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CD11c EXPRESSION IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA. A COMPARISON OF RESULTS OBTAINED WITH DIFFERENT MONOCLONAL ANTIBODIES

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ABSTRACT

In order to obtain more information on the pattern of CD11c-positivity in otherwise typical B-cell chronic lymphocytic leukemia (CLL), we analyzed immunological and clinico-pathological features of 99 such patients studied with two different monoclonal antibodies (MoAbs). Fifty-two out of 70 (74.2%) patients stained with IOM-11C MoAb and 3 out of 29 (10.3%) patients stained with Leu-M5 MoAb had more than 30% CD11c positive cells ($P < 0.0002$). The two groups were similar with regard to the expression of B-cell CLL-related antigens (CD5, CD20, CD23), as well as clinico-pathological features (i.e. Binet clinical stage and pattern of bone marrow involvement), thus suggesting that differences in CD11c expression were due to different reactivity patterns of the MoAbs utilized. In our experience, the use of different reagents may affect immunophenotyping results, thus providing conflicting data at times.

Key words: CD11c, B-CLL, expression pattern

CD11c, the 150kd molecular weight α-chain of a two-chain (150 and 95 kd) glycoprotein, belongs to the integrin family of glycoproteins that are members of the families of cellular adhesion antigens.[1] In its original description, CD11c showed strong reactivity with hairy cells[2] and, when coexpressed with CD22, was proposed as a unique marker of hairy cell leukemia (HCL).[3]

More recently, several studies have shown that CD11c is expressed in 13% to 78% of otherwise typical CLL patients; these results reflect differences in patient selection, in the monoclonal antibodies (MoAbs) used to detect CD11c, and in the technical sensitivity of flow cytometry instrumentations.[4,9] Keeping these concepts in mind, we analyzed CD11c-expression in 99 $CD5^+$ B-cell CLL patients diagnosed in two different periods. For the purpose of the present study the patient population was split into two groups according to the commercial MoAb used to detect CD11c (Leu-M5 or IOM 11c). Since the clinico-hematological features of the two groups were alike, differences in CD11c expression were considered suggestive of a different pattern of positivity of the MoAbs utilized.

Materials and Methods

Patient characteristics

Ninety-nine patients diagnosed as having CLL at our institution during the period June 1990 to March 1994 form the basis of this study. The mean age was 66 years (SD, 8.4) and the male to female ratio 61 to 38.

B-CLL was diagnosed according to generally accepted criteria that included peripheral blood lymphocytosis greater than 5×10^9/L and bone marrow (BM) lymphocytosis greater than 30%. Diagnosis of B-cell CLL was confirmed in all instances by analysis of surface Ig light chain and CD5 expression. All patients were staged according to the Binet clinical staging

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system[10] (stage A, 58; stage B, 23; stage C, 18), and the pattern of BM involvement was evaluated by means of BM biopsy in 62 of the 99 patients. Biopsy was not performed in the remaining 37 patients mainly due to lack of informed consent. Four histological patterns were recognized: interstitial, 32; nodular, 11; mixed, 4; diffuse, 15.[11,12] For the purpose of the present study, interstitial, nodular and mixed were grouped together as the non-diffuse pattern (Table 1).

Immunophenotype analyses

Fresh peripheral blood samples were used for immunological analyses. Mononuclear cells were separated from whole blood by Fycoll-Hypaque density gradient centrifugation, and cells were stained using both direct and indirect techniques. In the latter cases, a second-layer reagent of fluorescence (FTIC)-conjugated F $(ab')_2$ fragment of goat anti-mouse immunoglobulins (DAKO, Denmark) was used.

Monoclonal antibodies employed along with their CD as defined by the 4th International Workshop on Leukocyte and Differentiation Antigens, were as follows: HLA-DR (anti MHC-class II), OKT3-CD3 (T-lymphocytes), OKCLL-CD5 (T-lymphocytes, B-lymphocyte subsets, B-CLL cells), OKT26a-CD25 (activated T and B cells, activated macrophages), OKBCALLA-CD10 (lymphoid progenitors, C-ALL, granulocytes), Leu12-CD19 (immature and mature B-lymphocytes, malignant B-cells), Leu16-CD20 (B-lymphocytes, malignant B-cells), Leu20-CD23 (activated B-cells, B-CLL), κ and λ light chains (SIg) (Ortho, Raritan, NJ).

As far as CD11c is concerned, two different commercial MoAbs were used. In 70 patients diagnosed during the period June 1990 to March 1993, we utilized an IOM-11c MoAb (Immunotech, Marseille, France); in the remaining 29 patients, diagnosed between August 1993 and March 1994, Leu-M5 MoAb (Bekton-Dickinson, Mountain View, CA) was utilized. Flow cytometric measurements using simple color immunofluorescence were made by means of a CYTORON cytofluorograph (Ortho Diagnostic System).

Because of the different right and forward angle light-scattering properties of blood cells, only lymphocytes were used to separate this cell fraction from others. Controls were provided by cells incubated directly with FTIC-labeled second reagent alone, or by cells incubated with an unrelated MoAb with an isotype identical to that of the test MoAb (IgG1, CD41; IgG2a, CD10; IgG2b, CD69).

Leukemic cells were counted via the expression of CD19 and residual T-cells were identified through the expression of surface CD3 molecules. According to this criteria, the mean value of $CD19^+$ was 81.7±13.3% and that of residual T-cells 14.6±11.5%.

Thus, the requirement for CD11c positivity was expressed by at least 30% of positive leukemic cells. In 22 out of 99 patients who had a residual T-cell population greater than 20%, CD11c-positivity was assessed with double fluorescence. For this analysis phycoerythrinated (CD19/PE) and fluorescinated MoAbs (CD11c-FTIC) were used. Mean fluorescence intensity (MFI) was assessed on the basis of the mean channel fluorescence of each positive sample (linear acquisition, 0 to 250 channels).

Table 1. CD11c expression in B-CLL. Immunological and clinico-pathological characteristics of patients studied with IOM-11C MoAb (Group A) and Leu-M5 MoAb (Group B).

	Group A	*Group B*	*P*
N. patients	70	29	
Clinical stage			
A	40 (57.1 %)	18 (62%)	
B	17 (24.2%)	6 (20.6%)	NS
C	13 (18.5%)	5 (17.2%)	
BM histology			
Non diffuse	36 (75%)	11 (78.5%)	NS
Diffuse	12 (25%)	3 (21.4%)	
Cases			
$CD11c^+$	74.2%	10.3%	<0.0002
$CD5^+$	100%	100%	NS
$CD20^+$	87.2%	89%	NS
$CD23^+$	92.2%	95.6%	NS
κ^+	65.9%	72.2%	NS
λ^+	23.4%	13.6%	NS
SIg BDL*	10.6%	13.6%	NS

*SIg BDL: SIg below detection level

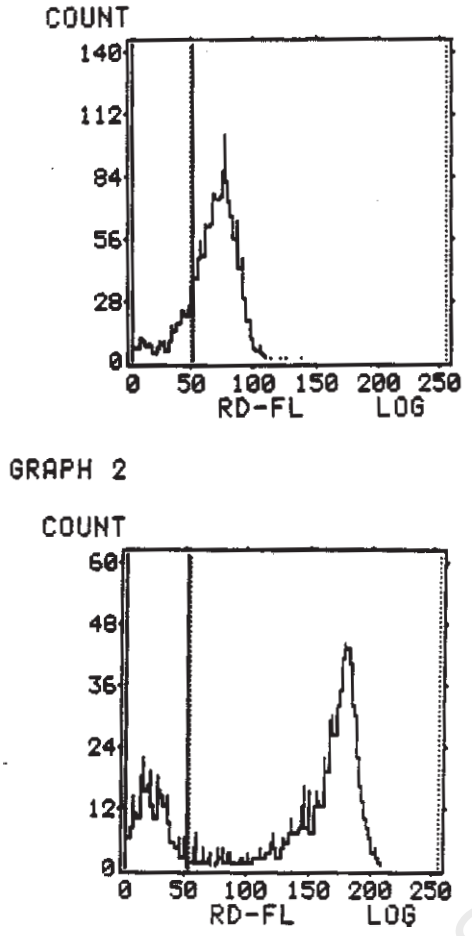

Figure 1. CD11c expression in B-cell CLL patient (A; MFI, 88.9 + 9.9) and hairy cell leukemia patient (B; MFI, 168.3 + 28.4).

Statistical analyses

The Student's t-test was used to evaluate differences among the means of various phenotypic groups. When dealing with discrete variables, statistical analyses were carried out by means of the chi-square test.

Results

Fifty-two out of 70 (74.2%) patients stained with IOM-11c MoAb and 3 out of 29 (10.3%) stained with Leu-M5 MoAb had more than 30% CD11-positive cells ($p<0.0002$). When patients were stratified according to the MoAb utilized for CD11c detection, it was clear that the two groups were alike with respect to the expression of CLL B-lineage antigens such as CD19, CD20, CD23, and light chains. This was also true when clinico-pathological comparisons were made according to clinical stage and histological pattern of BM involvement.

CD11c always displayed low fluorescence intensity (MFI, 86.5±15.3). This pattern of CD11c positivity can be distinguished from that of HCL cells. Indeed, this differentiation is easily made when CD11c histograms from a B-CLL and an HCL patient, respectively (Figure 1), are compared.

Discussion

The expression of CD11c in CLL is debated, and CD11c antibodies are heterogeneous and give varying results with CLL cells. In this setting we compared the results of CD11c expression obtained with two different commercial MoAbs. Since the two patient populations were alike with regard to clinico-hematological as well as immunological features, differences in CD11c expression were considered representative of different reactivity patterns of the MoAbs utilized. Our results are in keeping with those of literature; overall 140 out of 378 (37%) B-cell CLL patients analyzed with Leu-M5 and 108 out 140 (77.1%) analyzed with IOM-11c MoAb expressed CD11c ($p < 0.0002$).[3,9]

Finally, the present report provides information that is lacking in the literature on the positivity pattern of different CD11c MoAbs in B-cell CLL. In our opinion, when immunophenotyping results are compared it is mandatory to take into account the reagents used.

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