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

Khalil A. Aziz, MD, PhD.

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.


From the Regional Department of Immunology, Heartlands Hospital, Birmingham, United Kingdom.

Address correspondence and reprint request to: Dr. Khalil A. Aziz, Senior Clinical Scientist, Regional Department of Immunology, Birmingham Heartlands Hospital, Bordesley Green East, Birmingham, B9 5SS, United Kingdom. Tel. +44 (121) 4240185. Fax. +44 (121) 4243229. E-mail: khalil.aziz@heartsol.wmids.nhs.uk
with specific ligands on the surface of the endothelium and the extracellular tissues.\textsuperscript{7,9} 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 \( \alpha_4 \beta_1 \) 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.\textsuperscript{9} The third stage which involves migration of lymphocytes across the endothelium and subsequent localization in tissues is also mediated by the \( \alpha_4 \beta_1 \) receptor interacting, not only with VCAM-1, but also with the extracellular matrix proteins including fibronectin and collagen.\textsuperscript{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,\textsuperscript{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 \( \beta_1 \)-integrin family including \( \alpha_4 \beta_1 \), \( \alpha_5 \beta_1 \), and \( \alpha_5 \beta_1 \). Moreover, HCs were shown to utilize \( \alpha_4 \beta_1 \) and \( \alpha_5 \beta_1 \) to bind firmly to fibronectin and \( \alpha_5 \beta_1 \) to bind to vitronectin. Furthermore, fibronectin and vitronectin were shown to be expressed in the HCL bone marrow and the splenic red pulp.\textsuperscript{14}

<table>
<thead>
<tr>
<th>Receptors</th>
<th>Ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B2-integrins</strong></td>
<td></td>
</tr>
<tr>
<td>CD11a/CD18 (LFA-1, ( \alpha_4 \beta_2 ))</td>
<td>ICAM-1/2/3</td>
</tr>
<tr>
<td>CD11b/CD18 (CR3, ( \alpha_3 \beta_2 ))</td>
<td>ICAM-1</td>
</tr>
<tr>
<td>CD11c/CD18 (CR4, ( \alpha_4 \beta_2 ))</td>
<td>ICAM-1</td>
</tr>
<tr>
<td><strong>B1-integrins</strong></td>
<td></td>
</tr>
<tr>
<td>( \alpha_3 \beta_1 ) (VLA3)</td>
<td>Fibronectin/collagen</td>
</tr>
<tr>
<td>( \alpha_5 \beta_1 ) (VLA4)</td>
<td>Fibronectin</td>
</tr>
<tr>
<td>( \alpha_5 \beta_1 ) (VLA5)</td>
<td>Fibronectin/vitronectin</td>
</tr>
<tr>
<td>( \alpha \beta_1 )</td>
<td></td>
</tr>
<tr>
<td>Selectins:</td>
<td></td>
</tr>
<tr>
<td>L-selectin</td>
<td>CD15</td>
</tr>
<tr>
<td>CD15</td>
<td>CD34, GlyCAM</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
</tr>
<tr>
<td>CD44</td>
<td>Hyaluronan/fibronectin</td>
</tr>
</tbody>
</table>

These results clearly indicated that HCs had the potential to interact with fibronectin and thus localize within the bone marrow through the \( \alpha_4 \beta_1 \) and \( \alpha_5 \beta_1 \) adhesion receptors, while in the splenic red pulp, HCs would localize through the interaction of their \( \alpha \beta_1 \) 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 \( \alpha_4 \beta_1 \) receptors and migrate across the endothelium to the interstitial tissues.\textsuperscript{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 fibronectin could interact with the \( \alpha_4 \beta_1 \) adhesion 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,\textsuperscript{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. The principal ligand for CD44 is the extracellular matrix carbohydrate component, hyaluronan (HA). Many solid 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. 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. Accumulation of lymphocytes in tissues not only depends on adhesion molecules, but also depends on growth factors and chemokines. 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. 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.

Since the effects of IL-8 on HCs were unexpected, we...
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. 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 αβ1 and IL-8 enhanced such motility. 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.

**Figure 1.** In contrast, vitronectin was absent in the bone marrow, but present in the splenic red pulp. Interleukin-8 was present in all the tissues examined, both from normal and HCL. 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 αβ1 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 fibronectin 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. 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. 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). 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. 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 αβ1 integrin receptors. In contrast, HCs in the spleen would interact and move on vitronectin and VCAM-1 through the engagement of the αβ1 and αβ1 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.

**Acknowledgment.** This work was supported by the North West Cancer Research Fund and the Leukemia Research Fund, United Kingdom.

**References**


29. Aziz KA, Zuzel M, Cawley JC. Autocrine b-FGF induced by hairy-cell interaction with hyaluronan (HA) is responsible for the distinctive bone marrow fibrosis of hairy-cell leukaemia. *Br J Haematol* 2001; 113: 64.