Differential Expression of Very Late Activation Antigen-3 (VLA-3)/VLA-4 in B-Cell Non-Hodgkin Lymphoma and B-Cell Chronic Lymphocytic Leukemia

By Luca Baldini, Lilla Cro, Rossella Calori, Lucia Nobili, Ilaria Silvestris, and Anna T. Maiolo

The expression of β1 (very late activation antigens, VLA 1-6) and β2 integrins (leukocyte adhesion molecules [Leu-CAM]) in cell suspensions from the peripheral blood of 70 patients with B-cell chronic lymphocytic leukemia (B-CLL), 15 patients with leukemic lymphocytic lymphoma of intermediate differentiation (IDL), as well as from the lymph nodes of 20 patients with low/intermediate-grade non-Hodgkin’s lymphoma (NHL) was studied with the aim of characterizing their adhesive phenotype and evaluating its relationship to clinical behavior. CD11a (LFA-1) was more expressed in NHL and IDL than in B-CLL (P = .047), although it was demonstrable in 74.2% of cases; CD11c was more expressed in B-CLL (P < .0001), and its expression was preserved in almost all of the cases of small lymphocytic lymphoma. In NHL patients, including the cases of IDL, VLA-3 expression was observable in 8 of 35 cases (although always at a low level of intensity), while VLA-4 was almost constantly expressed in a way that was similar to its expression in control normal B cells. On the contrary, in B-CLL patients, VLA-3 was expressed (prevalently at high levels) in 87.1% of cases and VLA-4 only in 37.1%. No correlation was found between adhesion molecule patterns and the clinical features of the diseases. The biofunctional significance of the imbalance of VLA-3 and VLA-4 expression in B-CLL is not easy to explain, but it has undoubted intrinsic value as an additional marker for distinguishing B-CLL from, in particular, those B-cell neoplasms (such as IDL) that share many of the immunocyto-morphologic characteristics and the putative normal counterpart (the mantle zone) of B-CLL.

INTEGRINS ARE A superfamilly of heterodimeric glycoproteins, consisting of various α (1 through 11) and β (1 through 6) subunits, whose function is to mediate cell-cell and cell-matrix adhesion in a number of cell types.1,2

The different integrins are classified by their β-chain structure: β1 integrins are very late activation antigens (VLA) that have molecules with the same β subunit but various α-chains (α 1 through 6); β2 integrins are leukocyte adhesion molecules (Leu-CAM) whose molecules have the same β-chain (CD18) and three different α-chains, CD11a, (LFA-1), CD11b (MAC-1), and CD11c (P-150.95);3-5 β3 integrins include the GPIIb/IIIa complex and the vitronectin receptor α5β3.

β2 integrins can be found in all leukocytes;3-5 β1 integrins, homogeneously expressed in nonhematopoietic cells, are considerably modulated in leukocytes according to cell type and functional condition.3-6 Their distribution in normal lymphoid tissue has also been defined.7 Given the functional role of integrins in a number of cell systems, great interest has been shown in their possible involvement in pathologic conditions such as neoplasias, where it is reasonable to postulate adhesive phenotype alterations in neoplastic cells.8,9

Relatively few studies concern chronic lymphoid neoplasms, and these are limited to the expression of β2 integrins that would seem to condition the leukemic diffusion of malignant lymphoid cells.10-12

The aim of the present study was to evaluate the β1 and β2 integrin expression in both the lymph node and peripheral blood in non-Hodgkin’s lymphoma (NHL) (including cases in leukemic phase) and in B-cell chronic lymphocytic leukemia (B-CLL).

MATERIALS AND METHODS

Patients. Seventy patients with B-CLL, 20 patients with a histologic diagnosis of low/intermediate-grade B-cell NHL, and 15 patients with leukemic lymphocytic lymphoma of intermediate differentiation (IDL) were included in the study. All of the B-CLL showed the classical phenotype13: low or undetectable M and/or D class Ig and CD19, CD5 and CD23 positivity. There were 42 men and 28 women, with a mean age of 60.2 years (range, 45 to 78 years); Rai staging distribution was 12 stage 0, 25 stage I, 26 stage II, and seven stage III-IV. Twenty-nine patients had received chemotherapy before the start of the study.

Table 1 shows the clinico-pathologic characteristics of the NHL patients (including those with IDL). In 20 patients, the histotype was defined according to the Working Formulation. The other 15 NHL cases were defined as leukemic IDL on the basis of morphologic and immunologic characteristics14; these cases presented monoclonal circulating B cells greater than 5,000/µL with these phenotypical characteristics: high SIg density expression, SIg class heterogeneity (4M, 4MD, 3G, 4MG), constant CD1c positivity, variable CD5 expression (eight cases), and low reactivity for CD23 (three cases). As shown in Table 1, the lymphocytes were polymorphous, consisting of small, medium, or large cells with slight nuclear irregularities and occasional clefts, often with one or more nucleoli.

Control samples were B-lymphocyte–enriched normal spleen and tonsil cell suspensions; the Epstein-Barr virus (EBV)-immortalized B-lymphoblastoid CB33 cell line, and the c-myc transfected (SVmyc2.3)-UHI 10-1 cell line, negative for CD11a, were used as controls.15

Phenotype studies. Mononuclear cells were separated from blood (B-CLL and IDL patients), lymph node (NHL patients), and the spleen and tonsils of normal donors on Ficoll-Hypaque gradient, suspended in RPMI 1640 medium, and incubated with AB human serum 10% at 37°C for 30 minutes. Afterwards, they were washed and resuspended in phosphate-buffered saline–fetal calf serum (PBS-FCS) 1%. Direct and/or indirect fluorescence
procedures were used for monoclonal antibody (MoAb) staining and cytofluorimetric analysis.13

Assays were performed using the following MoAbs: anti-Leu1-fluorescein isothiocyanate (FITC), anti-Leu2-FITC, and anti-Leu20-phycocerythrin (PE; Becton Dickinson, Mountain View, CA) for CD5, CD19, and CD23, respectively; TEC-CALLA and TEC-IL2R (Techno Genetics, Milan, Italy) for CD10 and CD25, respectively; and anti-IOT6c (Immunotech SA, Marseilles, France) for CD1c.

Polyclonal anti-heavy chain IgM, IgD, and IgG, and anti-κ and anti-λ chain (Dakopatts A/S, Glostrup, Denmark) were used for S Ig analysis.16

For the study of adhesion molecules, the following MoAbs (all supplied by Immunotech) were used: IOT16 for CD11a (LFA-1 a chain), IOM1 for CD11b (Mac-1), IOM11c for CD11c (p.150.95), IOP49b for CD49b (a2-VLA chain), IOP49d for CD49d (a4-VLA), IOP49e for CD49e (α5-VLA chain), IOP49f for CD49f (α6-VLA chain). MoAb P1B5, specific for the α3-VLA chain, was generously provided by Dr Giorgio Inghirami (Columbia University, New York, NY). For α1-VLA chain detection, MoAb VLA-1 (T Cell Sciences Inc, Cambridge, MA) was used. FITC-labeled goat antimouse IgF(ab')2 (Techno Genetics) was used as the second step in indirect immunofluorescence analysis.

T lymphocytes and monocytes present in cell suspensions were reduced to less than 5% by means of immunomagnetic separation by using magnetic polystyrene beads with goat antimouse IgG covalently bound to the surface (Dynabeads M-450; Unipath SPA, Milan, Italy) and by pretreating the cells with anti-Leu3 and anti-ReMu3 (Becton Dickinson), specific for CD3 and CD14, respectively, as previously described.17 Using this procedure, the percentage of mononuclear neoplastic B cells, evaluated as of S Ig analysis.16

RESULTS

Leu-CAM expression in the analyzed samples was constant for CD11b, but heterogeneous for CD11a and CD11c (Table 2). In particular, there was a greater reactivity for CD11a in NHL (regardless of the degree of leukemic diffusion) than in B-CLL patients (P = .047), where in any case the positivity for this integrin was 74.2%. On the contrary, CD11c was more expressed in B-CLL (P < .0001); all except one of the NHL CD11c-positive cases were of the small lymphocytic type.

All of the patients were VLA-1, VLA-2, and VLA-6 negative and VLA-5 positive, regardless of the pathology. However, there was a significant differentiation in VLA-3

Table 1. Clinico-Pathologic Characteristics of the 35 Patients With NHL (Including IDL)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Cases</th>
<th>% Positive Cells for</th>
<th>No. of Cases Expressing</th>
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<tbody>
<tr>
<td>B-CLL</td>
<td>70</td>
<td>&gt;20-&lt;50</td>
<td></td>
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<tr>
<td>NHL</td>
<td>20</td>
<td>&gt;20-&lt;50</td>
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<tr>
<td>Leuk IDL</td>
<td>15</td>
<td>&gt;20-&lt;50</td>
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<td>Controls</td>
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*P < .0001 (B-CLL v NHL + IDL; χ² test).
†P < .0003 (B-CLL v NHL + IDL; χ² test).

Table 3. VLA-3 and VLA-4 Expression in B-Cell Lymphoproliferative Diseases

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Cases</th>
<th>% Positive Cells for</th>
<th>No. of Cases Expressing</th>
</tr>
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<tbody>
<tr>
<td>B-CLL</td>
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*P < .0001 (B-CLL v NHL + IDL; χ² test).
†P < .0003 (B-CLL v NHL + IDL; χ² test).
and VLA-4 reactivity. As shown in Table 3, B-CLL patients were highly VLA-3 positive (87.1%), but had reduced VLA-4 expression (37.1%). In particular, 38 expressed only VLA-3, 23 cases coexpressed VLA-3 and VLA-4, six cases expressed neither VLA-3 nor VLA-4, and three cases expressed only VLA-4 (Fig 1).

An opposite reactivity pattern was observed in both NHL and IDL, where the global expression of VLA-4 (97.1%) and VLA-3 (22.8%) was very similar to that in control normal B cells. In particular, among the NHL patients, five cases coexpressed the VLA-3 molecule (three cases of small lymphocytic, one of small cleaved cell, and one of small/large cell lymphoma), and only one case was VLA-4 negative; of the IDL patients, three coexpressed VLA-3 and VLA-4 (Fig 2).

In the 17 cases with partial VLA-3 or VLA-4 expression (11 B-CLL, four NHL, and two IDL), reactivity was certainly due to the neoplastic cells insofar as the percentage of monoclonal malignant B cells was always greater than 85% and CD5/VLA3 or VLA-4 coexpression (in the 14 CD5-positive cases) could be demonstrated by means of two-color staining.

The degrees of adhesion molecule expression in terms of fluorescence intensity are shown in Table 4. The degree of intensity of CD11a was less in B-CLL than in NHL. The pattern was the opposite for CD11b, its degree of reactivity varying in NHL patients according to the extent of leukemic diffusion.

VLA-3 expression in B-CLL was prevalently grade 2, while in the rare cases of VLA-3-positive NHL cases, fluorescence intensity was grade 1. On the contrary, no difference in the intensity of VLA-4 expression could be shown between NHL/IDL and B-CLL-positive cases (Figs 1 and 2). Patterns 1, 2, and 3 (Fig 1) were characteristic of B-CLL; while patterns 4 and 5 (Fig 1) and the absence of VLA-3 and VLA-4 expression were also observed in a small percentage of NHL and IDL cases (Fig 2, panels 3 and 4).

Evaluation of the agreement percentage of reactivity of VLA-3, VLA-4, CD11a, and CD11c in the B-CLL patients showed no consensus of expression (in all cases, the K Cohen test gave < .5). Two cases were negative for CD11a, CD11c, VLA-3, and VLA-4.

No statistically significant correlation emerged between
the different expressions of VLA-3, VLA-4, CD11a, and CD11c in B-CLL patients and their clinico-hematologic characteristics (age at diagnosis, sex, period of follow-up, Rai-Binet stage, leukocyte count, organomegaly, or the presence/absence of therapy). Given its heterogeneity, no similar statistical evaluation was possible within the NHL group.

**DISCUSSION**

In recent years, a lot has been learned about the biology of the adhesion molecules responsible for intercellular and cell-environment relationships. One of the main reasons for the interest in these molecules is the possibility that they may play a determining role in neoplastic transformation and diffusion. The relationships between cell adhesiveness, transformation, tumor development, and the metastatic behavior of malignant cells suggest that tumor cell membrane has fewer general adhesive properties than normal cells.8,9

In particular, in lymphoproliferative syndromes, a lack of cell adhesiveness or an abnormal adhesion pattern in transformed lymphocytes may free these cells from regulation and thus contribute towards the development of leukemia and lymphoma.4

In fact, following the observation that Burkitt's lymphoma (BL) cells lack the expression of CD11a, as well as that of CD11b and CD11c,19 Inghirami et al showed that the deregulation of c-myc oncogenes (a characteristic feature of such cells) causes the downregulation of CD11a.15

Furthermore, some investigators have reported an absence or lower level of expression of LFA-1 in B-CLL than in other B-cell proliferative syndromes, such as follicular or small lymphocytic NHL, and have hypothesized that the adhesion molecule phenotype of malignant cells might affect their circulation patterns as lymphomas or leukemias.10-12

Our results in B-CLL do not seem to support these observations. Although confirming a higher expression of LFA-1 (in terms of the percentage of cases and fluorescence intensity) in NHL, regardless of the leukemic phase (IDL), significant positivity was also found in classical phenotype B-CLL. Our finding is supported by the number of examined cases, the exclusion of cases of variant pheno-
type, and the B-cell enrichment and repeated findings of CD11a negativity in the UH1(10-1)sv myc 2.3 cell line used as a control.

The degree of expression of CD11c in B-CLL observed in our study is comparable with that reported by Inghirami et al., and did not correlate with any particular clinical variable, including the presence of splenomegaly (38.2% CD11c positive vs 45.4% CD11c negative).20

However, the most interesting result concerns the distribution of VLA-3 and VLA-4 reactivity in B-CLL, in comparison with that found in the other B-cell disorders considered. These two molecules were antithetically expressed in the two groups of B-cell neoplasms (B-CLL vs NHL/IDL), regardless of the nature of the normal counterpart (germinal center or mantle zone) or the nodal and/or leukemic phase of NHL.

It is known that, in normal lymphoid tissue B areas, VLA-4 is widespread and particularly concentrated at the level of the mantle zone, while VLA-3 is present only in a few germinal center cells.7 Furthermore, VLA-4 seems to be widely distributed in all of the lymphocytic subpopulations deriving from spleen, tonsil, and peripheral blood, while VLA-3 expression seems to be limited to activated T lymphocytes (NK and LAK cells) and monocytes/macrophages; furthermore, it is also quite strongly expressed on many malignant hematopoietic cell lines.3

The high expression of VLA-4 and the very low level of VLA-3 reactivity found by us in NHL patients is thus compatible with their normal lymphoid tissue distribution.

But what was unexpected is such a high degree of VLA-3 reactivity in B-CLL, along with a marked reduction in VLA-4 expression. Of 70 cases, only three had a VLA-3/VLA-4 pattern similar to that of the mantle zone, 23 coexpressed the two molecules and six expressed neither; but a good 38 cases only expressed VLA-3.

It is difficult to interpret this result on the basis of existing knowledge about the physiology and pathologic expression of these molecules. One possible hypothesis is that VLA-3+/VLA-4- or ± is specific to a CD5+ B subpopulation present in a very small percentage at the periphery of the germinal center of normal adult lymph nodes, and that this could represent the normal counterpart of B-CLL.

If this were the case, it would fit well into the picture of the phenotypic complexity of lymphocytic mantle cells, which, as is known, variably coexpress antigens of the T-cell line (CD1c, CD5, and CD27) and/or activating antigens (CD23 and CD25), probably as an expression of functional dynamism.13 On the other hand, the correlation between CD5 and VLA-3 expression is rather limited: of the 15 of 35 NHL cases that were CD5 positive (eight IDL, four small lymphocytic lymphomas, and three small cleaved cell lymphomas), only five coexpressed VLA-3.

Furthermore, in the spleen and tonsil tissue used by us as controls, where the percentage of CD5/CD19-positive cells varied from 15% to 25%, no VLA-3 reactivity could be demonstrated.

In considering the functional role of these molecules, it may be that their altered expression is somehow involved with the biologic behavior of leukemic cells. A possible role of VLA-3 in hematopoietic cell neoplasias has already been outlined.3 By means of an epitope other than the fibronectin receptor, VLA-4 reacts with VCAM-1 (a vascular cellular adhesion molecule that is induced by inflammatory cytokines) on the nonlymphoid endothelium, thus constituting an additional and/or alternative route to the CD11a–ICAM-1 system for lymphocyte recruitment.21

However, we feel that an imbalance in VLA-3/VLA-4 expression is not enough to justify an alteration in the neoplastic B-cell adhesive phenotype, nor that it can be considered responsible for the particular anatomic site of neoplastic involvement.

Firstly, the adhesive phenotype of lymphocytes is the result of a complex network that also includes other adhesion molecules (CD44, CD54–ICAM-1, CD58–LFA-3, VLA-5) normally expressed in the cases studied by us (data not shown). Furthermore, leukemic diffusion as an event in itself does not seem to correlate with the particular pattern of VLA-3/VLA-4 expression; the majority of IDL patients analyzed presented a normal distribution of the two molecules. However, it is true that, unlike in NHL, leukemic diffusion is a constantly present feature at B-CLL diagnosis.

Finally, the NHL histotype that most frequently expressed VLA-3, even though it was always associated with VLA-4 expression, was small lymphocytic lymphoma (3 of 5 cases), which, as is known, is considered the lymph node counterpart of B-CLL. However, the limited number of small lymphocytic lymphoma cases analyzed makes it impossible to draw any conclusions concerning the possible role of the pattern of VLA-3/VLA-4 expression in the anatomic site of involvement of small B-lymphocytic neoplasms.

But beyond any possible interpretative hypothesis concerning our results, there remains the evidence that a high expression of VLA-3 and a reduced expression of VLA-4 are characteristic of B-CLL, thus providing an additional marker for the differential phenotypical characterization of B-cell lymphoproliferative syndromes beginning from the lymphocytic mantle.

ACKNOWLEDGMENT

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