

Expression of cell surface markers after human B lymphocyte activation

(phenotypic changes/differentiation)

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ABSTRACT The fate of two recently described human B lymphocyte-specific antigens (B1 and B2) was studied after B-cell activation *in vivo* and *in vitro*. Whereas both B1 and B2 were present on virtually all B cells from normal lymph nodes, B2 was absent from approximately 50% of B cells from hyperplastic lymph nodes. When B cells from spleen, tonsil, or peripheral blood were stimulated *in vitro* with pokeweed mitogen, activated cells were found to lose B2 (days 4–5) and subsequently B1 (days 6–7). Temporally, B2 loss was accompanied by loss of surface IgD, expression of T10, and the development of intracytoplasmic IgM; B1 loss was correlated with the acquisition of surface IgG and the appearance of intracytoplasmic IgG. Peripheral blood B cells, on which B2 is normally only weakly expressed (B1⁺⁺⁺⁺B2⁺) in contrast to B cells from secondary lymphoid organs (B1⁺⁺⁺⁺B2⁺⁺), exhibited a transitory increase in B2 expression to the B1⁺⁺⁺⁺B2⁺⁺ phenotype prior to B2 disappearance during activation. Taken together with other findings, this observation suggests that peripheral blood may contain a relatively immature subpopulation of B cells.

In several previous studies (1–3), two human B cell-specific antigens, designated B1 and B2, were identified and characterized with the use of monoclonal antibodies. B1 is present on >95% of B cells in peripheral blood and lymphoid organs; it is absent from resting and activated T cells, monocytes, and granulocytes (1). B1 is expressed on virtually all tumors of B-cell origin with the exception of myeloma, and it is also found on a proportion of non-T non-B acute lymphatic leukemia cells which possess “pre-B” cell characteristics (2). B2 has a similar pattern of cellular distribution; however, in contrast to B1, B2 is only weakly expressed on peripheral blood B cells and on some but not all lymph node cells. B2 is strongly represented on tonsil and spleen cells (3). In addition, B2 shows heterogeneous expression on B-cell malignancies. B2 is present on most chronic lymphatic leukemia cells (90%), approximately 50% of both diffuse and nodular poorly differentiated lymphomas, and 10% of acute lymphatic leukemia cells but is absent in Burkitt lymphoma, Waldenström macroglobulinemia, and myeloma (4). Both B1 and B2 are distinct from standard B-cell markers, including Ig isotypes, C3 and Fc receptors, and DR (Ia-like) antigens (1, 3). Molecular weights of 30,000 and 140,000 have been assigned to B1 and B2, respectively (3).

The differential representation of B2 and, to a lesser extent, B1 on B-cell tumors and the diminished reactivity of B2 on B cells from peripheral blood and some lymph node samples has led us to investigate the possibility that B1 and B2 are human B-cell differentiation antigens. In the present studies, the fate of B1 and B2 was studied subsequent to B-cell activation and differentiation, both *in vivo* by examination of normal and reactive lymph node tissue and *in vitro* by using the pokeweed

mitogen (PWM)-induced differentiation of B cells to antibody-forming cells. In addition, the expression of B1 and B2 was correlated with other B-cell markers—including immunoglobulin isotypes, a newly described antigen shared by thymocytes, activated lymphocytes, and plasma cells (T10) (4)—and the production of intracytoplasmic Ig (cIg).

MATERIALS AND METHODS

Monoclonal Antibodies. The production and characterization of monoclonal anti-B1 and anti-B2 have been described (1, 3). Additional monoclonal antibodies used included: T3 (5) (anti-T cell); Mo1 (6) (anti-monocyte, anti-peripheral mononuclear leukocyte), and Mo2 (6) (anti-monocyte), kindly provided by R. Todd (Sidney Farber Cancer Institute, Boston); I-2 (7) [anti-DR (Ia-like) framework antigen]; antibodies specific for human κ or λ light chain, IgM, IgG, and IgD, kindly provided by Vic Raso (Sidney Farber Cancer Institute). All antibodies were utilized in the ascites form.

Normal Human Tissue. Human tissue specimens were obtained during surgery. Tonsil cells were obtained at the time of routine tonsillectomy. Lymph node tissue, taken for diagnostic biopsy, was considered to be nonmalignant based on histologic features and cell surface markers. Splenocytes were obtained from patients who had sustained traumatic rupture. All tissue specimens were immediately placed in medium containing 5% fetal calf serum, finely minced with forceps and scissors, and made into single-cell suspensions by extrusion through stainless steel mesh. Cell samples were cryopreserved and thawed as needed.

Isolation of Lymphocyte Populations. Human peripheral blood mononuclear cells were isolated from healthy volunteer donors by Ficoll/Hypaque centrifugation. Unfractionated mononuclear cells were separated into surface Ig-positive (sIg⁺) (B) and sIg⁻ (T plus null) populations by anti-F(ab')₂-Sephadex G-200 chromatography (8) with modifications designed to minimize monocyte retention by the column. In brief, purified rabbit anti-human F(ab')₂ was pepsin-digested and chromatographed on Sephadex G-150 to remove undigested material. The F(ab')₂ anti-human F(ab')₂ fragments were then coupled to CNBr-activated Sephadex G-200. Peripheral blood mononuclear cells were preincubated at 37°C for 1 hr to remove cell-bound serum IgG (9), and cells were then applied to the anti-F(ab')₂ column and fractionated as outlined (8). The sIg⁺ (B) population was obtained from the column by competitive elution with normal human gamma globulin. B-cell preparations were routinely >90% sIg⁺ and <5% E (sheep erythrocyte) rosette-positive, and contained approximately 5% monocytes as judged by appearance, latex ingestion, and reactivity with the

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Abbreviations: FACS, fluorescence-activated cell sorter; cIg, intracytoplasmic immunoglobulin; sIg, surface immunoglobulin; PWM, pokeweed mitogen.

monocyte-specific antibody Mo1. T cells were recovered by rosetting the sIg^- population with 5% sheep erythrocytes. The rosetted mixture was layered over Ficoll/Hypaque, and the recovered rosette-positive pellet was treated with 0.155 M NH_4Cl to lyse erythrocytes. The T-cell population obtained was $<2\%$ sIg^+ by methods described (10).

Cell Cultures. Unfractionated cryopreserved tonsil or spleen cells were thawed, washed three times, and resuspended in tissue culture medium (RPMI-1640 containing 10% fetal calf serum, 12.5 mM Hepes, 4 mM L-glutamine, and gentamycin at 25 $\mu g/ml$). In experiments with peripheral blood B cells, fractionated B cells and mitomycin C-treated T cells were mixed in a 1:1 ratio. All cells were cultured in the presence or absence of PWM (25 $\mu g/ml$) at a concentration of $2 \times 10^6/ml$ in 24-well culture plates (Falcon) and were maintained in a 95% air/5% CO_2 humidified atmosphere. At varying times after initiation, cultured cells were subjected to Ficoll/Hypaque centrifugation in order to remove dead cells prior to phenotypic analysis. In experiments designed to test Ig isotype after activation, cells were also incubated at 37°C for 1 hr (9) in the presence of normal rabbit IgG (1 mg/ml) in order to displace any secreted IgG that may have become bound to cells during the culture period.

In experiments designed to test the stimulatory capability, if any, of the anti-B1 and anti-B2 antibodies, peripheral blood B cells were cultured in the presence of varying dilutions of experimental or control antibodies (1:10 to 1:10,000) for 4 or 7 days. Cultures were carried out in microculture plates, with 1×10^5 cells per well. Sixteen hours prior to termination, cultures were pulsed with 0.25 μCi (1 Ci = 3.7×10^{10} becquerels) [3H]thymidine. Cultures were harvested with an automatic cell harvester (Brandel, Gaithersburg, MD) onto glass filter strips, dried, and assayed by scintillation spectroscopy (Packard). Additionally, cultured cells were examined after 7 days for the antibody-induced development of Ig-secreting cells by reverse hemolytic plaque assay. In separate studies, the ability of anti-B1 or anti-B2 to inhibit PWM-induced differentiation was also tested. Peripheral blood B cells were cultured in the presence and absence of PWM, in media containing varying dilutions of anti-B1, anti-B2, or control antibodies. Cells were examined after 7 days *in vitro* for the enumeration of Ig-secreting cells by reverse hemolytic plaque assay.

Indirect Immunofluorescence. Cryopreserved cells from lymphoid tissues or cultured cells from activation studies were analyzed for surface phenotype by indirect immunofluorescence as described (11). In brief, $0.5-1 \times 10^6$ washed cells were treated with 100 μl of a 1:500 dilution of specific or control antibodies, incubated at 4°C for 20 min, and washed three times. Cells were then treated with 100 μl of a 1:40 dilution of goat anti-mouse IgG conjugated with fluorescein isothiocyanate (Meloy Laboratories, Springfield, VA), incubated at 4°C for 20 min, washed three times, and analyzed on a fluorescence-activated cell sorter (FACS-I) (Becton Dickinson, Mountain View, CA). At least 20,000 cells were analyzed. The approximate num-

ber of cells staining with a given reagent was determined by subtraction of cells staining with an isotype-matched control antibody.

Assays for Ig-Producing Cells. Cells containing cIg were counted by indirect immunofluorescence by a modification of a described technique (12). Cyto centrifuge preparations of cultured, Ficoll/Hypaque-separated cells were fixed in 95% ethanol at 4°C for 20 min. Slides were then washed in phosphate-buffered saline for 20 min, exposed to control or class-specific anti-Ig monoclonal antibodies at a 1:200 dilution, and incubated for 20 min at room temperature in a humidified atmosphere. Slides were then washed for 2 hr in buffered saline and stained with a 1:50 dilution of fluorescein-conjugated goat anti-mouse IgG for 20 min. After additional 2-hr washing in buffered saline, slides were mounted in 50% (vol/vol) glycerin in phosphate-buffered saline, and cells containing cIg were counted with a fluorescent microscope. At least 200 cells were counted per slide. Fewer than 1% of cells were found to stain with control antibody preparations. In experiments carried out to test the stimulatory or inhibitory capacity of anti-B1 and anti-B2, Ig-secreting cells were counted by reverse hemolytic plaque assay as outlined (1, 13).

RESULTS

Reactivity of Anti-B1 and Anti-B2 on Lymphoid Tissues. The reactivity of anti-B1, anti-B2, T3, Mo1, Mo2, I-2, anti- κ , and anti- λ on various lymphoid tissues was examined by indirect immunofluorescence on the FACS-I. In all cases, the percentage of cells positive for B1 was similar to the percentage positive with a mixture of anti- κ and anti- λ , although slightly fewer cells were found to stain with B1 than with anti- κ/λ in lymph node (Table 1). In contrast, fewer cells stained with anti-B2 than with anti- κ/λ for all B cell-containing tissues. These differences were significant for lymph node ($P < 0.005$), spleen ($P < 0.05$), and tonsil ($P < 0.05$); a similar trend was also seen with peripheral blood. However, it should be noted that precise enumeration of B cells staining with anti-B2 in blood was difficult due to the low representation of B2 on this tissue. No significant staining of thymus was seen with any B-cell reagent. At least on a gross level, therefore, we find evidence for heterogeneity of reactivity of anti-B2 on the B-cell population from various lymphoid organs.

The results for lymph node cells in Table 1 were pooled from a total of 10 samples, of which 5 were derived from normal nodes and 5 were from hyperplastic nodes. Hyperplastic or reactive lymph nodes were defined as nodes with enlarged, active germinal centers that contained increased numbers of mature lymphocytes, many of which showed mitotic figures or increased numbers of reticulum cells. An informative picture emerged when results were retabulated as a function of the histopathologic status of the node from which cells were obtained (Table 2). In normal nodes the percentages of $B1^+$ and $B2^+$ cells were

Table 1. Reactivity of anti-B1 and anti-B2 with normal lymphoid organs

Tissue	Tested, no.	% of cells reactive with antibody						
		B1	B2	κ/λ	T3	Mo1	Mo2	Ia
Peripheral blood	8	9 \pm 2	6 \pm 1	10 \pm 2	66 \pm 4	20 \pm 4	9 \pm 4	13 \pm 1
Lymph node	10	32 \pm 6	23 \pm 4	35 \pm 5	39 \pm 8	5 \pm 3	ND	28 \pm 7
Spleen	4	37 \pm 8	26 \pm 4	36 \pm 7	22 \pm 5	7 \pm 2	ND	43 \pm 2
Tonsil	5	52 \pm 5	45 \pm 5	53 \pm 5	8 \pm 2	ND	ND	48 \pm 3
Thymus	5	<5	<5	<5	8 \pm 2	<5	<5	<5
Bone marrow	6	<5	<5	<5	<5	23 \pm 12	12 \pm 5	7 \pm 3

ND, not determined. Data are shown as mean \pm SD.

Table 2. Reactivity of anti-B2 with normal and reactive lymph node cells

Lymph node	Tested, no.	% cells reactive with antibody				
		B1	B2	κ/λ	T3	Ia
Normal	5	27 \pm 5	28 \pm 5	31 \pm 4	40 \pm 6	29 \pm 7
Reactive	5	36 \pm 7	17 \pm 3	39 \pm 6	38 \pm 10	26 \pm 6

Data are shown as mean \pm SD.

identical and closely similar to the percentage of sIg⁺ cells. In contrast, for reactive nodes, B2⁺ cells were less frequent than B1⁺ or sIg⁺ cells. Thus, in a reactive lymph node, presumably actively undergoing an immune response, B-cell heterogeneity was clearly present with respect to the expression of B1 and B2 antigens.

Loss of B1 and B2 During PWM-Induced Differentiation. The above findings suggested that phenotypic changes accompany activation *in vivo* and led us to attempt to mimic these events *in vitro*. PWM has been shown to induce the differentiation of some fraction of B cells to antibody-containing or -secreting plasma cells (14), a function that is T-cell dependent in man (15). The fate of B1 and B2 subsequent to PWM stimulation was therefore investigated.

Mononuclear spleen cell preparations were cultured with and without PWM and examined at various times thereafter for cell surface phenotype. A representative experiment is depicted in Fig. 1. Approximately 60% of the starting spleen cell population expressed both B1 and B2 strongly. Approximately 20–25% of the spleen cells were T cells (staining with T3) and 10% were macrophages (reactivity with Mo1) (data not shown). After PWM stimulation, however, the intensity of staining with both anti-B1 and anti-B2 initially remained stable (days 1–3, data not shown) and then both diminished, until finally both antigens became undetectable on stimulated cells. What fraction of B cells in the starting culture were stimulated by PWM to undergo these events is unknown. Nevertheless, B2 loss was initiated

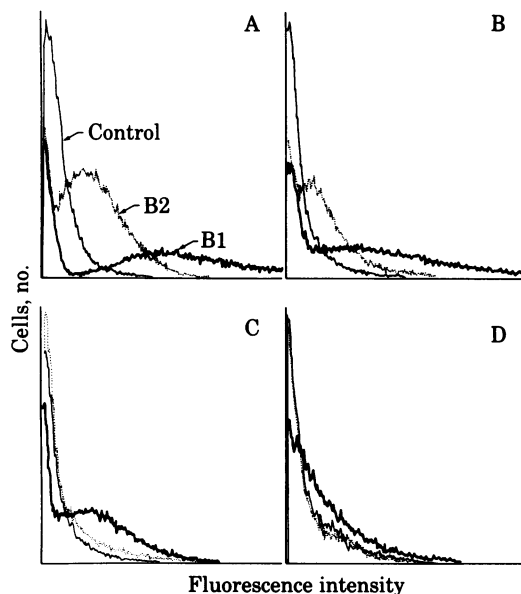


FIG. 1. Kinetics of disappearance of B1 and B2 from PWM-activated human spleen cells. Unfractionated spleen cells were cultured in the presence of PWM, and viable cells were analyzed at various times for the expression of B1 (heavy solid line) and B2 (dotted line) by indirect immunofluorescence on the FACS-I. Background staining in the presence of an isotype-matched unreactive antibody is also indicated (light solid line). (A) Day 0; (B) day 4; (C) day 5; (D) day 6.

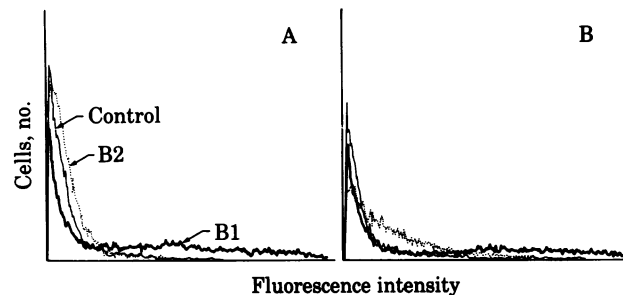


FIG. 2. Increased expression of B2 on PWM-activated peripheral blood B cells. Viable cells were analyzed by indirect immunofluorescence on the FACS-I. B1, heavy solid line; B2, broken line; control antibody, light solid line. (A) Day 0; (B) day 3.

on day 3–4 of culture and was virtually complete by day 5. B1 was lost beginning on days 4–5 and was nearly undetectable by day 6. Spleen cells cultured for 6 days without PWM continued to express both antigens at their original levels. Similar results were obtained with activated tonsil lymphocytes ($n = 3$).

Evidence exists supporting the hypothesis that peripheral blood B cells may in fact represent a relatively immature lymphocyte population, in contrast to B cells found in lymphoid organs (16). Stimulation of peripheral blood B cells with PWM was also carried out ($n = 2$), utilizing a 1:1 ratio of purified B cells and mitomycin C-treated T cells as described (17). The expression of B2 on peripheral blood cells is weak compared to spleen, lymph node, and tonsil cells, whereas B1 expression is as strong on peripheral blood cells as on B cells from solid lymphoid tissues (1, 3). Once again, PWM-stimulated, but not unstimulated, B cells lost both antigens, with B2 disappearing prior to B1. The kinetics of antigen loss were nearly identical compared with the results from spleen and tonsil. However, one important difference was noted: staining with anti-B2 first increased after stimulation (Fig. 2) before diminishing and finally disappearing. Although this finding could reflect the increased size of B cells undergoing activation, no such biphasic expression was seen for B1. Thus, the differentiation of peripheral blood B cells is accompanied by even more dynamic changes in the expression of B1 and B2 than are observed with splenic or tonsillar B cells, in which relatively linear loss of antigen is observed. Activated peripheral blood B cells pass through successive stages in which they first express large amounts of B1 but little B2 (B1⁺⁺⁺⁺, B2⁻), then acquire relatively large amounts of both antigens (B1⁺⁺⁺⁺, B2⁺⁺), lose B2 (B1⁺⁺, B2⁻), and finally bear neither B1 nor B2. B cells with the B1⁺⁺⁺⁺, B2⁺ phenotype predominate in unstimulated peripheral blood but are less numerous in solid lymphoid organs, where B1⁺⁺⁺⁺ B2⁺⁺ cells are more highly represented (Fig. 1).

Correlation of B1 and B2 Loss with Surface Phenotype and cIg Production. Several experiments were carried out to determine whether B1 and B2 disappearance might correlate with

Table 3. Correlation of sIg isotype with expression of B1 and B2 on peripheral blood B cells stimulated with PWM

Days in culture	Isotype*						
	B1	B2	sIgD	sIgM	sIgG	T10	Ia
0	++++	+	++	++	0	0	++++
4	+++	±	+	++	±	+	++++
7	0	0	0	++	+	++	++++

* Mean fluorescence intensity: +++++, strong, similar to anti-B1 (Fig. 1A); +++, moderate-strong, similar to anti-B1 (Fig. 1B); ++, moderate, similar to anti-B2 (Fig. 1A); +, weak, similar to anti-B2 (Fig. 2A); 0, negative staining; ±, questionable staining.

other changes in B cell surface phenotype—specifically, the expression of sIg isotype (sIgM, sIgD, and sIgG), Ia, and T10. B cells from peripheral blood were stimulated with PWM as above and analyzed on day 0 and at several subsequent times for the fate of B1, B2, and other phenotypic markers. Precultured peripheral blood B cells (day 0) were found to bear both sIgM and sIgD (Table 3), as found previously (18, 19), as well as large amounts of Ia antigen (20). In contrast, sIgG and T10 were absent or were expressed on fewer than 10% of B cells. PWM-stimulated cells were considerably different. As expected, by day 4 *in vitro*, the expression of both B2 and B1 was decreasing, indicating the activated nature of the B cells under study. Similarly, there was a decrease in the number of sIgD⁺ cells but no change in the number of sIgM⁺ or sIgG⁺ cells. In contrast, T10 antigen had become clearly detectable. On day 7, sIgD was undetectable, and sIgM and sIgG were clearly present. T10 was now strongly represented. The levels of Ia antigen did not change throughout the culture period. Virtually identical results were obtained with stimulated tonsil cells, with the exception that some T10 antigen was already detectable on the starting cell population. This may indicate the more activated nature of tonsil compared to peripheral blood B cells. The differentiation of B cells to plasmacytes in this system thus appears to be accompanied not only by loss of B2 and subsequently B1 but also by disappearance of sIgD, appearance of sIgG, and acquisition of T10.

Activated splenic and peripheral blood B cells were also analyzed for the presence of cIg (Table 4). For spleen, few cIg⁺ cells were found until day 4, when cIgM⁺ but not cIgG⁺ cells were detected. By day 6, large numbers of both cIgM⁺ and cIgG⁺ cells were found, together adding up to >100% which suggests that at least some double-producing cells were present. The sequential development of plasma cells containing IgM and IgG could therefore be induced under the conditions of culture used. Temporally, the development of cIgM⁺ cells occurred concurrently with B2 loss (days 4–6); the appearance of cIgG⁺ cells correlated with B1 disappearance (days 5–7).

Because B1 and B2 are lost during the differentiation process, the possibility that these antigens might play a demonstrable role in B-cell activation was also investigated. It was found that neither anti-B1 nor anti-B2 directly stimulated the proliferation or differentiation of peripheral blood B cells to Ig-secreting cells when incorporated into culture at dilutions ranging from 1:10 to 1:10,000. Additionally, neither antibody blocked PWM-induced proliferation or maturation to Ig-secreting cells under the same conditions.

DISCUSSION

Qualitative and quantitative changes in the expression of cell surface antigens are commonly found to accompany cellular

differentiation (21, 22). For human B lymphocytes, many phenotypic markers have been used toward this end, including sIg and cIg isotype(s) (23, 24), Fc and C3 receptors (25, 26), and Ia-like (DR) antigens (26, 27). In the present studies, the phenotypic alterations of B cells after stimulation by PWM to activation and differentiation to Ig-producing cells were investigated. Particular attention was directed toward the postactivation fate of two newly described B cell-specific antigens, B1 and B2 (1–3), and their relationship to other cell surface markers including sIg, Ia, and T10. The findings reported here demonstrate that profound phenotypic changes occur during B-cell activation. These alterations include loss of B2 and subsequently of B1, loss of sIgD, and acquisition of sIgG and T10. B cells from all sources tested, including peripheral blood, tonsil, and spleen, exhibited a similar picture with respect to both the kinetics and order of cell surface antigen loss and expression. These findings therefore provide a general framework within which human B-cell differentiation may be analyzed.

The dynamics of B1 and B2 expression after *in vitro* activation were of special interest. B2 loss inevitably occurred prior to that of B1 for all B cells examined: i.e., by day 5, compared to days 6–7 for B1. Temporally, B2 loss was accompanied by several additional changes, including loss of sIgD, acquisition of T10, and development of cIgM. sIgG was undetectable or present in low quantities until the disappearance of B1, at which time cIgG⁺ cells also appeared. Complementary results have been obtained in this laboratory from phenotypic analyses of human plasmacytomas. Malignancies of the IgM-producing Waldenström type have been found to express B1 but not B2; IgG-secreting myelomas lack both B1 and B2 (2, 3). This is not to imply that only Ig-secreting cells are B1⁺B2⁻. Indeed, cell-sorting experiments indicate that most B cells from reactive lymph nodes that lack B2 have lymphocytic morphologic features and only approximately 5% of these are cIg⁺ (unpublished data). An additional finding of importance was that T10 antigen appeared beginning about day 4 *in vitro*. This antigen, which is also present on immature thymocytes, appears to be the human equivalent of the thymus-B cell common antigen (Th-B) described in the mouse (28). Th-B is found on thymocytes, plasma cells, and a subpopulation of non-cIg⁺ B cells.

Whereas B1 expression is strong on normal B cells from all sources, B2 is well represented on solid lymphoid organs but only weakly expressed on peripheral blood B cells (2). Peripheral blood B cells may therefore constitute a unique, relatively homogeneous, lymphocyte subset. Along these lines, functional studies have shown that subpopulations of peripheral blood B cells are distinct in their responsiveness to polyclonal activators as compared with B cells from lymphoid organs (16). Moreover, the majority of B cells in blood bear both sIgM and sIgD (18, 19). Although there is controversy concerning the relationship

Table 4. Kinetics of appearance of cIg-positive cells in cultures of PWM-stimulated spleen and peripheral blood B cells

Days in culture	Spleen				Peripheral blood			
	% positive		Staining intensity		% positive		Staining intensity	
	cIgM ⁺	cIgG ⁺	B1	B2	cIgM ⁺	cIgG ⁺	B1	B2
0	4 ± 2	7 ± 4	++++	++	0	0	++++	+
2	2 ± 1	2 ± 2	++++	++	0	0	++++	+
3	ND	ND	ND	ND	0	0	++++	++
4	32 ± 7	1 ± 1	+++	+	9 ± 2	0	+++	+
5	ND	ND	++	±	23 ± 3	2 ± 2	++	±
6	60 ± 10	55 ± 6	+	0	ND	ND	ND	ND
7	ND	ND	0	0	14 ± 4	7 ± 2	0	0
9	71 ± 9	50 ± 8	0	0	ND	ND	ND	ND

ND, not determined.

of sIg isotype to state of B-cell differentiation (23, 24), several aspects of isotype acquisition appear to be generally accepted. Specifically, during ontogeny, B cells first express sIgM alone (19, 29, 30) and subsequently express both sIgM and sIgD (31, 32) and, in a proportion of cells, a third isotype as well (23), apparently independent of encounter with antigen. Cells bearing both sIgM and sIgD may secrete either IgM or IgG upon primary antigenic (or mitogenic) stimulation (33), during which time sIgD is the first isotype to be lost (34–36). In contrast, memory cell precursors of IgG-secreting cells have been shown to lack sIgD (33, 36) and instead appear to possess sIgG predominantly (33, 37, 38).

Although no extensive investigation of surface isotype vs. differentiation stage was carried out in these studies, our findings are consistent with these data. The predominant isotypic change observed after activation was from cells expressing sIgM and sIgD to cells expressing sIgM and sIgG. Loss of sIgD occurred at approximately the same rate as loss of B2; acquisition of sIgG was a relatively late event (Table 3). The finding (Fig. 2) of a transient increase in B2 expression by peripheral blood B cells after activation provides a further suggestion that these cells are relatively immature in that they can be stimulated to differentiate to the B1⁺⁺⁺⁺, B2⁺⁺ phenotype typical of B cells in secondary lymphoid organs. In this regard, tissue sections of tonsil or appendix stained with anti-B1 or anti-B2 demonstrate clearly that a distinct compartmentalization of B cell subsets exists in B-cell areas, with mantle zone cells reflecting the peripheral blood B cell phenotype and germinal center cells being more differentiated (ref. 39; A. Bhan, personal communication). In addition, mantle zone cells, but not B cells in germinal centers, express sIgD whereas the reverse pattern is seen with sIgG. Thus, mantle zone cells may represent recirculating B cells of the peripheral blood phenotype; cells within germinal centers may be more mature, perhaps a mixture of activated and memory B cells.

The present findings suggest that the B1 and B2 antigens, used in conjunction with conventional B cell markers, can provide useful tools for both experimental and clinical purposes. These include analysis of the process of normal human B lymphocyte differentiation, identification of functional B-cell subpopulations, and categorization of tumors of B-cell lineage.

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