CELL SURFACE IMMUNOGLOBULIN

XVIII. Functional Differences of B Lymphocytes Bearing Different Surface Immunoglobulin Isotypes*

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B-cell mitogens such as lipopolysaccharide (LPS)[1] can stimulate murine splenocytes in vitro to proliferate and differentiate into IgM-secreting plasma cells (1–3). The response of these stimulated lymphocytes is manifested by an increase in DNA synthesis, blastogenesis, a polyclonal antibody response, and an increased rate of IgM synthesis and secretion (1–5). It is not known whether all of these responses are due to a single subpopulation of B cells or whether some B cells proliferate, whereas others differentiate.

In this regard, there are several subpopulations of B lymphocytes which can be distinguished by the presence of different surface Ig classes. Thus, recent immunofluorescence studies indicate that approximately 20–30% of splenic B cells have only IgM on their surface, 30–40% have only an IgD-like molecule,[2] and the rest (40–50%) bear both isotypes (6–7). In contrast, very few cells in the spleen bear surface IgG (8). Those cells which express only IgM appear to consist predominantly of large lymphocytes, while the other two subclasses (IgD only and IgM plus IgD) are primarily small lymphocytes (9). It has been hypothesized that the double bearers are precursors of IgM-secreting cells, whereas the cells bearing IgD only are precursors of IgG-secreting cells (10). To explore this possibility further, subclasses of B lymphocytes have been exposed to LPS and the proliferative, blastogenic, and polyclonal response of each subpopulation determined. The results indicate that two distinct subpopulations are responsible for the LPS-induced response: cells bearing IgM or IgM plus IgD give rise to the polyclonal response and IgM secretion, whereas cells bearing IgD only proliferate and undergo blastogenesis.

Materials and Methods

General Approach. Murine splenocytes were treated with C' and either anti-Ig, anti-μ, or medium (control) to eliminate all Ig$^+$- or IgM$^+$-bearing cells, respectively. By a combination of

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[1] *Abbreviations used in this paper:* BAθ, rabbit antimouse brain serum; BSS, balanced salt solution; FCS, fetal calf serum; HRBC, horse erythrocytes; LPS, lipopolysaccharide; 2ME, 2-mercaptoethanol; NP40, Nonidet P40; PBS, phosphate-buffered saline; PFC, plaque-forming cells; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

[2] For the sake of simplicity, we will call this molecule IgD. Definitive classification awaits a demonstration of antigenic cross-reactivity with human δ-chain and amino acid sequencing.

radioiodination and purification of viable cells on 1 g gradients (9, 11) it was demonstrated that when cells were treated with anti-μ plus C' a population of cells representing 30–40% of the B cells bearing IgD only remained. When cells were treated with anti-Ig plus C' <10% of the B cells remained. The IgD only cells, the Ig population or the untreated population were cultured 1–4 days with LPS and the resultant population assayed for IgM synthesis and secretion (incorporation of [³H]leucine into intracellular and secreted Ig), a proliferative response ([³H]thymidine incorporation), a polyclonal response (direct plaque-forming cells [PFC] to two antigens) and changes in cell size (automated size analysis of the resultant cells).

Preparation of Antisera

RABBIT ANTIMOUSE μ-CHAIN. A single rabbit was injected at monthly intervals with 50 μg of purified μ-chain [(isolated from MOPC-104E IgM) (8)] mixed in complete Freund's adjuvant. The μ-chain was the generous gift of Doctors McWilliams and Lamm, Department of Pathology, New York University Medical School, New York. The purity of the immunogen was demonstrated by radioiodination and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the μ-chain. The rabbit was bled at intervals and each bleeding was individually assayed by Ouchterlony analyses against IgG, IgM, IgA, and whole serum and by "sandwich" immunoprecipitation (12) using purified radioiodinated myeloma proteins and lysates of radioiodinated splenocytes (which contain IgM and IgD). The high titer antisera which reacted only with IgM were further tested in a cytotoxic assay with A/J or BDF₁ splenocytes using preselected low background rabbit or guinea pig C' (Pel-Freez Bio-Animals, Inc., Rogers, Ark.). In all assays, the anti-μ serum killed 26–44% of the splenocytes. To test the specificity of the antiserum further, several bleedings were pooled and an aliquot was exhaustively absorbed with either MOPC-104E IgM (μ, λ), HP76 IgM (μ, κ), or purified IgG (γ, κ, λ). The former two absorptions completely eliminated the ability of the antiserum to kill splenocytes and to bind cell surface IgM in an indirect immunoprecipitation. The absorption with IgG had no effect on the cytotoxicity or precipitating capacity of the antiserum.

GOAT ANTIRABBIT Ig (GARIg). This serum was prepared against DEAE-cellulose-purified rabbit IgG and reacted with γ- and L-chains.

RABBIT ANTIMOUSE Ig. This serum contained specificities against mouse μ-, γ-, α-, κ-, and λ-chains and was prepared by immunizing rabbits with highly purified myeloma and normal serum Igs [MOPC 104E (μ, λ), TEPC 15 (α, κ), and serum IgG]. This antiserum was capable of immunoprecipitating all classes of Ig (including IgD) and was cytotoxic for 41–52% of splenocytes but not thymocytes (less than 10%). Its activity against splenocytes but not against thymocytes could be completely absorbed by the combination of proteins used for immunization. It will be referred to as anti-Ig in the text.

Anti-Ig or Anti-μ plus C' Treatment of Splenocytes. Unlabeled or radioiodinated BDF₁ or A/J spleen cells were suspended at 10⁸ cells/ml in either Eagle's minimal essential medium (MEM) (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 10% fetal calf serum and nonessential amino acids or in RPMI 1640 with supplements. Cells to be assayed for the polyclonal response or for cell enlargement were first pretreated with Tris ammonium chloride buffer (13) (to remove erythrocytes) and rabbit antimouse brain serum (BAθ) (14) at 0.1 ml/10⁸ cells for 30 min at 0°C, followed by a 1/4 dilution of guinea pig C' for 30 min at 37°C (to remove T cells). The latter treatment eliminated 30% of the cells. Pretreated or untreated cells were then incubated at 37°C with an optimal amount (0.5 ml/10⁸ cells) of heat-inactivated rabbit anti-μ or rabbit anti-Ig and either rabbit C' or guinea pig C' at a final dilution of 1/5 or 1/2, respectively. Controls received medium and C'. Cells were examined after 20 min and viabilities assessed by trypan blue exclusion.

Polyclonal and Blastogenic Response. Treated cells (anti-BAθ plus C' and either anti-μ plus C' or anti-Ig plus C') were washed twice in balanced salt solution (BSS), suspended in improved MEM with zinc and 0.5% fetal calf serum (FCS) (International Scientific Industries Inc., Cary, Ill.) (15), and cultured with supplements and 5 × 10⁻⁵ M 2-mercaptoethanol (2ME) (16, 17). The final density was 3 × 10⁶ viable nucleated cells/ml. Some cultures received 10 μg/ml LPS from Salmonella typhosa (Difco Laboratories, Detroit, Mich.). After 42 h, cultures were harvested and assayed for direct PFC to horse erythrocytes (HRBC) and TNP-HRBC using a slide modification assay described by Jerne and Nordin (18). Additional aliquots were mixed with filtered 0.04%

trypan blue and submitted to live cell size analysis using the cytograph 6300A equipped with pulse height analyzer (J. Kettman and J. Rudin, unpublished observations).

Proliferative Response. Treated cells (anti-μ plus C' or anti-Ig plus C') were washed three times in BSS and suspended at 5×10^6 viable nucleated cells/ml in RPMI 1640 with supplements (19) and 5% heat-inactivated FCS (GIBCO). 0.1 ml of the cell suspension was added to a microtiter plate well (Falcon Plastics, Div. of BioQuest, Oxnard Calif.) using three replicates per group. LPS from *Escherichia coli* (055:B5 Difco Laboratories) was diluted with the same medium and added at a final concentration of 10 μg/ml. The microtiter plate was placed in an airtight box containing 10% CO_2 at 37°C. The cells were usually pulsed on the 2nd day of culture with 1 μCi of [³H]thymidine (Amersham/Searle Corp., Arlington Heights, Ill.) and harvested 5 h later using a MASH II apparatus (Microbiological Associates, Bethesda, Md.). The results are expressed as mean counts per minute per culture.

Ig Synthesis and Secretion. Treated (anti-μ plus C' or anti-Ig plus C') or untreated cells were cultured for 4 days with or without LPS as described above for the proliferative response. Cells were then washed and a total of 2×10^7 viable cells suspended at 5×10^6 cells/ml in Eagle's MEM lacking leucine and containing 10% FCS, 2% antibiotic-antimycotic mixture, and nonessential amino acids. Cultures were labeled for 3 h at 37°C in a moist CO_2 incubator with 50 μCi/ml [³H]leucine (55 Ci/mmol; Amersham/Searle Corp.). At the end of the incubation period, cell lysates and secretions were prepared and dialyzed for 16 h at 4°C against 0.01 M phosphate-buffered saline (PBS), pH 7.3. Lysates were centrifuged and the acid and immunoprecipitable radioactivity determined (12, 20). Duplicate aliquots of the dissolved immunoprecipitate were electrophoresed on 7.5% SDS-PAGE as described previously (12).

Internal markers consisting of ¹²⁵I-μ and ¹²⁵I-L chains were mixed with one of the duplicate samples. Gels were fractionated and counted. In plotting the patterns from different gels, the marker proteins were aligned.

Fractionation of Radioiodinated Cells on 1 g FCS Gradients. $1-2 \times 10^8$ splenocytes were radioiodinated and washed as described previously. Cells were then suspended in medium and treated with anti-μ plus C', anti-Ig plus C', or C' alone, for 20 min at 37°C. In these experiments cells were treated with 1/2 the usual volume of anti-μ serum or anti-Ig serum used for unlabeled cells since preliminary experiments had shown that labeled cells are more sensitive to lysis. After the incubation period, viabilities were assessed and cells were washed and suspended in 3% FCS. Cells were fractionated on a 1 g gradient as previously described (9, 11). 10-ml fractions were collected, centrifuged, and both viabilities and size distributions determined by automated cytograph analysis. The clumped dead cells appeared in the large cell fraction [cells which bear only IgM (9) and sediment faster than 6 mm/h] and unclumped dead cells (2 mm/h) were recovered at the top of the gradient. Small viable cells from each gradient (sedimenting between 2.5-3.0 mm/h) were pooled, counted, and lysed in 0.5% Nonidet P40 (NP40) (Shell Chemical Co., New York). The lysate was supplemented with FCS to a final concentration of 10% to prevent proteolysis of the IgD (21). Lysates were dialyzed, centrifuged, and immunoprecipitated as described previously. Immunoprecipitates were dissolved in 1% SDS containing 8 M urea, reduced with 2ME, and mixed with ³H-μ and ³H-L chains. Samples were electrophoresed on SDS-PAGE and gel fractions counted. In plotting gel patterns, marker proteins were aligned.

Results

Killing with Anti-Ig or Anti-μ. Experiments were designed to establish whether treatment with anti-μ plus C' or anti-Ig plus C' eliminated all IgM[3] and Ig-bearing cells, respectively, from the population. Thus, radioiodinated cells were treated with C' and the appropriate antiserum or medium (control) and the dead cells eliminated by fractionation on a 1 g gradient. Viable small lymphocytes recovered from these gradients were lysed and the lysates precipitated with anti-Ig. Precipitates were analyzed by SDS-PAGE. In a representa-

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[3] Cells which bear either IgM or IgM plus IgD will be referred to as IgM+. Cells which bear only IgD will be referred to as IgD-only.

tive experiment, treatment of radioiodinated cells with anti-Ig plus C' resulted in a net loss of 52% of the cells. When these cells were separated on a 1 *g* gradient, 25% of the viable cells were recovered as small lymphocytes. When these cells were lysed and the resultant lysates immunoprecipitated with anti-Ig, there was an 87% decrease in immunoprecipitable counts relative to the C' control. No H- or L-chain peaks were observed when a sample of this precipitate was reduced and electrophoresed on SDS-PAGE (Fig. 1). These results indicate that the treatment eliminates cells bearing surface Ig detectable by radioiodination. In contrast to the results with anti-Ig, in this same experiment, anti-μ plus C' resulted in a net killing of only 27% of the splenocytes (Table I). Of the viable cells loaded on a 1 *g* gradient, 18% were recovered as viable small lymphocytes. The radioactivity from these lysed cells which could be precipitated with anti-Ig was reduced by 51% compared to the C' control. SDS-PAGE of the immunoprecipitate (Fig. 1) showed complete loss of μ-chain and partial (35%) reduction of δ-chain radioactivity relative to that immunoprecipitated from the lysates of the complement control. In a total of three experiments, an average of 53% (35–75%) of the δ-chain was depleted by eliminating the IgM$^+$ cells. It was, therefore, concluded that approximately 50% of the radioiodinated IgD is present on small IgM-bearing cells. The exact figure may vary depending on the age and strain of the animals.

LPS Stimulation of Protein and Ig Synthesis and Secretion by Subpopulations of B Cells. In order to determine whether IgM$^+$ or IgM$^-$ B cells were responsible for IgM secretion after stimulation with LPS, cells were treated with C' and either anti-μ or anti-Ig. Remaining cells were cultured for 4 days with or without LPS and then labeled with [³H]leucine to examine incorporation of the isotope into protein and Ig. The decrease in incorporation after elimination of IgM-bearing cells was defined as the contribution of IgM-bearing lymphocytes. The difference between the decrease in incorporation after elimination of IgM-bearing cells and elimination of Ig-bearing cells was defined as the contribution of IgD-only cells. Incorporation into Ig after treatment with anti-Ig and C' was surmised to be due to either (a) incomplete killing of the B lymphocytes, (b) persistent plasma cells, or (c) precursor Ig$^-$ B cells which differentiate during culture. As seen in lines 1, 3, and 5 of Table II A and Fig. 2, treatment with either anti-Ig plus C' or anti-μ plus C' resulted in a substantial and similar loss of protein synthesis and secretion by surviving cells. Ig synthesis and secretion, in particular, was suppressed by 60–70%. It was thus concluded that IgM-bearing cells are responsible for the majority of Ig synthesis and secretion before stimulation with LPS. As seen in lines 2, 4, and 6 of Table II A and summarized in II B, after stimulation with LPS the aliquots, which had been pretreated with C' and either anti-Ig or anti-μ, showed no significant increase in Ig synthesis or secretion. In contrast, the control showed a substantial increase in both protein and Ig synthesis and secretion. It was, therefore, concluded that IgM-bearing cells are the subpopulation which synthesize and secrete IgM before and after stimulation with LPS. IgD-only cells, in contrast, are not stimulated to produce IgM.

LPS Stimulation of the Polyclonal Response in Subpopulations of B Cells. As described above, IgM-bearing cells are stimulated by LPS to synthe-
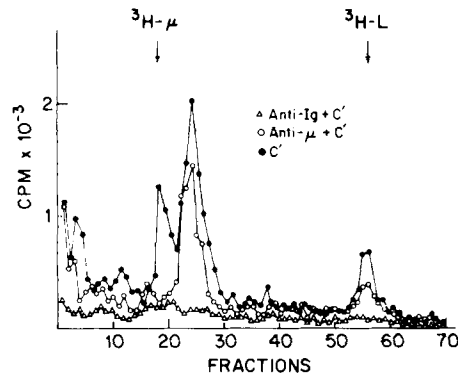
Fig. 1. Effect of eliminating IgM$^+$ cells on recovery of cell surface Ig from surviving small lymphocytes. Radioiodinated cells were treated with antibody plus C' and the dead cells and large cells eliminated on 1g gradients. The viable small lymphocytes were lysed and lysates immunoprecipitated with anti-Ig. Precipitates were dissolved, reduced, mixed with ^{3}H-μ and ^{3}H-L chains, and electrophoresed for 16 h at 4 M A/gel on SDS-PAGE. By calculating the areas under the H-chain peaks it was determined in this experiment that 35% of the δ-chain was depleted by removing μ-bearing cells. The range of depletions for several experiments was 35–75%.

TABLE I

Preparation of Radioiodinated IgM Small Lymphocytes by Treatment of Splenocytes with Anti-μ + C' Followed by Isolation of Viable Small Lymphocytes on a 1 g Gradient*

Treatment	Dead cells	Number of viable cells loaded on a 1 g gradient‡	Number of small lymphocytes recovered§	Radioactivity recovered from small viable cells	
				Acid precipitable	Immuno-precipitate
	%	cells × 10^7	cells × 10^6	cpm × 10^6	cpm × 10^3
Anti-Ig + C'	65	1.6	4	1.1	56
Anti-μ + C'	43	2.8	5	1.4	193
Medium + C'	16	4.2	9	2.5	426

* A representative experiment of five that were done. Cells were radioiodinated before treatment.
‡ This figure represents the viable cells remaining after treatment of 5 × 10^7 cells.
§ Fractions containing >95% viable cells were assayed for size distribution using the cytograph.

size and secrete IgM at a markedly increased rate. In these experiments we determined whether this synthesis of IgM is responsible for the polyclonal response by assaying the response to two antigens. As seen in Fig. 3, when C'-treated B cells are cultured with LPS for 2 days, a substantial polyclonal response to the two antigens was observed. In three experiments, when the cells were pretreated with anti-μ plus C' before culture, the polyclonal response was reduced an average of 85% relative to the C' control. A similar reduction of 88% (not shown) was seen when cells were treated with anti-Ig plus C'. These results indicate, as do those from the above experiment, that the polyclonal IgM response depends predominantly on stimulation of IgM$^+$ precursors with mitogen. Thus, IgD-only cells do not give rise to the polyclonal response.

    LPS-Induced Proliferative Response in Subpopulations of B Cells.   After

TABLE II

Synthesis and Secretion of Protein, Including Ig, by LPS-Stimulated A/J Splenocytes

			A. A REPRESENTATIVE EXPERIMENT*			
Line	Treatment	Days in culture	Protein-associated radioactivity cpm × 10³		Radioactivity in Ig cpm × 10³	
			Lysate	Secretion	Lysate	Secretion
1	Anti-Ig + C'	0	1,620	150	19	8
2	Anti-Ig + C'	4	2,592	144	25	10
3	Anti-μ + C'	0	1,860	260	21	10
4	Anti-μ + C'	4	2,360	170	31	11
5	C'	0	2,531	520	51	46
6	C'	4	31,652	5,630	466	230

	B. SUMMARY OF FOUR EXPERIMENTS‡				
Treatment	Average change in radioactivity incorporated into protein§		Average change in radioactivity incorporated into Ig§		
	Lysate	Secretion	Lysate	Secretion	
Anti-Ig + C'	1.6	0.8	1.3	1.2	
Anti-μ + C'	1.3	0.8	1.6	1.2	
C1	14.2	10.9	10.2	5.8	

* 10 Viable cells/culture.
‡ Three experiments using anti-μ plus C' and one using anti-Ig plus C' followed by 2 days of culture LPS.
§ cpm day 4/cpm day 0/10⁷ cells.

stimulation with LPS, B cells divide as assessed by a marked increase in thymidine incorporation. In order to determine which subpopulations of B cells divide, an approach similar to that described in the previous section was employed. As seen in Table III (A, lines 1 and 5 and B, line 2), when anti-Ig plus C'-treated cells were cultured without LPS, the background incorporation was decreased >90% relative to the C' control. When these cells were cultured in the presence of LPS, there was a twofold increase in [³H]thymidine incorporation in both the anti-Ig plus C' and C'-treated cells (Table III A; lines 1, 2, 5, and 6). However, the net counts per minute per culture was decreased 90% compared to that seen in the C' control (Table III A, lines 1, 2, 5, and 6; and B, line 2). These results indicate that the Ig⁺ cells in the spleen account for >90% of the proliferative response. The remaining 10% is probably due to stimulation of precursor (Ig⁻) B cells or some residual B cells.

When cells were treated with anti-μ plus C', the background incorporation was reduced by 75% (Table III A, lines 3 and 5; and B, line 1). This decrease is proportional to the percent of IgM⁺ B cells in the spleen (60-70%). When the treated cells were cultured with LPS, the stimulation index was 3.7 (Table III A, lines 3 and 4) compared to 1.7 in the C' control (Table III A, lines 5 and 6). The average increase in counts per minute per culture after LPS stimulation was
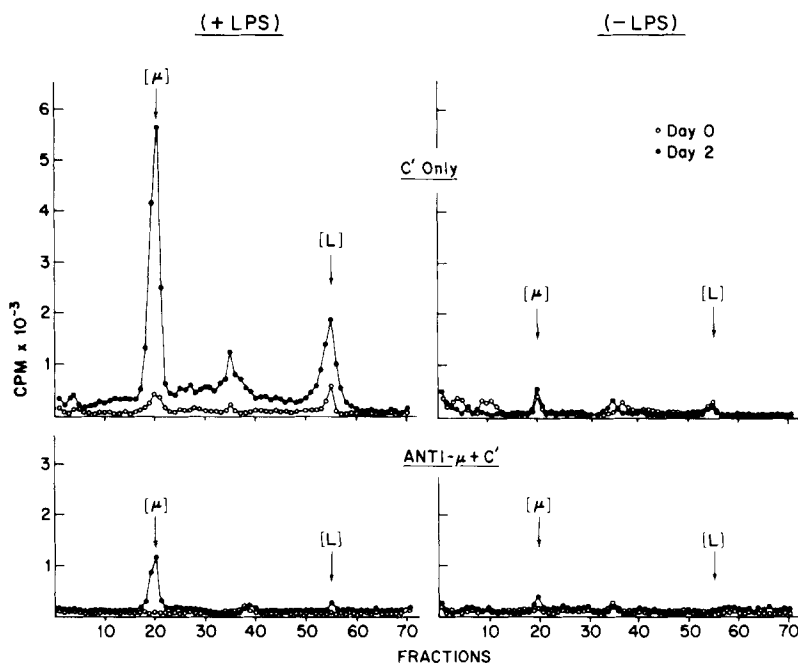
FIG. 2.   Effect of prior elimination of IgM⁺ cells on the subsequent synthesis of Ig by surviving cells after 2 days of culture with or without LPS. After the 2-day culture period, 2 × 10⁷ cells were labeled for 180 min with [³H]leucine. Cells were lysed and processed as described in Fig. 1. In order to obtain quantitative comparisons of ³H radioactivity, only one aliquot of each dissolved precipitate was electrophoresed with ¹²⁵I-μ- and ¹²⁵I-L-chain markers. The positions of the markers on the duplicate aliquots are indicated in brackets.

48% of that seen in the control (Table III B, line 1), although in one experiment it was as high as 85% (Table III A, lines 3-6). These results suggest that the IgD-only cells account for an average of 67% of the proliferative response even though these cells represent only 30-40% of the B cells. In contrast, the IgM⁺ cells account for only 23% of the response although they constitute 60-70% of the B cells. Thus, in response to LPS, the IgD-only cells proliferate but do not secrete IgM. In contrast, the IgM⁺ cells which give rise, after stimulation, to virtually all IgM secretion, are less active in the proliferative response. These experiments do not determine, however, whether proliferation of IgM⁺ cells is obligatory for the elaboration of a polyclonal response. Indeed, there could be heterogeneity among the IgM⁺ cells in regard to whether proliferation accompanies differentiation to IgM secreting cells.

Analysis of Cell Size in LPS-Induced Responses.   Another parameter for assessing mitogen stimulation is the increase in cell size after stimulation. This increase can be a result of either blastogenesis or the differentiation of cells into plasma cells. To determine whether the IgM⁺ and/or the IgD-only cells enlarge after stimulation, cells were pretreated as described previously. Surviving B cells were then evaluated for increases in size by automated cytograph analysis. As seen in Fig. 4, which is a representative experiment of three that were done, when untreated cells were cultured for 2 days in the absence of LPS the majority
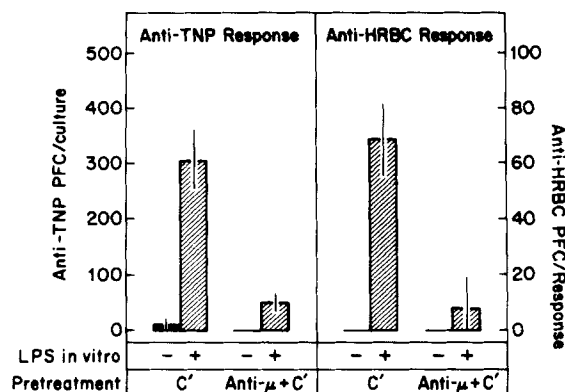
FIG. 3. Effect of prior elimination of IgM+ cells on the in vitro polyclonal response. Anti-TNP and HRBC PFC are shown after 2 days of culture with or without LPS.

TABLE III

Effect of Treatment with Anti-μ + C' or Anti-Ig + C' on Incorporation of [³H]Thymidine by LPS-Treated Cells after 2 Days of Culture

A. A REPRESENTATIVE EXPERIMENT*

Line	Treatment	LPS	Mean' cpm ± SEM/ culture	Net* cpm/ culture	% Inhibition of the response relative to C' control‡
1	Anti-Ig + C'	–	6,315 ± 802	7,527	88
2	Anti-Ig + C'	+	13,842 ± 3,781		
3	Anti-μ + C'	–	19,478 ± 1,117	53,055	15
4	Anti-μ + C'	+	72,533 ± 4,503		
5	C'	–	83,330 ± 10,485	62,043	–
6	C'	+	145,373 ± 11,038		

B. SUMMARY OF FOUR EXPERIMENTS**

Line	Treatment	% Incorporation relative to C' control after 2 days		% of control response‡‡
		–LPS‖	+LPS¶	
1	Anti-μ + C'	25	48	67
2	Anti-Ig + C'	8	10	12

* Line 2-line 1; line 4-line 3; line 6-line 5.

‡ 100|(line 2-line 1)/(line 6-line 5)] and 100|(line 4-line 3)/(line 6-line 5)].

§ BDF₁ mice.

‖ |(Mean cpm in antibody + C'-treated cultures without LPS)/(Mean cpm in C'-treated culture without LPS)] × 100.

¶ Same as (‖) only with LPS.

** Two experiments with BDF₁ spleens and two with A/J spleens.

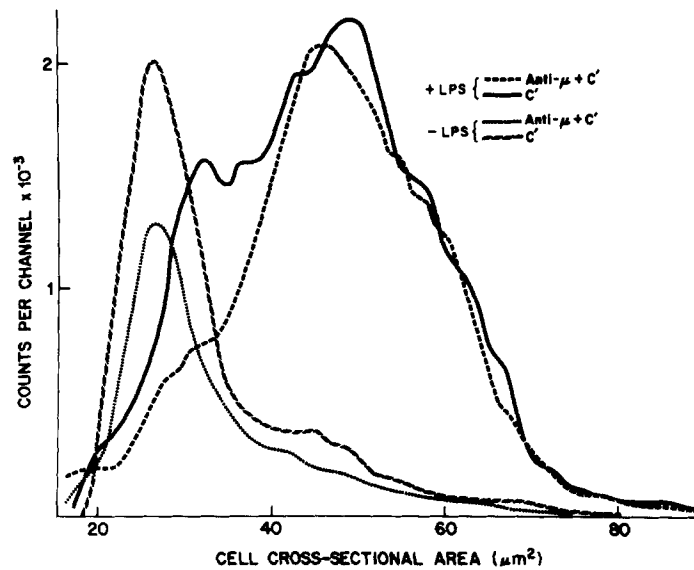‡‡ 100|(Net cpm/culture for antibody + C'-treated sample)/(Net cpm/culture for C' control)].

FIG. 4. Automated size analysis of cells surviving treatment with anti-μ plus C' after 2 days of culture with or without LPS. Small lymphocytes are 25 μm^2 and large cells 35-50 μm^2.

of cells have a cross-sectional area of approximately 25 μm^2. After 2 days of culture with LPS there is a shift in size to two major populations, one of approximately 35 μm^2 and one of approximately 50 μm^2. When cells are treated with anti-μ plus C' and cultured for 2 days without LPS, the cell size is similar to that seen when the untreated population is cultured under the same conditions. However, the cells remaining after 2 days in the presence of LPS are 45-50 μm^2. The population of 35 μm^2 is decreased by approximately 60%. These results indicate that most of the cells bearing surface IgM increase in size from 25 μm^2 (small lymphocytes) to 35 μm^2. The majority of those bearing IgD-only become even larger cells with a cross-sectional area of 45-50 μm^2. Thus, both IgM$^+$ and IgD-only cells appear to enlarge after LPS stimulation. However, in general they give rise to cells of two different sizes, although these populations overlap to some extent. Based on the results in the previous section, one possible interpretation is that the IgM$^+$ cells differentiate mainly into plasma cells (35 μm^2) while the IgD-only cells become enlarged blasts (45-50 μm^2) which proliferate.

Discussion

These studies were designed to delineate the functions of subpopulations of B lymphocytes bearing different receptor isotypes on their surface. Since anti-mouse δ-antiserum is not yet available, the approach used was to examine the effects of anti-Ig plus C' vs. anti-μ plus C' on the polyclonal, proliferative, blastogenic, and biosynthetic response of B cells after 1-4 days of culture in the presence of the B-cell mitogen, LPS. The decrease in function after treatment with anti-Ig plus C' was used as a means of measuring activity of Ig$^+$ B cells. The decrease in response after treatment with anti-μ plus C' was attributed to

IgM$^+$ cells; i.e., those bearing only IgM or those bearing both IgM and IgD. The difference in decrease in response after anti-Ig plus C' and anti-μ plus C' is interpreted as being due to cells bearing only IgD.

The results indicate that treatment with anti-Ig plus C1 eliminates virtually all Ig-bearing cells and abrogates approximately 90% of the responsiveness by each of the criteria mentioned above to a B-cell mitogen, LPS. These observations confirm earlier findings (1–5). In contrast, treatment with anti-μ plus C' results in the selective survival of a subpopulation of B cells that represents an estimated 1/3 of the cells (or 17% of splenocytes) but which bears about 50% of the iodinatable surface IgD. This subpopulation enlarges and proliferates after LPS stimulation, but the cells do not give a polyclonal response or synthesize and secrete IgM. The IgM-bearing cells, in contrast, are responsible for virtually all IgM synthesis and secretion, and the elaboration of a polyclonal response. The latter findings are consistent with previous studies by Andersson et al. (22) who showed that treatment with anti-μ eliminated synthesis and secretion of IgM. This finding is also consistent with a model of B-cell differentiation which suggests that cells which bear both isotypes (IgM and IgD) can be triggered to give rise to an IgM response (10). However, our studies do not establish whether the cells bearing IgM or the cells bearing both IgM plus IgD are the precursors.

These studies, therefore, establish that B-cell responsiveness to mitogenic doses of LPS is heterogenous, involving some cells which differentiate into IgM-secreting plasma cells and others which divide but do not secrete IgM. Moreover, the subpopulations of responding cells can be distinguished by the presence of different receptor isotypes on their surface. Since other studies have suggested that receptor isotypes undergo changes during maturation of B cells (20, 23), the nature of the mitogenic response may be related to the stage of maturation of the B cells in question. This possibility was suggested by Grono-wicz and Coutinho (24–26) in a series of experiments with different B-cell mitogens. In addition, Kearney and Lawton (27, 28) have shown that in cultures of fetal tissues or adult bone marrow, LPS stimulation results in a polyclonal response and appearance of IgM-secreting cells but not in a proliferative response. Several studies have established that in fetal tissues and adult bone marrow, in contrast to adult spleen, there is a paucity of cells bearing both IgM plus IgD and no IgD-only cells (references 27 and 28; and J. Quebbeman, D. Spiva, E. S. Vitetta, and J. W. Uhr, unpublished observations). Thus, by analogy with these studies, the IgD-only cells are induced by LPS to proliferate and elaborate another function. It has been previously hypothesized that, upon further stimulation, IgD-only cells become IgG-secreting plasma cells and, therefore, that the IgD-only cells are "memory" cells (10). Recent studies by Pernis and co-workers support this possibility (29, 30). Since it has been demonstrated that, by using modified conditions of culture during LPS stimulation, IgG-secreting cells appear after 5–7 days (27, 28) this concept will be tested by using long-term stimulation of IgD-only cells with LPS.

Summary

Three populations of murine splenic B lymphocytes have been characterized previously (6, 7, 9) as those bearing only IgM, those bearing only IgD, and a

population bearing both isotypes. These studies were designed to test the response of the IgM⁺ cells (IgM-only or IgM plus IgD) vs. the IgD-only cells to the B-cell mitogen, lipopolysaccharide. Results suggest that after 1-4 days of culture, in the presence of mitogen, the IgM⁺ cells enlarge and elaborate an IgM polyclonal response. The IgD-only cells, in contrast, do not exhibit an IgM polyclonal response, but instead undergo blastogenesis and proliferation.

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