Surface Membrane Immunoglobulin Expression in Chronic Lymphocytic Leukemia

D.		Brohée
P.		Cauchie
P.		Nève

CHU André Vésale, Montigny-le-Tilleul, Belgium

Dr. D. Brohée, Department of Internal Medicine, CHU Vésale, rue de Gozée, 706, B-6110 Montigny-le-Tilleul (Belgium)

Chronic lymphocytic leukemias (CLL) can be classified on morphological grounds as classical (less than 11% of prolymphocytes), mixed cell types (CLL/PL, 11 to 55% of prolymphocytes), and prolymphocytic (PL, more than 55% of prolymphocytes) [1, 2]. It is currently presented that PL lymphocytes express surface membrane immunoglobulins (Sm Ig) very brightly in contrast to the weak expression on CLL lymphocytes [1-7]. In most of the cases, Sm IgM alone or, less frequently, Sm IgM and IgD are expressed [5, 6]. It has been suggested that kappa chains were expressed more often [1, 4, 5, 8]. However, analysis of positivity has relied mainly on fluorescence microscopy and subjective quantitation.

We used a FACScan™ 488 nm argon flow cytometer (Becton Dickinson) to determine antigen density on a semiquantitative basis, in 20 consecutive patients (M/F 13:7, mean age 74 years) observed in our community hospital. The data were acquired in list-mode files using the Simultest™ software after automatic setting of the cytometer with the AutoComp™ software. Further analyses were performed with the Lysis™ software. Gate accuracy was checked with Simultest LeucoGATE™ anti-CD45 and CD14 monoclonal antibodies. The negative control was determined using Simultest Control γ 1/ γ 2a™ irrelevant antibodies. For Sm Ig determination, the following antibodies were used: Simultest Anti-Kappa (FITC)/Anti-Lambda (PE)™ after Fc-receptor blocking with rabbit serum, FITC-labelled antihuman IgG, IgA, IgD and IgM, and in some selected cases, PE or FITC-labelled anti-kappa or anti-lambda Fab'2 rabbit fragments (DAKO). Fluorescence intensity was quantified in arbitrary units (AV) on a scale from 1 to 10,000 (256 channels). The number of positive cells was expressed as the percentage of lymphocytes with a fluorescence intensity above the negative control (typically above 10 AU). Raw data are displayed in table 1. In only one case was no Ig determinant found (5%). In contrast with previous studies [1, 4, 5, 8], but in good keeping with a recent one [9], we observed a lambda predominance (55%). No Sm IgG or IgA were detected. Heavy chain expression was remarkable, in contrast with the current opinion, showing IgM alone in 10%, IgD alone in 25%, both IgD and IgM in 45%, and none in 20%. If we applied a threshold of 20% for positivity, the corresponding figures would be 5, 40, 15 and 40%. To our knowledge, this is the first report demonstrating that Sm IgD are the most frequently expressed immunoglobulins (at least in 55%, and 70% without threshold restriction). It should be noticed that the percentages of positive cells

for IgD and IgM were concordant in only 4 cases. Discordant results were also found in fluorescence positivity or intensity, between light chain and heavy chain expression.

A bright Sm Ig expression (any Ig determinant with intensity > 50 AU) was observed in the PLL case, but also in 3 CLL/PL (60%) and 4 classical CLL (29%).

We conclude that the Sm Ig phenotype is not a reliable marker for CLL classification. If it had to be part of a universal CLL typing system, a panel of reference antibodies against specified Ig epitopes should be defined and provided to the community.

In our series, the predominant IgD heavy chain expression suggests a later stage of B-cell commitment than generally recognized in CLL [6, 7] or a greater disruption of the normal programme of cell maturation.

Incidentally, we found that the light chain of the 4 serum monoclonal proteins observed in our series, was different than that expressed at the surface of the CLL cells. In another study, the same light chain had been found in serum and on leukemic cells [10], and, accordingly, it is generally held that the monoclonal proteins in CLL are di-

Table 1. Phenotype determination and semiquantitative evaluation of surface membrane immunoglobulin expression in 20 consecutive chronic lymphocytic leukemias

Sm Ig expression is presented as the percentage of positive cells (fluorescence intensity above the upper normal limit of the negative control), with, between parentheses, the mean fluorescence intensity. The symbols + (?) are used for very faint positivity and unfair gate determination.

rect secretory products of the tumor cells. Our cases could be examples of neoplastic phenotype shift [11]. Rather, we suggest that these gammopathies are secondary to the CLL-related immunodeficiency and abnormal T/B immunoregulation [6, 7], and are examples of Rads type 3b gammopathies [12]. Monoclonal proteins in CLL might be of various origin, secreted by the CLL malignant clone or by unrestrained benign clones.

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