THE SWITCH FROM IgM TO IgG SECRETION IN SINGLE MITOGEN-STIMULATED B-CELL CLONES

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B-lymphocyte mitogens, such as lipopolysaccharide (LPS) (1) or lipoprotein (2) stimulate murine B cells to growth and maturation to Ig secretion (3). Every third B cell of spleen is stimulated by LPS or by lipoprotein to grow to a clone of IgM-secreting, plaque-forming cells (PFC)[1] (4, 5). Mitogenic stimulation also results in the appearance of cells secreting IgG (6, 7). IgM- and IgG-secreting cells are detected by the protein A sheep erythrocyte-plaque assay (8). We determine in this paper the frequency of mitogen-stimulated B-cell clones which develop IgG-secreting, PFC under culture conditions which will let every growth-inducible B cell grow into a clone of cells (4, 9). Since we can limit the number of mitogen-reactive growth-inducible B cells to one per culture, we can investigate how many of the clones containing IgM-secreting PFC will develop IgG-secreting PFC, i.e. switch the class of Ig they produce, and how many clones develop IgG-secreting PFC only.

Materials and Methods

Animals. C3H/Tif/BOM 6–8 wk of age, were obtained from Dr. Bomholtgaard, Ry, Denmark. C57BL/6, DBA/2, and BALB/c mice, 6–8 wk of age, and Lewis strain rats, 4 wk of age, were from the Institut für Biologisch-medizinische Forschung AG., Füllinsdorf, Switzerland.

Cells, Mitogens, and Culture Conditions. Spleen cells were grown at concentrations indicated in the Results section in RPMI-1640 medium containing 2-mercaptoethanol (2-ME, 5×10^{-5}M), fetal calf serum (concentrations and batches indicated in the Results), and 3×10^6/ml of rat thymus filler cells in plastic tissue culture dishes or tubes (4, 5, 9).

Small, resting lymphocytes were purified from larger cells containing dividing and PFC (10) by 1 *g* velocity sedimentation (11).

LPS-S (EDTEN 18735 and S435/188049) was kindly prepared for us by Doctors C. Galanos and O. Lüderitz, Max-Planck-Institut für Immunobiologie, Freiburg i. Breisgau, West Germany and used at 50 μg/ml in culture. *Escherichia coli* lipoprotein, a gift from Dr. V. Braun, Mikrobiologie II, Universität Tübingen, Tübingen, West Germany, was used at 2 μg/ml (2).

Plaque Assay for Ig-Secreting Cells. For the detection of all cells secreting IgM or IgG a modified hemolytic plaque assay was used (8). Protein A-coupled SRC and rabbit anti-Ig (either anti-IgM or anti-IgG) antisera were used in the assay as developing antibodies together with properly diluted complement (Bio-Cult, Irvine, Scotland). Protein A used for the coating of SRC was obtained from Dr. H. Wigzell, Biomedicum, University of Uppsala, Uppsala, Sweden. Rabbit anti-mouse IgM antibodies were raised against purified myeloma MOPC 104E 19 S IgM (λ,μ).

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[1] *Abbreviations used in this paper*: B, bone marrow derived-, bursa equivalent; H-(μ,γ), heavy chain (μ,γ); Ig(M,G), immunoglobulin (M,G); LPS, bacterial lipopolysaccharides; 2-ME, β-mercaptoethanol; PFC, plaque-forming cells; SRC, sheep erythrocytes.

Rabbit anti-mouse IgG antibodies were a mixture of antibodies raised against purified myeloma MOPC 21 IgG_1 (κ,γ) and against purified myeloma Adj-PC-5 IgG_{2a} (κ,γ_{2a}). Each antibody preparation was titrated for optimal developing capacity in the plaque assay (8), using myeloma cell lines producing the appropriate IgG subclasses. Since the two anti-IgG-antisera detected different populations of IgG-PFC (IgG_1 and IgG_{2a}), each of them was titrated separately and the mixture made up as a solution of both antisera at their independently optimal concentrations.

The subclass specificity of the two anti-IgG-antisera apparent in the protein A plaque assay was tested as follows: the anti-IgG_1-antiserum, at dilutions used for development of IgG_1-secreting PFC (tested with the X63AG8-myeloma cell line), did not develop plaques with 5563- and Adj-PC-5-myeloma cells (secreting IgG_2), with MOPC 315 cells (secreting IgA), with MOPC 104 E cells (secreting IgM), and with MOPC 41 and 46 cells (secreting K-L-chain). The anti-IgG_2 antiserum, at dilutions used for the development of IgG_2-secreting cells (tested with the 5563-myeloma cell line), did not develop plaques with the IgG_1-secreting X63-AG8-myeloma line nor with any of the other myeloma cells secreting IgA, IgM, or K-L-chains mentioned above.

Results

Mitogen-Stimulated Development of IgG-Secreting PFC in Cultures Containing Limiting Numbers of Reactive Precursors

FREQUENCY OF MITOGEN-REACTIVE B CELLS IN SPLEEN DEVELOPING TO CLONES OF IgG-SECRETING PFC. We have developed culture conditions with which each growth-inducible B cell will grow and maturate into a clone of Ig-secreting cells under the stimulatory influence of a mitogen (4, 5, 9). This was achieved by adding 3×10^6 thymus filler cells, either from syngeneic or allogeneic mice or from xenogeneic rats, into the culture medium. We can, therefore, dilute the number of reactive B cells until they become limiting in cultures. This allows us to determine frequencies of reactive B cells. According to Poisson's distribution, one reactive B cell is present in that number of cultured cells, which will let 37% of all cultures appear negative in a measured response. With such limiting dilution analyses we have previously determined that one LPS-reactive or one lipoprotein-reactive B cell yielding a clone of IgM-secreting PFC is present in three to four splenic B cells of 6–8 wk old C3H/Tif mice (5).

The results of limiting dilution analyses of splenic B cells of 6–8 wk old C3H/Tif and of C57BL/6J mice for the frequencies of precursor B cells yielding IgG-PFC is shown in Fig. 1. The linear digression of the curve, plotted as numbers of cultured cells against the logarithm of the fraction of nonresponding cultures, indicates that precursor B cells reactive to either LPS or to lipoprotein yielding IgG-PFC were limiting the cultures. Earlier results on the frequencies of reactive B cells yielding an IgM-PFC response (4, 5) could be confirmed (Table I). The frequencies of LPS or of lipoprotein reactive B cells in either C3H/Tif or in C57BL/6J-spleen cells yielding a clone of IgG-secreting PFC were one tenth of those yielding a clone of IgM-secreting PFC. In these in vitro cultures, therefore, and stimulated by the B-cell mitogens LPS or lipoprotein, not all mitogen-reactive B cells have the capacity to develop into IgG-secreting PFC. We determine below whether the IgG-secreting PFC clones originate from IgM-synthesizing and secreting clones or whether they never synthesize or secrete IgM during their clonal development after stimulation.

CLONAL DEVELOPMENT OF IgM-SECRETING AND IgG-SECRETING PFC WITH TIME OF STIMULATION. The development of single clones of reactive cells yielding

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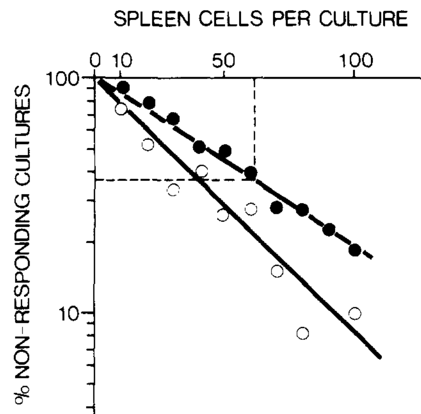

FIG. 1. Titration of LPS-reactive B cells yielding IgG-secreting clones. Abscissa indicate the number of input spleen cells per culture of C3H/Tif mice (●) or C57BL/6 mice (○). The cultures all contained a constant number of rat thymus cells (3×10^6 cells/ml) together with 50 μg/ml LPS. The assays for IgG-secreting PFC were done at day 7 of culture. The dotted line points to the number of C3H/Tif spleen cells per culture at which 37% of all cultures did not yield a response, i.e., which, according to Poisson's distribution, contained one mitogen-reactive B cell.

TABLE I

Frequencies of Mitogen-Reactive Splenic B Cells Yielding a Clone of IgM- or of IgG-PFC

Mouse strain	Mitogen	Absolute frequencies of mitogen-reactive B cells yielding clones of			
		IgM-Secreting PFC		IgG-Secreting PFC	
		LPS	Lipoprotein	LPS	Lipoprotein
C3H/Tif		1 in 3	1 in 4	1 in 30	1 in 40
C57BL/6J		1 in 2	1 in 3	1 in 25	–

IgM-secreting PFC or yielding IgG-secreting PFC could be followed by diluting the reactive B cells to near one per culture, i.e., to 6 C3H/Tif-spleen cells per culture for IgM-secreting PFC clones, and to 60 spleen cells per culture for IgG-secreting PFC clones. IgM-secreting PFC clones were assayed for 3 days between days 4 and 6 of culture, IgG-secreting PFC clones for 5 days between days 4 and 8 of culture. Table II summarizes the experimental data.

In agreement with our studies on the development of IgM-secreting PFC clones (4) the fraction of nonresponding B-cell cultures with time of stimulation remained constant, while the average number of IgM-PFC per culture increased steadily, doubling approximately every day. IgG-secreting PFC clones were practically not detectable at day 4 of growth, where IgM-secreting PFC clones were well detectable. IgG-secreting PFC clones began to appear from day 5 on, reaching a constant value for the fraction of nonresponding cultures at day 6. The average number of IgG-secreting PFC, however, continued to increase until day 7. This indicated that between days 6 and 7 of culture, IgG-secreting PFC

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TABLE II

Development of IgM-Secreting PFC-Clones and of IgG-Secreting PFC Clones with Time of LPS-Stimulation under Culture Conditions Limiting the Number of Reactive B-Precursors to Near One

Assay (time of culture)	Number of positive cultures per total*	Fraction of negative negative (nonresponding) cultures	Average number of PFC per culture
IgM-secreting PFC clones: 40 cultures each at 6 C3H/Tif-spleen cells‡ per culture			
Day 4	18/40	0.55	8.6
Day 5	18/40	0.55	15.0
Day 6	19/40	0.525	28.0
IgG§-secreting PFC clones: 40 cultures each at 60 C2H/Tif spleen cells‡ per culture			
Day 4	2/40	0.95	0.9
Day 5	11/40	0.725	18.4
Day 6	18/40	0.55	29.5
Day 7	19/40	0.525	75.0
Day 8	18/40	0.55	75.0

* In medium containing 25% fetal calf serum.

‡ Small cells purified from background PFC by 1 *g* velocity sedimentation.

§ IgG_1 + IgG_{2a}.

TABLE III

Frequencies of Background PFC Secreting IgM or IgG_{1+2} in Spleen of Different Inbred Strains of Mice

Mouse strain	Frequency* (per number of spleen cells) IgM-Secreting	IgG_{1+2} Secreting
C57BL/6J	1 in 800	1 in 7,000
C3H/Tif	1 in 150	1 in 1,800
BALB/c	1 in 180	1 in 1,200
DBA/2	1 in 400	1 in 4,500

* Average of five mice.

clones were growing in a constant fraction of all cultures.

This is only so when purified small, resting spleen cells are used for mitogenic stimulation. With unfractionated cells, also containing large, background PFC, a fraction of which secrete IgG, measurable IgG-PFC responses are detected early in culture as well. In fact, IgG-secreting, background PFC were found to amount to approximately 10% of the number of IgM-secreting, background PFC in 6–8 wk old mice of several inbred strains (Table III).

DEPENDENCE ON THE CONCENTRATION OF FETAL CALF SERUM IN CULTURE. We have previously found in experiments with cultures containing excess reactive B cells (9) that the length in time of development and the magnitude of the mitogen-induced IgG-secreting PFC response was dependent on the concentration of fetal calf serum in culture. We can now reinvestigate whether higher

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TABLE IV

Dependence on the Concentration of Fetal Calf Serum of the Frequencies of LPS-Reactive B-Cells Developing to Clones of IgM-Secreting and to IgG-Secreting PFC Clones, and of the Size of these Clones, in Cultures Limiting the Number of Precursor B-Cells to Near One

Fetal calf serum concentration	Number of positive cultures per total	Fraction of negative (nonresponding) cultures	Average number of PFC per culture
%	IgM-secreting PFC clones: 24 cultures each, with 6 C3H/Tif spleen cells per culture		
2	2/24	0.91	1.5
5	14/24	0.41	26.7
10	14/24	0.41	59.0
20	15/24	0.38	59.5
30	15/24	0.41	56.0
	IgG-secreting PFC clones: 40 cultures each, with 60 C3H/Tif spleen cells per culture		
2	0/40	1.0	0
5	5/40	0.875	3.2
10	22/40	0.45	30.0
20	28/40	0.350	103.0
30	29/40	0.325	100.5

concentrations of fetal calf serum increase the frequencies of reactive B cells yielding an IgG-PFC clone and/or the size of the IgG-secreting PFC clones, and, thereby, the length of growth of these clones. Spleen cells from 6 to 8 wk old C3H/Tif mice were grown at 60 cells per culture in the presence of LPS and of different concentrations of fetal calf serum. Assays of all cultures were done at day 7 of culture. The responses of these spleen cells were controlled by measuring the IgM-secreting PFC response at six spleen cells per culture at the different fetal calf serum concentrations at day 5 of culture. Table IV summarizes the experimental results.

A constant fraction of all splenic B cells (six cells per culture) responded between 5 and 30% fetal calf serum with an IgM-secreting PFC response. The clone size indicated by the average number of IgM-secreting PFC per culture reached a constant number at 10%. Only 2% fetal calf serum was clearly not sufficient to let a constant fraction of all reactive B cells respond with measurable number of IgM-secreting PFC. Higher concentrations of fetal calf serum, between 20 and 30%, were needed in culture before a constant fraction of all spleen cells (60 cells/culture) responded with the development of IgG-secreting PFC. At those concentrations the size of the IgG-secreting PFC clones also reached a constant number of IgG-PFC per culture. Slightly lower frequencies of reactive B cells were measured with lipoprotein as stimulating B-cell mitogen (data not shown). These results indicate that there is no considerable recruitment of IgG-secreting cell clones from the resting pool late after stimulation, and therefore suggests that all the IgG-secreting clones which we detect at day 6 or 7 start to grow at time 0 of stimulation.

DISTRIBUTION OF THE NUMBER OF IgM-SECRETING PFC AND OF IgG-SECRETING PFC IN INDIVIDUAL CULTURES OF LPS-STIMULATED SPLENIC B CELLS. The absolute number of IgM- and IgG-secreting PFC was determined at a dilution of

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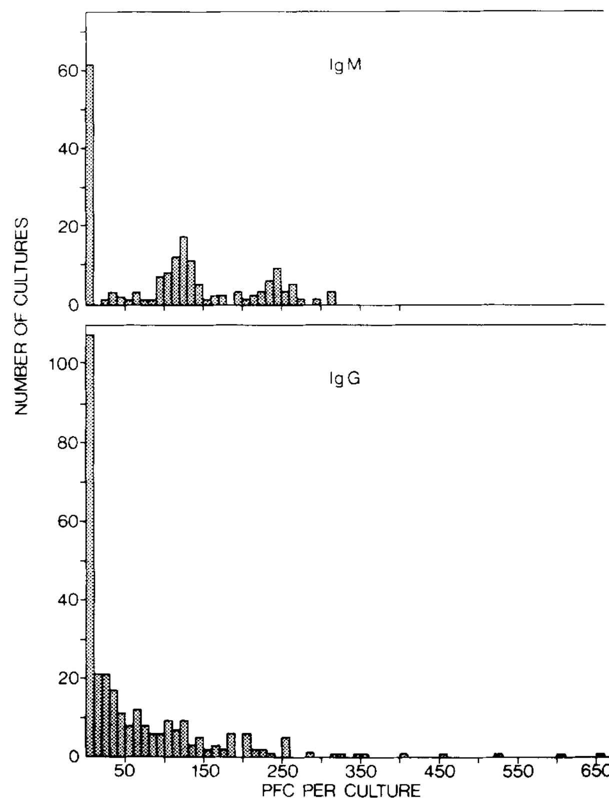

FIG. 2. Distribution of IgM- and IgG-secreting PFC in individual cultures of splenic B cells. Small spleen cells of C3H/Tif mice were distributed in individual cultures at six spleen cells per culture (for IgM responses) or at 60 spleen cells per culture (for IgG responses—in both assays yielding approximately 37% nonresponding cultures) with 50 μg/ml LPS and 3×10^6 thymus cells per ml. The total number of IgM-secreting PFC was determined at day 5 while the number of IgG-secreting PFC was determined at day 7 of culture.

LPS-reactive splenic B cells where approximately 37% of all cultures did not yield a PFC response. Small, resting splenic lymphocytes purified by velocity sedimentation and stimulated by mitogen show a constant frequency of responding cells when assayed for IgM- (4) and IgG- (Table II) PFC clones at different times of growth. During these times of clonal growth the absolute number of IgM-secreting PFC found in single cultures shows a discontinuous distribution (Fig. 2, top) which, according to Poisson's distribution, indicates that individual cultures contain either none, one, or two LPS-reactive B cells capable of developing into a clone of IgM-secreting PFC (4). These results suggest that all reactive B cells start to grow at time 0 of mitogenic stimulation, that all cells in a growing clone divide and that all dividing cells in that clone secrete IgM.

A similar analysis of the absolute number of IgG-secreting PFC developing in single cultures after LPS stimulation does not show a discontinuous distribution (Fig. 2, bottom) under conditions where the frequency of responding IgG PFC producing clones is constant. Taken together with our previous findings (4) that during the first 5 days of culture every cell in a mitogen-stimulated B-cell clone

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TABLE V
Switch of IgM-Secreting B-Cell Clones to IgG Secretion

Number of cultures			Fraction of		
Tested in total*	Positive for IgM-‡ secreting PFC	Positive for IgG-§ secreting PFC	IgM-PFC-Positive cultures in total cultures	IgG-PFC-Positive cultures in total cultures	IgG-PFC-Positive cultures in all IgM-PFC-positive cultures
Exp. 1					
480	86	9	0.18	0.02	0.1
Exp. 2					
720	98	10	0.14	0.014	0.1

	Number of IgG-PFC-positive cultures	Fraction of IgG-PFC-positive cultures, also positive for IgM-PFC among all IgG-PFC-positive cultures
Exp. 1	9	1 (9 out of 9)
Exp. 2	10	1 (10 out of 10)

* Containing an average of one spleen cell per culture C3H/Tif/BOM, i.e. an average of 0.16 LPS-reactive B cells (4).

‡ Assay day 5, positive with more than 10 PFC per culture.

§ Assay day 7, positive with more than 6 PFC per culture.

divides and every dividing cell secretes sufficient IgM to produce a plaque, these results indicate that growing, IgM-secreting B cells within one clone can switch early or late, i.e., after different numbers of cell cycles, to IgG secretion.

CLONAL ORIGIN OF IGG-SECRETING PFC. The inhibition of mitogenic stimulation to IgG-secreting PFC development by anti-μ antibodies (13) had previously suggested that IgG-secreting PFC may originate from mitogen-sensitive precursor B cells, which have IgM on their surface. Stimulation of such surface IgM-positive precursor B cells by a B-cell mitogen might lead first to a clone of cells secreting IgM, which later, during its growth and maturation, switches to IgG secretion. These surface IgM-positive B precursors may, on the other hand, never secrete IgM, but develop directly to IgG-secreting PFC. This was tested in cultures limiting the number of precursor B cells yielding an IgM-PFC clone to one, assaying for IgM-PFC at day 5, and, in the same cultures, for IgG-PFC at day 7 of stimulation. According to Poisson's distribution 82–90% of all cultures will be nonresponding, 16.4–9% will contain one clone from one precursor, 1.6–0.45% two clones from two precursors, and 0.1–0.2% three clones from three precursors, if an average of 0.1–0.2 B-cell precursors is plated per culutre. According to our previous results (4), an average of 0.1–0.2 B-cell precursors yielding an IgM response is expected in an average of 0.6–1.2 spleen cells from C3H/Tif mice. Since only one tenth of all LPS-reactive B-cells developing into a clone of IgM-secreting PFC will yield an IgG-secreting PFC clone we expected every 50th and 100th culture to contain such an IgG-PFC clone and every 5th–10th culture to contain an IgM-PFC clone. Table V summarizes the results of two such experiments. Among the 1,200 cultures tested, 19 yielded an IgG PFC response. All these 19 cultures had previously contained IgM-secreting PFC.

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The probability that the IgM and IgG-secreting PFC clones would have arisen from independent precursors in these experiments can be calculated by using Fisher's exact test of independence. These probabilities were found to be 3.4×10^{-7} and 4.0×10^{-9}, respectively. This shows that the IgG-secreting cells, which develop during the first 7 days of mitogenic stimulation in culture are all members of clones which secrete IgM at an earlier time of clonal growth.

Discussion

The present results show that the frequency of mitogen reactive B cells which develop into a clone of IgG-secreting cells is only about 1.5% of the total number of spleen cells under conditions where the frequency of precursors of IgM clones is 15%. This is a minimum estimate under our best possible conditions for clonal growth and maturation to Ig secretion of mitogen-stimulated B cells (4, 5). The data show that this frequency of B precursor cells yielding IgG-secreting clones (i.e., approximately 1 in 60 spleen cells) is constant between days 6 and 7 of culture. Therefore, little or no recruitment of IgG-secreting PFC clones occur at this time of culture. However, improved culture conditions allowing growth beyond day 7 of culture will show more conclusively whether or not development of new IgG-secreting PFC clones will occur more frequently in more cultures at later times of growth.

Switching of mitogen-activated B cells to IgG secretion can also be observed with lymph node and thoracic duct lymphocytes at the same relative frequencies compared to IgM (unpublished observations, reference 9). Since the majority of lymphocytes from these organs are thought to belong to the mature, committed antigen-sensitive population of cells we think that the clones which we observed switching from IgM to IgG secretion derive from mature, committed, resting antigen- and mitogen-sensitive small B lymphocytes.

Strikingly similar relative numbers of IgM- and IgG-producing cells are obtained when background PFC in spleen are measured (see Table III). Again, the number of IgG-secreting background PFC is approximately one tenth of the number of IgM-secreting cells. It points to the possibility—previously discussed by us (14)—that background PFC are representative samples of the repertoire of mitogen-reactive precursors of a given lymphocyte population.

The results presented herein also demonstrate that IgG-secreting PFC clones, although only constituting 10% of the IgM-PFC clones, all arise in those cultures where previously an IgM secreting clone had developed. This finding can only be explained by an intraclonal "switch" in the class of Ig secreted by some of the cells in the clone. The alternative interpretation, namely that IgM- and IgG-secreting clones arise from distinct precursors but activation of IgG precursors necessarily requires the presence of an IgM-secreting clone does not fit our observations. Thus, if this were the case, the frequency of detectable IgG-secreting clones should be higher when every culture contains at least one IgM-secreting clone (as in the experiment shown in Fig. 1) than when over 80% of the cultures contain no such IgM-secreting clone (as in the experiments shown in Table V). This, however, is not the case since in both conditions around 1/60 cells gave rise to an IgG-secreting clone. Such a switch in the class of Ig secreted cells has already been suggested by others using different experimental systems (15–18).

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The absolute numbers of PFC secreting either IgM or IgG in single cultures containing on the average one mitogen reactive B-cell precursor for the development of IgM- or IgG-PFC clones show different distributions. Numbers of IgM PFC among individual clones show a discontinuous distribution compatible with a Poissonian assortment of mitogen-reactive precursors and doubling times of 18 h from time 0 of stimulation, thus indicating that all cells in a growing clone divide and that all dividing cells secrete IgM for the first 5–6 days of culture (4).

The absolute numbers of IgG-PFC in individual clones, on the other hand, show a continuous distribution. Since all IgG-secreting PFC arise from members of IgM-secreting clones, the detected IgG-PFCs are descendants of cells which all start to grow at time 0 of stimulation. Furthermore, the frequency of IgG-secreting PFC clones remain constant under our conditions which indicates that no recruitment of IgG-secreting clones occur after day 5 or 6 of stimulation. Under such conditions a continuous distribution of the absolute numbers of IgG PFC is best compatible with a switch occurring at different times after stimulation by mitogen, but before day 6 of culture, in different individual clones of growing cells. Our present data cannot give any information on whether switching from IgM to IgG secretion happens only once in one cell of a growing B-cell clone, or can repeatedly occur in different cells of one clone.

Our finding that only 10% of the mitogen-induced IgM-secreting PFC clones to IgG secretion is in agreement with previously published results obtained in vivo. By analyzing the clonal products of precursor cells responding to various antigens it was found that between 10 and 14% (15, 17) of the IgM-secreting clones also contained IgG-secreting cells by day 6 after stimulation. Furthermore, it was found that few or no IgG-secreting clones developed independently of IgM-secreting clone (17).

The low frequency of IgM clones which switch to IgG secretion in our experiments is compatible with the low or undetectable IgG production found in the immune response to thymus-independent antigens (19). The classical T-cell dependence of IgG responses may result from the postulated existence of a separate subset of B cells responding to T-cell derived factors (20–22). Such a subset could then partially be distinct from the subpopulation of B cells reactive to the mitogens LPS and lipoprotein in that these B-cell subpopulations show a much higher frequency of IgM secreting clones switching to IgG secretion.

The experiments by Nossal et al. (15) and Sterzl and Nordin (17), however, indicate that the same minor fraction, i.e. 10–15%, of all stimulated IgM-secreting B-cell clones will switch to IgG secretion. It is, therefore, conceivable that, for all known ways of B-cell stimulation – T-independent or T-dependent – only 10% of the reactive precursors are committed to switch to IgG-secretion upon stimulation. The final relative number of IgM- and IgG-secreting cells and, thus, the numbers of secreted molecules in serum, must then depend on regulatory events which occur after the induction of the originally responsive precursors and which alter the relative number of IgM to IgG-producing cells from 10:1 to some other ratio. T-dependent responses which apparently favor high IgG-responses, particularly upon repeated challenge, must, therefore, favor the propagation of those 10% reactive B-cell clones which can switch to IgG secretion.

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Summary

The frequency of mitogen-reactive B cells yielding an IgG plaque-forming cell (PFC) response has been determined in vitro by limiting dilution analysis under culture conditions which allow every growth-induced B cell to grow and mature into a clone of Ig-secreting cells. The frequencies of lipopolysaccharide (LPS)- and lipoprotein-reactive precursors for IgG-secreting cells in the spleen of 6–8 wk old C3H/Tif and of C57BL/67 mice were found to be between 1 in 30 and 1 in 40 B cells and, therefore, only one tenth of the frequencies of mitogen-reactive precursors of clones secreting IgM.

All IgG-secreting cells developed by switching in clones which previously contained IgM-secreting cells. This was shown in two experiments where the total number of mitogen-reactive precursor yielding IgM-secreting cell clones was limited such that 82 or 90% of all responding cultures originated from one precursor. Thus, of 480 cultures in the first and 720 cultures in the second experiment, 86 and 98 cultures were found positive, yielding IgM-secreting cells at day 5. When the same cultures were assayed at day 7 for IgG-secreting cells 9 and 10 cultures were found positive. All 19 cultures with IgG-secreting cells previously had contained IgM-secreting cells.

The probability that IgG-secreting cells and IgM-secreting cells would have arisen from independent precursors can be calculated using Fisher's exact test of independence. For the two experiments those probabilities are 3.4×10^{-7} and 4.0×10^{-9}. Since we have previously shown that each cell in a mitogen-stimulated, growing B-cell clone divides, and that each dividing cell secretes Ig, we conclude from these experiments that the large majority – in our experiments all – of the IgG-secreting cells in mitogen-stimulated B-cell clones develop by switch from IgM-secreting cells. IgG-secreting cells develop either early or late during growth of a single IgM-secreting cell clone. The switch to IgG secretion, therefore, is not fixed in the time of clonal growth after mitogenic stimulation.

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