HUMAN B LYMPHOCYTE SUBSETS

I. IgG-bearing B Cell Response to Pokeweed Mitogen*

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It is well established that B lymphocytes recognize the antigen to which they can respond by using antibody specific for that antigen as a surface receptor. In recent years it has become clear that most B cells display more than one class of immunoglobulin on their surface, although each of the two or three such molecules expressed have the same antigen specificity (1, 2). It remains unresolved why the B cell expresses more than one type of antigen receptor molecule. Considerable work in the murine system (3, 4) has suggested that the class of immunoglobulin expressed reflects the maturation state and/or response capabilities of the B cell. Direct investigation of this problem is possible through the use of the fluorescence-activated cell sorter (FACS).[1] Subsets of B lymphocytes can be defined according to their display of surface immunoglobulin and separated on the FACS in order to study their function in isolation.

These studies used the FACS to investigate the B cell subsets of man as defined by their expression of different classes of surface immunoglobulin. We have found that the small subset of B cells expressing IgG are responsible for the majority of the response to pokeweed mitogen (PWM) in an antibody-secreting cell (plaque) assay. Furthermore, these IgG-bearing cells gave rise to both IgG- and IgM-secreting cells. These findings have substantial implications for current theories of B cell differentiation, and because PWM is the polyclonal B cell activator most commonly used in human studies, we have chosen to investigate the human IgG-bearing B cell in detail.

Materials and Methods

Cell Preparation. Spleen cells were obtained from nonpathological spleens removed at surgery. They were disassociated by passage through a stainless steel screen (Bellco Glass, Inc., Vineland, N. J.). The cells were washed and held overnight in 150-cm^2 plastic tissue-culture flasks (Corning Glass Works, Science Products Div., Corning, N. Y.). Nonadherent cells were removed and erythrocytes and dead cells were sedimented in a Ficoll-Hypaque (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) density gradient, and interface cells were stored frozen in liquid nitrogen in McCoy's 5A medium (Grand Island Biological Co., Grand Island, N. Y.), containing 10% dimethylsulfoxide for up to a month before use. Spleen cells for each experiment were thawed, washed, and transferred to a 37°C 5% CO_2 incubator in tissue-culture flasks with Hanks' balanced salt solution (Grand Island Biological Co.) with

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[1] *Abbreviations used in this paper:* E_n^+, cells forming rosettes with neuraminidase-treated sheep erythrocytes; E_n^-, cells not forming such rosettes; FACS, fluorescence-activated cell sorter; FCS, fetal calf serum; GaRG, goat anti-rabbit globulin antibody; PWM, pokeweed mitogen.

0.1 M Hepes buffer (*N*-2-hydroxyethylpiperazine *N'*-2 ethanesulfonic acid) and 5% fetal calf serum (FCS; Microbiological Associates, Walkersville, Md.) for 1 h.

Lymphocytes were prepared from blood of normal adult human donors as previously described (5). Briefly, blood was defibrinated and incubated with carbonyl iron (lymphocyte separator reagent, Technicon Instruments Corp., Tarrytown, N. Y.), the erythrocytes were sedimented in 1.5% Dextran, and the monocyte-depleted lymphocytes were recovered at the interface of a Ficoll-Hypaque gradient.

Spleen cells or blood lymphocytes were separated into E_n^+ and E_n^- populations by rosetting with neuraminidase-treated sheep erythrocytes (6), followed by Ficoll-Hypaque gradient centrifugation. Sheep erythrocytes were lysed with 0.85% ammonium chloride solution. Cells were held overnight in plastic tubes containing 5×10^6 cells in 1 ml of RPMI-1640 (Grand Island Biological Co.) supplemented with 10% FCS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in a humid 5% CO_2 incubator.

E_n^+ cells were further purified by passage over a nylon wool column yielding >95% positive cells as assessed by staining with a monoclonal anti-human T cell antibody (17F12, a gift of Dr. R. Parkman, Harvard Medical School). E_n^- cells ranged from 50 to 90% B cells as determined by labeling with antibodies to IgM and IgD.

Cell Labeling. B lymphocytes were labeled before sorting by incubation of 5×10^6 cells in 0.2 ml containing 0.5 mg/ml of fluoresceinated $F(ab')_2$ fragment of rabbit anti-human IgG for 0.5 h at 0°C. The cells were washed three times before sorting.

To evaluate the expression of surface immunoglobulin isotypes on the sorted populations, the cells were incubated at 37°C overnight and then labeled using the same technique with fluoresceinated $F(ab')_2$ fragment of rabbit antibodies to human IgG, IgM, or IgD (N. L. Cappel Laboratories, Inc., Cochranville, Pa.). During the overnight 37°C incubation, the "capping" phenomenon (7) led to the loss of nearly all of the previous label. Total B cells were enumerated by labeling with a mixture of antibody to IgG, IgM, and IgD, or by using a monoclonal anti-human Ia antibody (New England Nuclear, Boston, Mass.).

All of the antibodies were used in doses determined in preliminary experiments to be saturating. The specificity of the antibodies used in labeling was confirmed in two ways; first by Ouchterlony immunodiffusion analysis using myeloma proteins as standards, and second, by treating B cells with one nonfluoresceinated antibody followed by labeling with each of the fluoresceinated antibodies and FACS analysis. By both methods, no reaction of the antibodies with the improper immunoglobulin class could be demonstrated. To enumerate the B cells reacting with each of the antibodies, the fluorescence-distribution curves obtained from the FACS were compared with those obtained from B cells labeled with fluoresceinated $F(ab')_2$ fragment of normal rabbit gammaglobulin, or with T cells treated with the same specific antibody. These two controls did not differ significantly and permitted the setting of an objective level of fluorescence above which the cells were considered to be "positive" for the immunoglobulin class in question.

Sorting. Labeling of B cells with antibody to IgG resulted in ~10–15% of the cells exhibiting fluorescence above control levels. These cells were sorted on the FACS (Becton Dickinson FACS II, Mountainview, Calif.) and were considered to be the IgG-positive population. The fluorescence-distribution curves did not clearly resolve this population, however. Thus, to reduce overlap with the IgG-negative population, an additional 10% of the intermediate staining cells was routinely discarded. The remaining 75–80% of the cells was sorted as IgG negative.

Plaque Assay. After sorting, cells were cultured for 5 d in 96 flat-bottomed multiwell plates (Linbro Chemical Co., Hamden, Conn.) in RPMI medium with 50 μg/ml gentamycin (Schering Corp., Kenilworth, N. J.) at 37°C in a 5% CO_2 humid air incubator. All cells were suspended at 10^6/ml and to each well 100 μl of sorted cells was added to an equal volume of the nylon wool-purified E_n^+ cells and 50 μl of PWM (Grand Island Biological Co.) at an optimal dilution of 1:20. Quadruplicate wells were pooled, washed, and used in a reverse hemolytic plaque assay.

Purified goat anti-rabbit gammaglobulin (GaRG) was prepared from hyperimmune serum by adsorption of the antibody to an insolubilized column of rabbit gammaglobulin and elution of the column with 4.5 M potassium iodide. This purified GaRG at a concentration of 1 mg/ml was coupled to sheep erythrocytes using $CrCl_3$ as previously described (8).

Cell viability by trypan blue exclusion at the time of plaquing was consistently 70–80% in all

cultures. The cells were diluted in RPMI medium to yield 5×10^5 viable cells per ml. 100 μl of cells was added to 20 μl of GaRG-coupled erythrocytes (33% by volume), 20 μl of developing antiserum (anti-human μ or γ chain) (N. L. Cappel Laboratories, Inc.) at an optimal dilution of 1:10 and 20 μl of guinea pig complement (Baltimore Biological Labs, Cockeysville, Md.) diluted 1:2. Just before plating, 340 μl of 0.5% agarose (L'Industrie Biologique Française, S.A.) in minimum essential medium supplemented with 10% FCS (Grand Island Biological Co.) was added to the cell mixture. The tubes were immediately mixed and uniformly spread onto a 100- × 15-mm polystyrene petri plate (Scientific Products, Inc., State College, Pa.). The dishes were incubated for 6 h at 37°C. Single cells surrounded by an area of erythrocyte lysis (plaques) were counted in a dissecting microscope. Means ± SE of two plates are reported as plaques per 10^5 original lymphocytes.

Preliminary experiments established that the formation of plaques could be completely inhibited by adding 10 μg/ml cycloheximide (Sigma Chemical Co., St. Louis, Mo.) to the cultures. The development of the plaques also depended on the addition of the developing antiserum.

Cell Irradiation. In those experiments involving irradiated cells, the cells received 2,000 rad from a General Electric Maximar 250-III X-ray unit (General Electric Co., Medical Systems Div., Milwaukee, Wis.).

Proliferation Assay. Cells were sorted and cultured under the same conditions as for the plaque assay described above, with the exception that half as many cells were added to each culture well. The cultures were maintained for 3–7 d and then each well received 0.4 μCi of tritiated thymidine (New England Nuclear) 18 h before harvesting with a multiple-sample harvester (Flow Laboratories, Inc., Rockville, Md.). The radioactivity incorporated into the cells was measured in a liquid scintillation counter (Beckman Instruments, Inc., Palo Alto, Calif.). The mean ± SE is reported for triplicate cultures.

Results

Labeling Patterns of Sorted B Lymphocytes. Splenic B lymphocytes were sorted into IgG-positive and IgG-negative populations. They were examined for their display of surface immunoglobulin isotypes immediately after sorting and after overnight culture. The results are reported as the mean ± SE percentage positive cells for 10 experiments (Table I). Based on this data, one can make the following basic observations about B cell subsets in man: (*a*) most splenic B lymphocytes have both IgM and IgD on their surface; (*b*) few of the B cells have only IgG on their surface; (*c*) the majority of cells having IgG also have another surface immunoglobulin isotype, either

TABLE I

Labeling Patterns of Splenic B Lymphocytes

Cells	Percent positive (± SE)			
	IgG	IgM	IgD	IgM and IgD
Original	14.2 ± 2.2	51.5 ± 6.6	61.7 ± 10.0	62.7 ± 8.7
IgG positive	80.3 ± 6.2*	67.4 ± 6.1	66.4 ± 10.0	87.0 ± 5.0
IgG negative	2.9 ± 0.5	42.4 ± 7.8	52.3 ± 8.9	62.5 ± 14.7

The percentage of positive cells (mean ± SE for 10 experiments) is shown. The cells were held overnight after sorting, and then labeled with the indicated antibodies. The original cells were held overnight without sorting. The period of overnight culture had no significant effect on the observed percentage for the original or IgG-negative cells (data not shown) but was necessary to properly analyze the labeling of the sorted IgG-positive cells. Analysis done immediately after sorting, the figure fell to 60.8% after overnight culture. Although the IgG-positive population contained 80% positive cells immediately after sorting, the figure fell to 61% after overnight culture.

IgM or IgD, or both. Incidental observations made during the course of these studies (data not shown) were: (*d*) the IgG-positive cells had slightly larger mean size (as judged by light scatter) than the IgG-negative B cells; (*e*) the IgG-positive cells were slightly more brightly labeled by antibodies to IgM and IgD than were the IgG-negative cells.

The purity of the IgG-positive population was >80%. Contamination with IgG-negative cells could be explained by the sorting of pairs of IgG-positive and IgG-negative cells as IgG positive, as well as by a small rate of sorting errors. The purity of the IgG-negative population averaged 97%. There was no significant change in the proportion of IgG-positive cells in the unsorted or the IgG-negative populations after overnight culture. However, the proportion of IgG-positive cells that could be relabeled with anti-IgG after overnight culture was lower than on the day of sorting. The most likely explanation of this is the failure of some IgG-bearing B cells to fully regenerate their surface IgG after its removal by antibody (7). The presence of passively bound IgG is very unlikely because the cells had undergone two periods of overnight culture before sorting. The IgM and IgD labeling of IgG-positive cells could not be evaluated immediately after sorting; however, after overnight culture, the majority of the fluorescent anti-IgG had been lost from the cells and they could be relabeled with antibodies to IgM and IgD.

Linearity of the IgM and IgG Plaque Assay. The comparison of the activity of IgG-positive and IgG-negative cells in their response to PWM depended on the linearity of the assay system, because different numbers of B cells were to be added to the cultures. The standard assay culture contained 50% T cells. The sorted B cell populations varied from 30% B cells in some preparations of IgG-negative cells to >90% B cells in some IgG-positive preparations. For this reason a dose response was done for the number of added B cells. The results (Fig. 1) showed that the response was nearly linear over the range of 10–75% B cells. In addition, T cells were not limiting in the assay until the proportion of B cells exceeded 75%, and the IgM and IgG responses did not differ in their T cell requirements until the T cells became limiting. At <25% T cells, the IgM response proved to be more T cell independent than the IgG response.

In the sorting experiments described, the proportion of B cells in the final assay cultures varied from 15 to 45%, which is on the linear portion of the dose response curve for both IgG and IgM plaques.

Plaque Response of the IgG-bearing Splenic B Cells. The IgG-positive and IgG-negative populations were compared with the entire splenic B cell population with regard to their ability to give IgG and IgM plaque responses to PWM (Fig. 2). Several experiments demonstrated that the labeling process with anti-human IgG did not affect the subsequent response of the B cells.

In all 10 experiments, the IgG-positive cells gave a dramatic response to PWM consisting of both IgG and IgM plaques. The IgG-negative cells were depleted in their overall response with marked depletion of the IgG response, and variable but always significant depletion of the IgM response. Although the levels of the IgM and IgG responses differed considerably, the distinction between IgG-positive and IgG-negative cells was consistent in every experiment. When expressed in terms of plaques per B cell, the enrichment of the IgG-positive cells and the depletion of the IgG-negative cells was striking (Table II).

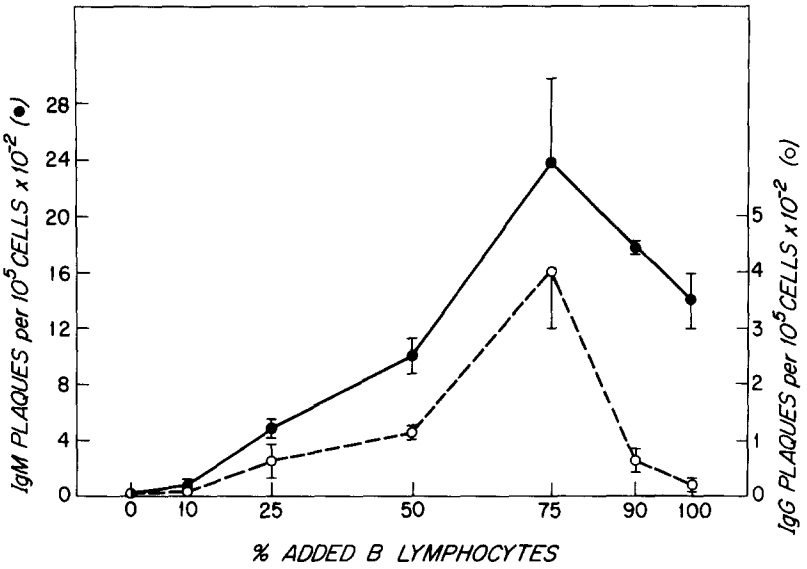

FIG. 1. IgG and IgM plaque responses as a function of the proportion of B cells in the culture. The percentage of B cells added to the cultures was varied from 0 to 100%. The remainder of the cells were purified T cells. Both the IgG and the IgM responses increased linearly with the proportion of B cells until the T cells became limiting at about 25% T cells. The IgM (●) response was more T cell independent than the IgG response (○).

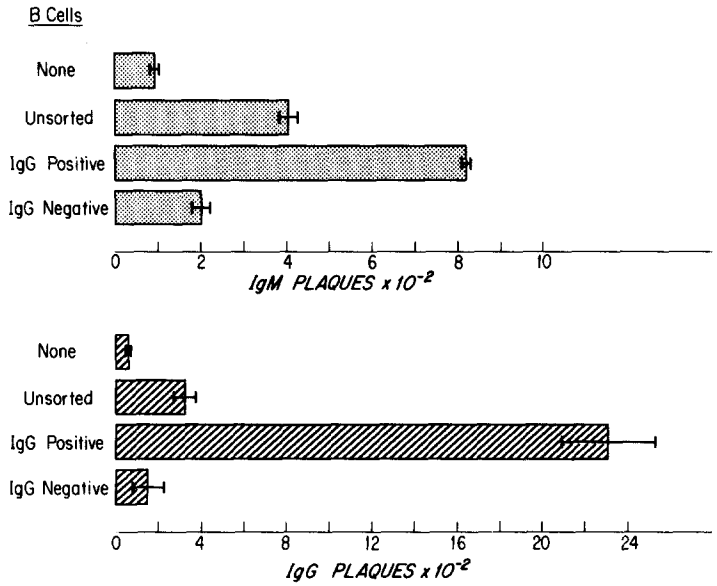

FIG. 2. IgG and IgM plaque responses of sorted splenic B cells. A typical experiment in which normal splenic B cells were sorted into IgG-positive and IgG-negative populations as described. The sorted populations are compared with cells that were labeled in the same way but were not sorted. The IgG-positive population was enriched for both IgG and IgM responses, while the IgG-negative population was correspondingly depleted of both.

TABLE II

Plaque Response of Sorted IgG-positive and -negative B Cells

Experiment	Cell population	B cells*	IgM plaques/ 10^5 B cells	IgG plaques/ 10^5 B cells
		%		
I	Unsorted	94	419	210
	IgG positive	97	998	1,555
	IgG negative	89	248	6
II	Unsorted	58	576	88
	IgG positive	84	1,784	1,210
	IgG negative	61	407	15

* As determined by labeling with antibody to IgM and IgD. Results of two experiments in which the data have been expressed as plaques per 10^5 B cells in order to illustrate the enrichment of IgG and IgM response in the IgG-positive cells and the corresponding depletion of both responses in the IgG-negative population.

Sorting of the IgG-bearing splenic B cells took place after two overnight culture periods (one before freezing and one after). As a result, the cell populations were extensively depleted of adherent macrophages, and passively bound IgG had been eluted. The IgG-positive cells constituted 10–15%, and the IgG-negative cells made up 75–80% of the entire B cell population. The IgM response of the IgG-positive population could not be explained by the 10–20% contamination of the IgG-positive cells with IgG-negative cells because the response of the IgG-negative cells was considerably smaller on a per-cell basis.

Response of IgG-bearing Blood B Cells. In five experiments, when blood B cells were prepared, passively bound IgG was removed by incubation at 37°C, and the IgG-positive B cells were sorted, the results were similar to those obtained from spleen B cells (Fig. 3). Again the IgG-positive cells gave a substantial IgM response which was in fact higher than that given by the IgG-negative cells.

The preparation of B cell subsets from blood is difficult because of the low overall frequency of B cells (5–10% of lymphocytes) and the fact that the IgG-positive cells are even less numerous in the blood than in the spleen. There is the added complication in blood of a population of lymphocytes that avidly bind serum IgG (9). These latter cells appear to be much less frequent in the spleen (unpublished observations).

Lack of Interaction between IgG-positive and IgG-negative B Cells. It was possible that the IgG-positive cells were responding to PWM much better than IgG-negative cells because some other cell type (perhaps a macrophage) was being selected into the IgG-positive population during sorting. This possibility was investigated by adding irradiated cells to the IgG-negative population in order to supply the missing accessory cell. The addition of irradiated IgG-positive cells (Fig. 4), or irradiated whole spleen lymphocytes (data not shown), in various ratios failed to improve the response of the IgG-negative cells.

Similarly, it was possible that the IgG-negative cells responded poorly because some aspect of the labeling or sorting procedure had resulted in active suppression of B cell responses in that population. This possibility was tested by mixing IgG-positive and IgG-negative cells in various proportions after sorting. Such mixtures of IgG-positive and IgG-negative cells gave the same response that would have been predicted from simple addition of the responses of the two populations alone. A 4:1 ratio of IgG-

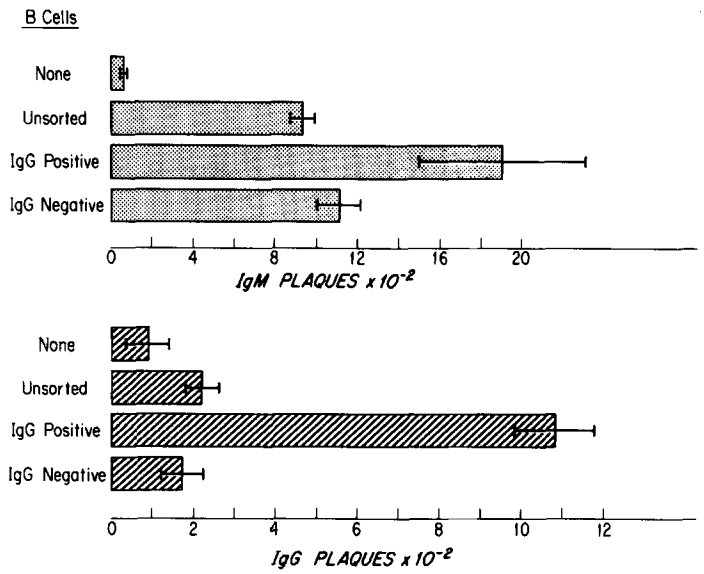

FIG. 3. IgG and IgM plaque responses of sorted blood B cells. Purified blood B cells were sorted into IgG-positive and IgG-negative populations as described. The IgG-positive population was enriched for both IgG and IgM responses. The IgG-negative population was depleted of IgG responsiveness but still gave a substantial IgM response.

negative to IgG-positive cells reconstituted the response to exactly that seen in the unsorted population (Fig. 5).

Proliferative Responses of IgG-bearing B Cells. There is evidence that the immunoglobulin synthetic response to polyclonal B cell activators requires cell division (10). Thus it was possible that the poor response of the IgG-negative population was due to an inability of these cells to divide in response to PWM. This was tested by experiments in which tritiated thymidine incorporation was used to assess the proliferative response of the IgG-positive and IgG-negative cells to PWM (Fig. 6). This response is T cell-dependent, as is the plaque response. The results showed that the IgG-negative cells were capable of mounting a proliferative response to PWM which was nearly as strong as that of unseparated B cells. The IgG-positive cells consistently gave a somewhat better response, which in various experiments amounted to 1.5–2.5 times as much thymidine incorporation as the IgG-negative cells. This difference was present when the proliferation was measured after 3, 5, or 7 d of culture with PWM. The peak proliferative response of the B cells was on day 5 for all populations, thus corresponding to the peak immunoglobulin synthetic response (data not shown). It is difficult to determine whether this difference in proliferation could account for the better immunoglobulin response of the IgG-positive cells. However, it is clear that the IgG-negative cells are not markedly deficient in their ability to respond to PWM by proliferation.

Discussion

The functional capabilities of various B cell subsets as defined by surface immunoglobulin isotypes have been studied in a number of ways. These include: the addition of antibodies against various surface immunoglobulin classes to in vitro

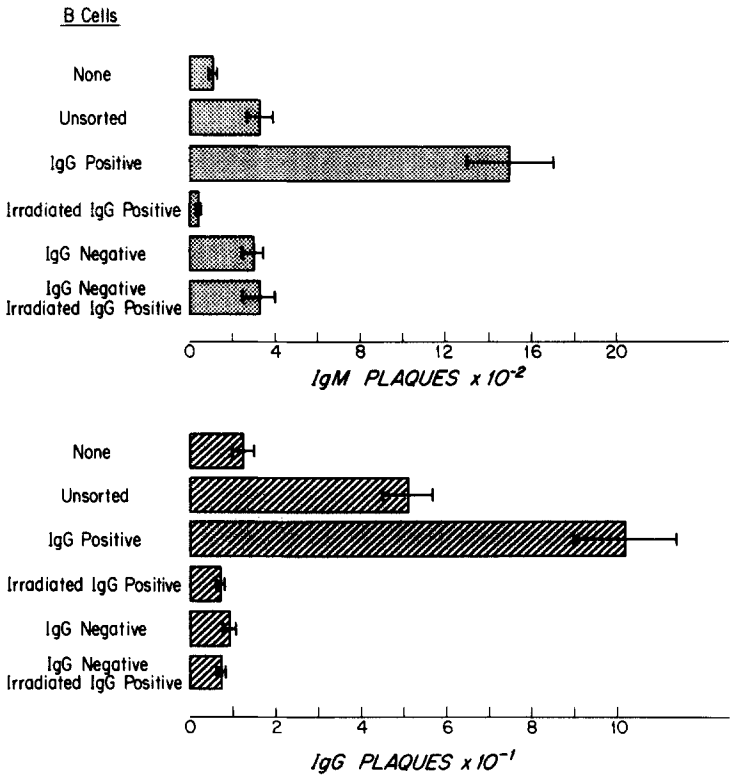

FIG. 4. IgG and IgM plaque responses of sorted splenic B cells. The effect of adding irradiated IgG positive cells to the IgG negative population. Splenic B cells were sorted as described. The IgG-positive cells were enriched, and the IgG-negative cells were depleted of both IgG and IgM responsiveness. Addition of irradiated IgG-positive cells to the IgG-negative population in equal numbers did not augment the response of the IgG-negative cells.

culture systems to inhibit or stimulate the function of subsets (11, 12); the use of antibody plus complement to eliminate subsets by cytotoxicity before culture or adoptive transfer (13); the use of specific antibody to block antigen receptors and thereby spare subsets from suicide by radioactive antigen (14); and the use of the FACS to separate subsets by positive and negative selection (15–20, and the present study). The advantage of the FACS is that it permits complete control over the separation process. It must be remembered that the binary distinction between "positive" and "negative" cells is an artificial one based on the limitations of most methods of cell separation. Similarly, the use by some investigators of the same criteria for sorting that are used in fluorescence microscope studies fails to take full advantage of the ability of the FACS to quantitate immunofluorescence.

We have chosen to use objective criteria for separating B cell subsets. Our studies have shown that the set of human B cells expressing surface IgG is capable of responding to PWM by the synthesis of both IgM and IgG and that in fact this minor subset (10–15%) of splenic B cells gives rise to the majority of the PWM response in both Ig classes. The deficient response of the vast majority of non-IgG-bearing cells is not due to lack of an accessory cell or to any suppressive influence that could be demonstrated. These results must be viewed in the light of several papers concerning

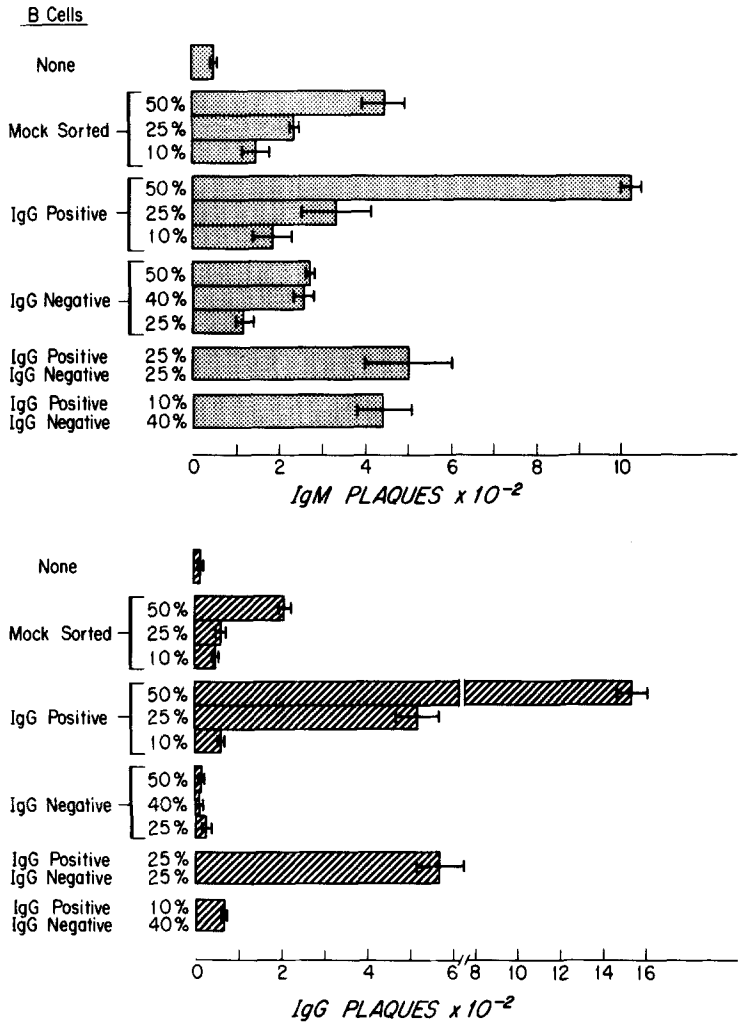

FIG. 5. IgG and IgM plaque responses of sorted splenic B cells. The effect of mixing IgG-positive and IgG-negative populations. After separation of IgG-positive and IgG-negative cells by sorting they were assayed by addition of varying proportions of the sorted cells to the cultures as indicated. The IgG-positive cells were enriched for both IgG and IgM responses, and the IgG-negative cells were depleted. Mixing IgG-positive and IgG-negative cells in either equal numbers or in a 4:1 ratio (similar to the normal ratio in the spleen) resulted in a total response that was nearly identical to what would be predicted by simple addition of the contributions of the isolated populations. In this experiment the control population was passed through the FACS in a "mock sort" before being placed into culture.

the response of B cell subsets. A series of studies by Zan-Bar et al. (16–18) using the FACS to separate cells before adoptive transfer in mice resulted in the following general conclusions: (*a*) IgG-bearing cells gave rise only to IgG responses; no IgM response could be detected with either primed or unprimed B cells; (*b*) after removal of IgG-bearing cells, the IgG-negative cells gave an increased IgM response and the addition of IgG-positive cells to other cell populations suppressed the IgM response of those cells; (*c*) removal of IgD-bearing cells prevented subsequent IgG production but

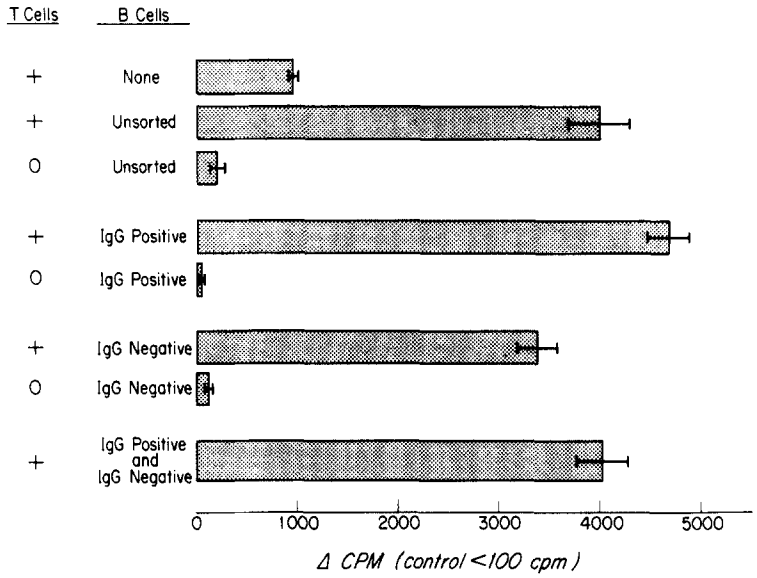

FIG. 6. The proliferative response of sorted splenic B cells. Separated IgG-positive and IgG-negative populations were cultured under conditions similar to those used for plaque assays. Incorporation of tritiated thymidine was used to measure proliferation after 5 d of culture. Both the IgG-positive and the IgG-negative populations gave an excellent response, which was T cell dependent. In this and other experiments the IgG-positive cells gave a somewhat better response than did the IgG-negative cells.

removal of IgM-bearing cells did not, implying that the cells giving rise to IgG expressed IgD but not IgM. These studies differ in several important aspects from those reported here. An antigen-driven, T cell-dependent, in vivo response in the murine system was being measured by serum antibody levels. The adoptive transfer protocol allowed for prolonged residence of the cells in the in vivo environment, which may have allowed extensive changes in surface immunoglobulin expression coincident with maturation of the cells. In addition, because antigen-specific responses were being measured, there is the possibility of feedback inhibition influencing the measured responses. For example, the suppressive effect of the IgG-bearing cells on the IgM responses could have been mediated by the antigen-specific IgG antibody produced by these cells rather than by any direct interaction between the subsets.

With these comments in mind it is useful to compare the implications of our results. First, we have shown that under the influence of the polyclonal activator PWM, IgG-bearing cells can give rise to a substantial number of IgM-producing cells. This implies that the expression of surface IgG does not reflect irreversible commitment to subsequent IgG production. A similar observation has been made in the murine system using lipopolysaccharide as an activator (21). Studies in man using antiimmunoglobulin as a stimulus for B cell proliferation have shown that anti-IgG is capable of stimulating IgM-bearing B cells, and that cells probably bearing both IgG and IgM are responsible for the majority of the proliferative response to anti-IgG (12). Second, the removal of IgG-bearing cells did not result in increased IgM responses on the part of the IgG-negative population. In fact the IgG negative population gave lower IgM responses than the unseparated cells. In mixing experi-

ments, we were unable to show any interaction between the IgG-positive and the IgG-negative populations. Third, the population of IgG-positive cells consisted to a large extent of cells also having IgM and IgD on their surface in amounts clearly above control levels. Triply endowed cells (IgG, IgM, and IgD) have been observed in man by others (4). The functional significance in man of small numbers of cells having IgG but not IgM or IgD is unknown.

It is very likely that the PWM-driven response we have measured differs in important ways from the antigen-driven response, and it is possible that in a normal antigen response the IgG-bearing cells give rise only to secreted IgG. However, from the physiological point of view it would seem that they or their progeny are quite capable of IgM production.

We have also shown that the IgG-positive and IgG-negative populations are both capable of a strong proliferative response to PWM, with the IgG-positive cells giving a slightly stronger response. Because the immunoglobulin synthetic response is dependent on cell division (10), this suggests that the deficiency found in the IgG-negative population is not due to a failure of the cells to divide but to a failure to complete terminal differentiation.

An additional important point must be made concerning the common practice of evaluating human B cell function by PWM-driven responses (22, 23). Such responses depend heavily on the function of only a minor subset of B cells and may not measure at all the capabilities of the vast majority of B cells. Full evaluation of the subset restriction of other polyclonal B cell activators in man will be necessary in order to be certain that all B cell function is tested when evaluating the human immune status.

Summary

The subset of B lymphocytes having IgG on their surfaces was purified from human spleen and blood using a fluorescence-activated cell sorter (FACS). This subset constituted about 15% of B lymphocytes. The remaining non-IgG-bearing B cells were also obtained for study. These two populations were examined for (*a*) their expression of other surface immunoglobulin isotypes, (*b*) their ability to give rise to IgG- and IgM-secreting (plaque-forming) cells in a pokeweed mitogen (PWM)-driven culture system, and (*c*) their ability to proliferate in response to PWM stimulation.

The results of these studies indicate that most IgG-bearing B cells also express surface IgM and IgD. Less than 15% had only IgG. The IgG-positive cells gave rise to both IgG and IgM plaque-forming cells when driven by PWM, and in fact were responsible for most of the total plaque response in both the IgG and IgM classes. The non-IgG-bearing B cells were depleted of both IgG and IgM responsiveness. The failure of the non-IgG-bearing B cells to give a strong response to PWM did not appear to be due to either depletion of accessory cells or to any suppressive influence. Finally, proliferation studies indicated that both the IgG-bearing and the non-IgG-bearing cells proliferated in the presence of PWM with a somewhat stronger proliferative response in the IgG-bearing cells.

These studies demonstrate that the IgG-bearing cell is not irreversibly committed to IgG production but can also give rise to IgM-secreting cells, and that human PWM-driven immunoglobulin secretory responses are predominately due to a numerically small subset of B cells.

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