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DO MYELOMATOUS PLASMA CELLS REALLY EXPRESS SURFACE IMMUNOGLOBULINS?

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ABSTRACT

Surface immunoglobulins (sIg) are traditionally considered to be absent in plasma cells (PC). However, it has recently been reported that up to one third of myeloma patients are positive for surface immunoglobulins. Nevertheless, this observation has not been confirmed in other studies. In order to assess whether or not sIg are really expressed by myelomatous PC and to exclude possible staining of either cytophilic or cytoplasmic Igs, simultaneous experiments were carried out with i) incubation at 37°C, ii) blocking with non-conjugated anti-Ig light chains, and iii) cytoplasmic staining after cell membrane fixation and permeabilization. Triple staining for CD38/κ/λ was used in all cases and the staining intensity was quantitated in MESF (molecule equivalents of soluble fluorochrome). In addition, 20 B-CLL cases and 10 healthy donors were used as reference controls. Our study shows that 7 out the 20 patients (35%) analyzed expressed sIg and that the surface staining was specific.

Key words: surface immunoglobulin, flow cytometry, plasma cell, multiple myeloma

Both normal and myelomatous plasma cells (PC) represent the latest stage of B-cell differentiation, although they frequently lack expression of the typical surface B-cell-associated markers such as CD20, HLA-DR, CD22, CD37 and CD21.[1] In contrast, it has been shown that they express cytoplasmic immunoglobulins (cIg) together with non-lineage restricted antigens like CD38 and CD9 among others.[2] Although surface Igs were classically considered to be absent in this last stage of B-cell differentiation,[3] Omedé *et al.*[4] have recently reported sIg reactivity in up to one third of all multiple myeloma (MM) cases, those considered immature myelomas (ie cases displaying lymphoplasmocytoid morphology and more immature phenotype) with a poor outcome. In spite of this interesting clinical implication, most immunophenotypic studies have failed to show the presence of sIg in MM patients. Moreover, when considering sIg expression in PC, several pitfalls (staining of cytophilic and cytoplasmic Igs due to poor membrane preservation) must be avoided. This is especially important in samples obtained more than 24 hours prior to staining and in which high amounts of monoclonal Ig molecules are usually present in both the serum of the patient and in the cytoplasm of the cells under study.

The aim of the present study was to assess whether the reactivity detected is really due to the presence of sIg in MM PC or to the staining of cytophilic and/or cytoplasmic Igs.

Materials and Methods

Patients and samples

A total of 20 patients (19 with MM and one patient with plasma cell leukemia) diagnosed according to the criteria of the Chronic Leukemia-Myeloma Task Force (1973) were included in the present study. There were 13 males and 7 females; the mean age of this series

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was 72.1±5.8 years. The serum monoclonal component was IgG in 15 and IgA in three, while the remaining two patients had Bence-Jones myeloma. The serum monoclonal Ig light chain was κ in 65% and λ in 35% of the cases. Fresh (<12 hours from the time of collection to preparation and analysis) EDTA-anticoagulated and phosphate buffered saline (PBS)-diluted (1/1 vol/vol) bone marrow samples were studied in all cases. In addition, PB samples from 20 patients with B-CLL and 10 healthy donors were obtained in order to have reference control values for the assessment of Ig molecules.

Immunophenotypical studies

For this study, triple labeling with the monoclonal antibodies (MoAb) anti-κ, anti-λ and anti-CD38 conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE) and the PE/Cyanin5 fluorochrome tandem were used in all cases. Anti-κ and anti-λ MoAb were purchased from Becton-Dickinson (San José, CA, USA) and anti-CD38 from CALTAG Laboratories (San Francisco, CA, USA). Samples were washed twice in 4 mL of PBS and centrifugated at 2000 r.p.m for 5 minutes prior to staining with the MoAb. Then they were incubated for 15 minutes, at room temperature in the dark, in the presence of 10 μL of each of the above mentioned MoAb. Afterwards, 2 mL of FACSlysing solution (Becton-Dickinson) was added and the samples were incubated for another 10 minutes under the same conditions. Then the tubes were centrifuged for 5 minutes at 2000 r.p.m. and the cell pellet washed again with 4 mL PBS. Finally, the cells were resuspended in 0.5 mL of PBS (tube 2) for flow cytometry analysis. An isotype-matched negative control was used in all cases (tube 1).

In order to exclude reactivity of the anti-Ig light chain antibodies with either cytophilic or cytoplasmic Igs, an experiment containing three additional tubes (#3, #4 and #5) was designed:

- tube 3 (to exclude cytophilic staining): sample preparation protocol identical to that described for tube 2, but preceded by incubation at 37ºC for 30 min with 4 mL of an Ig-free medium (PBS) in order to eliminate cytophilic Igs.[5]
- tube 4 (to exclude cytoplasmic staining): sample preparation was similar to that described for tube 2, but after washing and prior to staining with the direct-conjugated MoAb, the cell pellet was incubated for 15 min at room temperature with an excess (40 μL) of non-conjugated anti-Ig κ and λ light chain MoAb (Becton-Dickinson) from the same clones as the fluorochrome-conjugated reagents, and then washed in 4 mL of PBS;
- tube 5 (to assess cIg expression): prior to staining with anti Ig light chain MoAb conjugated with fluorochromes as described for tube 2, a fixation/permeabilization (Fix & Perm, Caltag) step was performed.

In all cases, data collection was carried out on a FACSort flow cytometer (Becton-Dickinson) equipped with an argon ion laser tuned to 488 nm and 15 mWatts. Information on a total of 20,000 events/tube was acquired and stored. The Pain-A-Gate Plus software (Becton-Dickinson) was used for data analysis. PC were identified according to their strong reactivity for CD38 and their light scatter distribution. Quantitation of positivity for the anti-κ and anti-λ MoAb was performed using QuickCal beads (Flow Cytometry Standards Corp.) and results were expressed as the mean number of specific MESF (*molecule equivalents of soluble fluorochrome*) obtained after substracting the values obtained for non-specific fluorescence (tube 1). The threshold for positivity was 1000 MESF for FITC and PE, respectively. Finally, in order to compare the intensity of reactivity for κ or λ in positive MM cases with those found both in normal B-lymphocytes and in other B-cell lymphoproliferative disorders such B-CLL, we carried out tubes 1 and 2 in 10 normal peripheral blood samples and in 20 B-CLL sIg^+ cases (10 κ and 10 λ).

Results and Discussion

Of the 20 patients analyzed, 7 showed reactivity for sIg (35%); the sIg light chain was κ in 4 cases and λ in 3. This incidence is similar to that reported by Omedé *et al*.[4] The plasma cell leukemia case studied proved to be negative for sIg staining. Table 1 shows the correlation

Table 1. Correlation between Ig isotype and reactivity for surface light chain immunoglobulins.*

Ig isotype	sIg (+)	sIg (–)
IgG	5	10
IgA	1	2
Bence-Jones	1	1

**Results expressed as number of positive cases for each category.*

Table 2. Comparison of intensity staining between normal lymphocytes, sIg^+ B-CLL and sIg^+ MM cases.*

	MESF	
	κ-FITC	*λ-PE*
Normal B-lymphocytes	40,938±17,587	46,845±17,587
B-lymphocytes from B-CLL	2,474±951	7,885±6,646
PCs from Multiple Myeloma	3,360±531	4,034±1,027

** Results expressed as mean ± standard deviation after correction of MESF values (fluorescence intensity in the tested sample minus the isotype-negative control value for the same sample). The intensity of sIg assessed by MESF was significantly lower in PC from multiple myeloma patients than in B lymphocytes from healthy donors (p= 0.001). No differences were observed between PC from MM and B lymphocytes from B-CLL patients (p= 0.1).*

Table 3. Expression of surface light chain immunoglobulins in a multiple myeloma patient.*

	Ig light chain	
Tube Number	*κ-FITC*	*λ-PE*
2 (sIg staining)	298 (–)	4,114 (+)
3 (sIg staining - cytophilic Ig)	448 (–)	5,840 (+)
4 (Ig staining - sIg)	74 (–)	199 (–)
5 (cIg staining)	618 (–)	432,673 (+++)

**Results expressed as specific molecule equivalents of soluble fluorochrome. Threshold for positivity was 1000 MESF for both FITC and PE.*

between Ig isotype and reactivity for sIg. The positivity was weak in all cases but one, which showed strong reactivity for λ light chain. Overall, the pattern of sIg staining intensity assessed by MESF in PC was similar to that observed in B lymphocytes from B-CLL and sig-

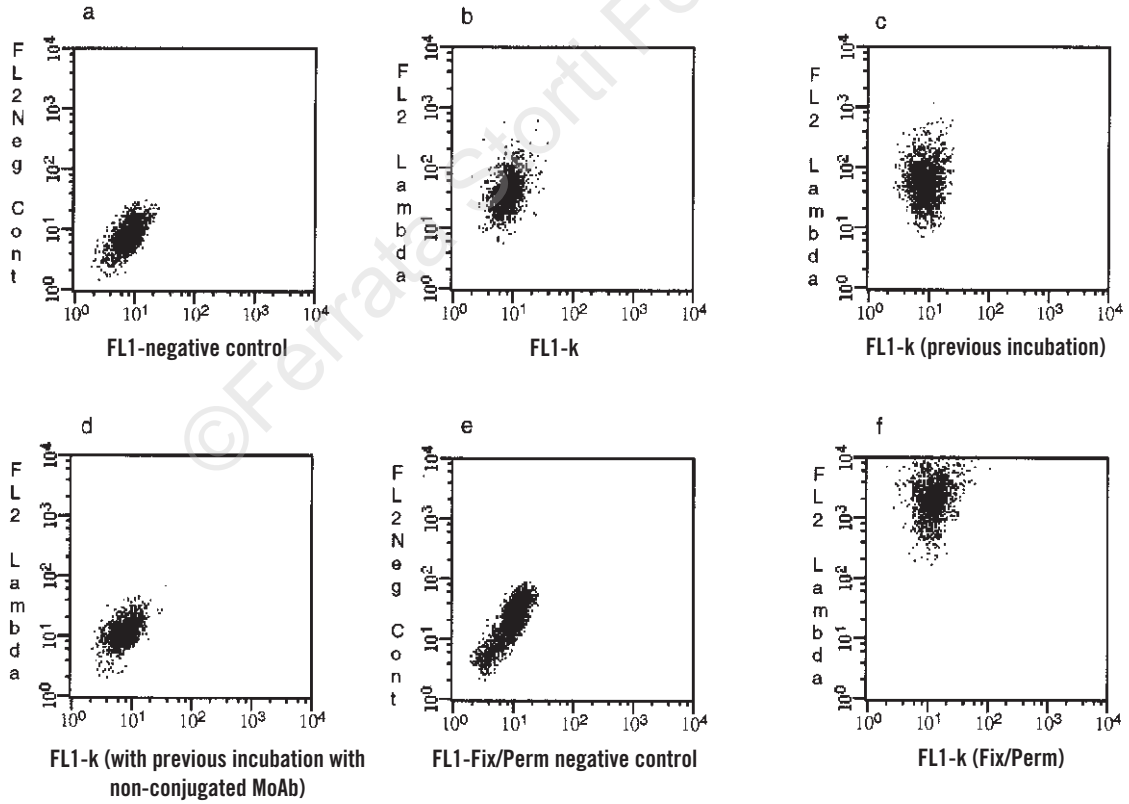

Figure 1. a) tube 1: isotype negative control; b) tube 2: sIg light chain staining with fluorochrome-conjugated MoAb showing positivity for λ light chains; c) tube 3: sIg light chain staining as described for tube 2 after incubation at 37ºC for 30 min, showing the same pattern of positivity as in b; d) tube 4: prior incubation with non-conjugated anti-Ig light chain MoAb makes the positivity seen in tube 2 disappear, leaving the same pattern as a; e) negative control tube using previous permeabilization step; f) tube 5: strong positivity for cytoplasmic λ light chains.

nificantly lower to that found in normal mature B-lymphocytes (Table 2). In all patients the sIg light chain isotype obtained was identical to that of the serum monoclonal Ig, as well as to that of the cIg present in the myelomatous plasma cells. Figure 1 shows an example of the reactivity obtained in tube 2 in a sIg λ positive case (Figure 1b) as compared to the negative control (tube 1, Figure 1a). Incubation of the sample at 37°C, which was performed prior to staining in order to eliminate cytophilic Igs (tube 3), showed no significant differences when compared to tube 2, in which this incubation/washing step was not performed (Figure 1c). Accordingly, Table 3 shows that the MESF values for Ig light chains do not change significantly between tubes 2 and 3. In addition, the remaining non-plasma cells (granulocytes) were also negative for sIg staining, thus excluding non specific staining due to cytophilic Ig (data not shown).

The second goal of this experiment was to rule out the possibility that the Ig reactivity detected was due to cIg staining. As shown in Table 3 and in Figure 1d, when the cells were incubated with a non-conjugated MoAb against the λ Ig light chain prior to incubation with the fluorochrome-conjugated reagents, positivity disappeared (tube 4). These findings indicate that an excess of non-conjugated MoAb used on non-permeabilized/non-fixed cells prevents detection of cell Ig by the fluorochrome-conjugated reagents. This would only be possible if the Ig molecules were on the cell surface, since the different reactivity detected between tubes 2 and 5 (Figure 1) indicates that without cell fixation/permeabilization saturation of the cIg molecules present in myelomatous plasma cells is not achieved. Moreover, it should be noted that the MESF values are significantly higher for cIg than for sIg (Table 3, Figure 1f). Accordingly, our results indicate that the reactivity detected in tube 2 is truly due to the presence of Ig light chain molecules on the external part of the cell membrane.

Consequently, it can be concluded from our study that PC from a relatively high proportion of MM patients do express surface Igs, and that the reactivity detected is not due to cytophilic or cytoplasmic immunoglobulins.

References

1. Ruiz-Argüelles G, San Miguel JF. Cell surface markers in multiple myeloma. Mayo Clin Proc 1994; 69: 684-90.
2. San Miguel JF, Gonzalez M, Gascón A. Immunophenotype heterogeneity of multiple myeloma: influence on the biology and clinical course of the disease. Br J Haematol 1991; 77: 185-90.
3. Caligaris Cappio F, Bertero MT, Bergui-L. Autoimmunity, autoimmune diseases and lymphoproliferative disorders. Haematologica 1994; 79:487-92.
4. Omedé P, Boccadoro M, Fusaro A, Gallone G, Pileri A. Multiple myeloma: "early" plasma cell phenotype identifies patients with aggressive biological and clinical characteristics. Br J Haematol 1993; 85:504-13.
5. Howitz DA, Lobo DI. Characterization of two populations of human lymphocytes bearing easily detectable surface immunoglobulin. J Clin Invest 1975; 56:1464-72.