytes and their corresponding ligands on the endothelium and the extracellular matrix.

Role of the integrins in hairy-cell localization in tissues. Although great advances have been made in the elucidation of the different mechanisms involved in the trafficking of normal leukocytes between blood and tissues, very little data existed on the mechanisms of leukemic cell trafficking and localization in tissues, particularly for HCs. Localization of leukemic cells in tissues gives great survival advantages and makes them less susceptible to current treatment. Therefore, elucidation of the mechanisms of leukemic cell localization in tissues would be of great clinical importance as it would open the way for devising ways of interfering with such mechanisms thereby keeping the cells in circulation and thus depriving them of the tissue survival potential and making them more susceptible to current treatment. We have been, for a number of years, involved in the elucidation of the mechanisms of HC localization in tissues, particularly the spleen and the bone marrow, and in the process of fibrosis of the latter organ which accompanies HC infiltration. Since HCs were observed histologically to interact with the extracellular matrix components,[2,12,13] the initial step in working out the mechanisms of tissue localization, was to examine for potential adhesion receptors on their surfaces that are capable of interacting with the extracellular matrix protein. Indeed, HCs were shown to express adhesion receptors belonging to the β1-integrin family including α4β1, αvβ1, and α5β1.[14] Moreover, HCs were shown to utilize α4β1 and α5β1 to bind firmly to fibronectin and αvβ1 to bind to vitronectin. Furthermore, fibronectin and vitronectin were shown to be expressed in the HCL bone marrow and the splenic red pulp.[14]

Table 1 - Adhesion receptors and their ligands.

Receptors	Ligands
B2-integrins	
CD11a/CD18 (LFA-1, αLβ2)	ICAM-1/2/3
CD11b/CD18 (CR3, αmβ2)	ICAM-1
CD11c.CD18 (CR4, αxβ2)	ICAM-1
B1-integrins	
α3β1 (VLA3)	Fibronectin/collagen
α4β1 (VLA4)	Fibronectin/collagen
α5β1 (VLA5)	Fibronectin
αvβ1	Fibronectin/vitronectin
Selectins:	
L-selectin	
CD15	CD34, GlyCAM
Others	
CD44	Hyaluronan/fibronectin

ICAM-1/2/3, intracellular adhesion molecule-1, 2 and 3;
VCAM-1, vascular cell adhesion molecule
VLA - very late antigen
LFA - leukocyte functional antigen

These results clearly indicated that HCs had the potential to interact with fibronectin and thus localize within the bone marrow through the α4β1 and α5β1 adhesion receptors, while in the splenic red pulp, HCs would localize through the interaction of their αvβ1 adhesion receptor with vitronectin. In order for HCs to localize in tissues, they must first migrate from the blood vessels to the extracellular tissues. In the spleen, the splenic artery empties directly to the splenic red pulp from which the cells percolate through the tissues and reach the splenic sinuses and back to the general circulation. This could be therefore, one mechanism by which HCs reach the splenic red pulp. The second route could be through migration from the splenic arterioles to the red pulp. Indeed, it was shown that the HCL splenic endothelium expresses a large amount of VCAM-1 and that HCs could interact with these ligands through their α4β1 receptors and migrate across the endothelium to the interstitial tissues.[15]

With regards to the bone marrow, it was shown that the fibrosis observed was due to the synthesis and deposition of fibronectin, rather than collagen which is found in other classical fibrotic conditions. This fironectin could interact with the α4β1adhesion receptor on HCs and thus play a role in the localization of the infiltrating cells in the bone marrow. However, since normal bone marrow does not express fibronectin,[16] it was not clear regarding the mechanisms involved in the initial stages of HC localization to this organ.

Role of CD44 in hairy-cell localization to the bone marrow. To investigate the potential mechanisms behind the initial stages of bone marrow localization by HCs, we started looking for other non-integrin adhesion

receptors; CD44 was a potential candidate. CD44 is normally expressed on the cell surface of many cells in an inactive form. Activation of cells leads to the activation of CD44, through conformational changes, and subsequent binding to its ligands.[17] The principal ligand for CD44 is the extracellular matrix carbohydrate component, hyaluronan (HA).[17,18] Many sold tumors have been shown to express the active form of CD44 and the level of such expression correlated with the invasiveness and the aggressiveness of such tumors.[19,20] We therefore looked initially at the surface expression of CD44 on HCs. The cells were shown to express a high level of the active form of CD44. Moreover, when HCs were added to hyaluronan-coated surfaces, the cells adapted a motile morphology and migrated by a CD44-dependent mechanism.[21,22] Accumulation of lymphocytes in tissues not only depends on adhesion molecules, but also depends on growth factors and chemokines.[9,23] One of the most active and frequently studied chemokine is interleukin-8 (IL-8). The latter chemokine is produced by a variety of cells including endothelial cells, macrophages and lymphocytes. Interleukin-8 acts as both chemokinetic and chemotactic factor for white blood cells including lymphocytes.[24,25] However, and unexpectedly, when we investigated the effect of IL-8 on HCs, we found that it abrogated the cell motility on HA. Moreover, IL-8 induced HCs to spread on HA and project numerous microvilli and such effects were shown to be due to increased F-actin polymerization.[21] Since the effects of IL-8 on HCs were unexpected, we

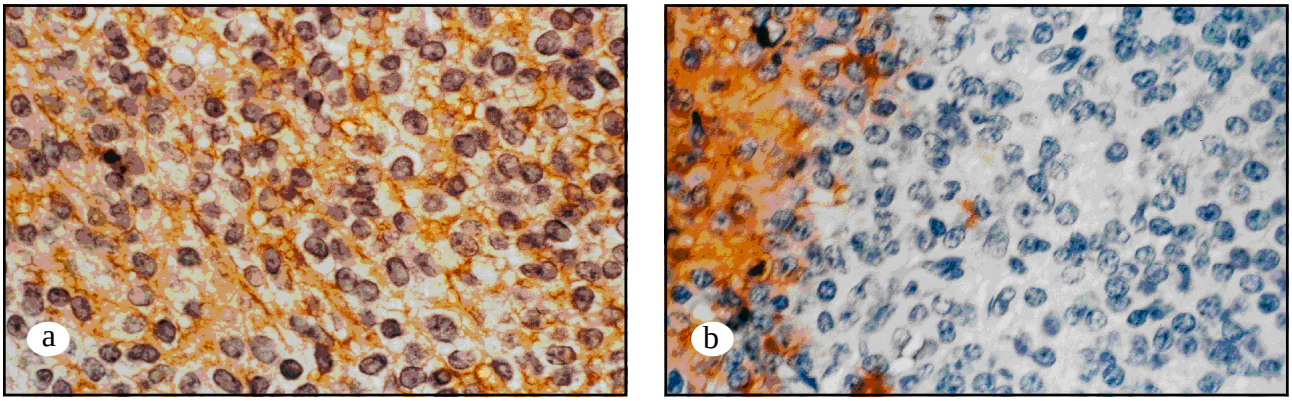

Figure 1 - Expression of hyaluronan (HA) in **(a)** HCL-bone marrow and **(b)** the spleen. The presence of HA was detected by peroxidase-conjugated HA-binding protein. Note HA is present throughout the bone marrow whereas in the spleen HA is restricted to the white pulp.

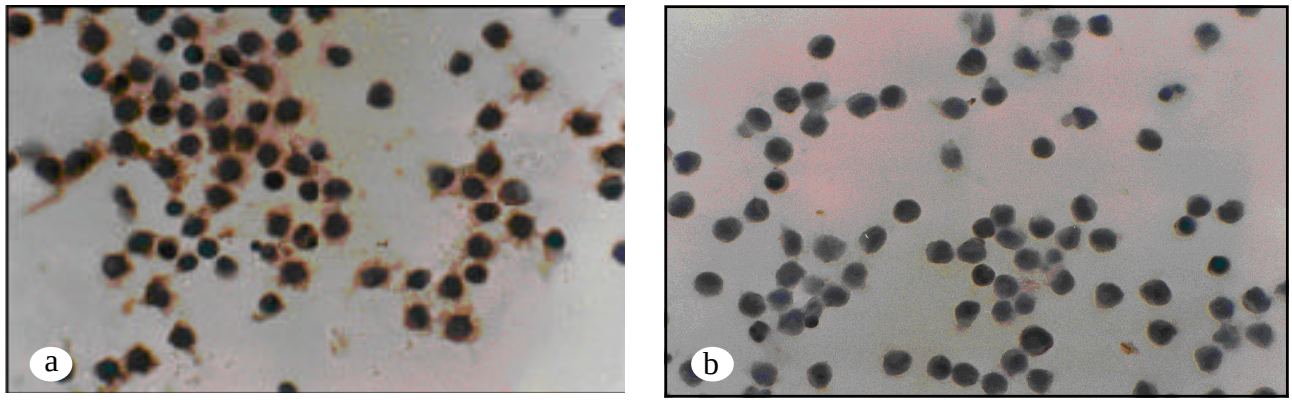

Figure 2 - Hairy cells cultured on **(a)** hyaluronan, but not on **(b)** bovine serum albumin, produced large amount of fibronectin. Fibronectin was detected by an anti-fibronectin peroxidase-conjugated monoclonal antibody.

further examined its effect on HC interaction with other extracellular matrix components including vitronectin. In contrast to its inhibitory effect on HCs migration on HA, IL-8 enhanced HC-motility on vitronectin.[22] It seems therefore that IL-8 could manifest dual effects on HC motility, which depended on the substrata used, and hence the engagement of a particular cell surface receptor. Thus, signals induced by IL-8 and CD44 caused inhibition of HC motility, while that induced by αvβ1 and IL-8 enhanced such motility.[21,22] In order to see whether these in-vitro findings could reflect the in-vivo situation, we first examined for the presence of HA in both normal and HCL tissues. We found that HA was abundant in the normal and HCL bone marrow and the splenic white pulp. However, HA was not detected in the splenic red pulp where HCs normally accumulate,[22] **Figure 1**. In contrast, vitronectin was absent in the bone marrow, but present in the splenic red pulp.[14] Interleukin-8 was present in all the tissues examined, both from normal and HCL.[22] These findings strongly indicate that HCs would be attracted from the circulation to the bone marrow by IL-8 or other chemotactic factors. Hairy cells would cross the blood vessels and migrate to the bone marrow through the engagement of their surface receptor α4β1 with VCAM-1 on the surface of the endothelium. Once in the bone marrow, HCs would interact with HA through CD44 and become immobilized by IL-8, leading to a gradual accumulation of cells. In contrast, HCs in the splenic red pulp would interact and move on vitronectin and such movement would be accelerated by IL-8.

Mechanism of hairy-cell leukemia bone marrow fibrosis. Bone marrow fibrosis is one of the main features of HCL and is associated with heavy infiltration of HCs. In contrast, infiltration of the splenic red pulp by HCs is not accompanied by such fibrosis. The reason for this preferential tissue fibrosis in these organs was not understood. Our observation of HA expression in both normal and HCL bone marrow, but not in the splenic red pulp, led us to speculate that HA could play a major role in the process of the bone marrow fibrosis in HCL. We hypothesized that HA interaction with HCs could stimulate the cells, through CD44, to synthesize and deposit fironectin in the bone marrow. To test this hypothesis, we proceeded to culture HCs on HA, vitronectin and a control substrata, bovine serum albumin (BSA). To our delight, we were able to demonstrate that HCs on HA, but not on vitronectin or BSA, produced large amount of fibronectin,[22] **Figure 2.** Moreover, blocking CD44 adhesion molecules on HCs with monoclonal antibodies, prior to addition to HA abrogated the production of fibronectin. This further proved our hypothesis that engagement of CD44 on HCs leads to fibronectin production.[21,22] Fibrogenesis in other primary fibrotic conditions has been attributed to the involvement of soluble growth factors, namely transforming growth factor-beta (TGF-β) and basic fibroblast growth factor (b-FGF).[26-28] To complete our work, we therefore proceeded to elucidate the mechanisms of fibronectin production by HCs at the molecular level. We were able to show that HCs cultured on HA, but not vitronectin or BSA, produced high levels of b-FGF, but not TGF-β. Moreover, blocking b-FGF with monoclonal antibodies abrogated the synthesis of fibronectin, while addition of purified b-FGF to HCs cultured on vitronectin or BSA, induced the production of fibronectin.[29] These results conclusively proved that b-FGF is the responsible factor for the induction of the observed fibronectin production by HCs.

In conclusion, our work has demonstrated that infiltrating HCs to the bone marrow would interact with HA through their surface receptors, CD44, and become immobilized by the chemokine, IL-8. Engagement of CD44 would induce HCs to produce b-FGF, which in turns would, in an autocrine fashion, stimulate the cells to synthesize and produce fibronectin. The newly synthesized and deposited fibronectin would induce further localization of HCs through the engagement of their α4β1 integrin receptors. In contrast, HCs in the spleen would interact and move on vitronectin and VCAM-1 through the engagement of the αvβ1 and α4β1 integrin adhesion molecules, Such movement would be further accelerated by the IL-8. It appears therefore that HCs in the spleen are in constant motion and that the apparent accumulation of the cells is due to the slow movement across the splenic red pulp. Our study spanning a number of years has lead to the full elucidation of the mechanism of HC accumulation in the spleen and the bone marrow, and more importantly, the mechanism of HCL bone marrow fibrosis.

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