Precursors of antibody-producing cells in the mouse are functional homologues of bursa-derived lymphocytes in birds (1); they have antibody-like immunoglobulin receptors (2-8) specific for limited antigenic moieties (6-16) on their cell membrane which are largely of the γM immunoglobulin (Ig) class in experimentally virgin animals of this species (16, 17). Combination of antigen with the specific receptor and the cooperation of specific thymus-derived lymphocytes triggers division and differentiation of these precursor cells into mature antibody-secreting cells (6, 18-23).

In the preceding paper (24), we demonstrated that antibody to mouse μ-chain specifically suppressed primary plaque-forming cell (PFC) responses of all Ig classes in mouse spleen cell cultures stimulated with heterologous erythrocytes. This suppressive effect was mediated through the precursors of antibody-producing cells and appeared to involve, for the most part, a potentially reversible saturation of antigen receptors with μ-chain determinants by the anti-μ and prevention of antigenic stimulation rather than elimination of these virgin precursor cells with surface μ-chains via cell death. Antibodies to mouse γ1 and γ2 heavy chains suppressed both γ1 and γ2, but not γM or γA primary PFC responses. The data are not incompatible with the concept that precursors of γG-synthesizing cells initially have antigen receptors with μ-chain determinants, hence suppression of γG responses by anti-μ; but at some time during maturation into γG-producing cells, these precursors also express γG determinants in antigen receptors. This explains suppression of γ1 and γ2 but not γM responses by anti-γ1 and anti-γ2 reagents.

Studies on changes in the Ig class of antigen receptors on γG precursors might be facilitated in spleen cell cultures from immunized mice where the precursor frequency is increased and the maturation of γG precursors in the in vivo environment is accentuated.

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Abbreviations used in this paper: "γG" is used to refer to γ1, γ2δ, and γ2ε immunoglobulins in aggregate; HBSS, Hanks' balanced salt solution lacking sodium bicarbonate; HRBC, horse red blood cells; Ig, immunoglobulin(s); MEM, minimal essential medium; PFC, plaque-forming cell(s); RBC, red blood cells; SRBC, sheep red blood cells.
ated. Thus, it might be predicted that immunization would generate populations of precursor cells with γG or γA receptors rather than γM receptors which are readily stimulated by specific antigen, but whose stimulation is not blocked by anti-μ. Support for this prediction is derived from other experimental systems where immunization increases the frequency of specific antigen-binding cells (14, 25–27), results in increased affinity of antibody produced (28–31), and favors development of γG antibody responses after subsequent antigenic challenge (31–36). Thus anti-μ should have little or no suppressive effect on γG or γA PFC responses in cultures of spleen cells from immunized mice, but the suppressive effect on γM responses should remain.

In the experiments described in this paper, mice were immunized with sheep erythrocytes (SRBC); spleen cells were obtained at various intervals after immunization for study of their capacity to produce Ig class-specific PFC responses in vitro. The suppressive effect of Ig class-specific antiglobulins on these responses was examined.

Materials and Methods

Animals and Immunization.—Mice, rabbits, and goats were maintained as described previously (24). SRBC and horse erythrocytes (HRBC) were prepared for immunization, use in the hemolytic plaque assay, or for stimulation of spleen cell cultures as described previously (24, 37, 38). C57BL/6 male mice, 2–4 months old (Jackson Laboratories, Bar Harbor, Maine) were immunized by a single intraperitoneal injection of 10 × 10^8 SRBC at specified intervals before initiation of spleen cell cultures.

Myeloma Proteins and Preparation of Antiglobulins.—Myeloma proteins or their Fe fragments and light chains were purified from ascitic fluid or urine of BALB/c AnN mice bearing appropriate plasma cell tumors as described in detail elsewhere (24, 37). Antiglobulins used for development of γG and γA PFC in the hemolytic plaque assay were raised in rabbits (35, 37); antiglobulins and anti-ferritin for addition to spleen cell cultures were raised in goats (24). All antisera, myeloma proteins, and light chains were rendered nontoxic and specific by absorption and sterilized as described in detail previously (24).

Spleen Cell Cultures and Hemolytic Plaque Assay.—10 × 10^6 spleen cells from normal or immunized mice were incubated with 3–6 × 10^6 SRBC or HRBC/culture in 1 ml of completely supplemented Eagle's minimal essential medium (MEM) (24, 37–39), containing 10% fetal bovine serum, and 50 units each/ml of penicillin and mycostatin and 50 μg of streptomycin, in 35 × 10 mm plastic dishes (Falcon 3005 [Falcon Plastics, Div. of Bioquest, Oxnard, Calif.]) in a water-saturated atmosphere of 7% O2, 10% CO2, and 83% N2 at 37°C in an air-tight box on a slowly rocking platform. Cultures were supplemented daily with fetal bovine serum and twice-concentrated medium (38). Antiglobulins and myeloma proteins were diluted as necessary in Hank's balanced salt solution (HBSS) and added at the initiation of the cultures in 0.05 ml volumes.

Cells were harvested after 5 days' incubation; Ig class-specific PFC were enumerated in a modified Jerne hemolytic plaque assay (37, 40). γM PFC were enumerated after incubation of indicator RBC and spleen cells in semisolid agarose in HBSS with guinea pig complement (Baltimore Biological Laboratories, Baltimore, Md.). Ig class-specific γ1, γ2, or γA PFC were developed with monoclonal rabbit antiglobulins and complement in assay preparations in which development of all γM PFC has been inhibited by goat anti-μ (37).

Results are calculated per 10^6 recovered cells; data are from experiments with comparable cell recoveries within experimental groups and are expressed as the geometric mean of PFC/10^6 recovered cells or as percent of control responses from three to five experiments. The coefficient of variability among experimental groups is less than 20%. Table 1 displays, for each
| PFC response | Anti-globulin | Days after immunization |
|--------------|--------------|------------------------|
|              | 0            | 3          | 5          | 10         | 14         | 21         | 28         |
|              | %* R.M. 2%  | R.M.       | R.M.       | R.M.       | R.M.       | R.M.       | R.M.       |
| γM           | —            | 1.00       | 100.0      | 65.4       | 28.5       | 4.5        | 2.8        | 1.8        | 0.8        |
| Anti-μ       | 4            | 0.04       | 15.0       | 23.0       | 32.0       | 9.1        | 0.8        | 17.0       | 0.5        | 13.0       | 0.2        | 8.0        | 0.06       |
| Anti-γ1      | 93           | 0.93       | 98.0       | 103.0      | 67.4       | 117.0      | 33.3       | 101.0      | 4.6        | 95.0       | 2.7        | 98.0       | 1.8        | 93.0       | 0.7        |
| Anti-γ2      | 95           | 0.95       | 98.0       | 95.0       | 62.1       | 91.0       | 25.9       | 97.0       | 4.4        | 103.0      | 2.9        | 110.0      | 2.0        | 91.0       | 0.7        |
| Anti-γA      | 107          | 1.07       | 101.0      | 102.0      | 60.2       | 96.0       | 27.4       | 103.0      | 4.6        | 106.0      | 3.0        | 103.0      | 1.9        | 89.0       | 0.7        |
| γ1           | —            | 1.00       | 5.7        | 4.6        | 5.7        | 2.9        | 2.3        | 2.0        | 1.8        |             |            |            |            |            |            |
| Anti-μ       | 13           | 0.13       | 25.0       | 33.0       | 1.5        | 52.0       | 3.0        | 97.0       | 2.8        | 48.0       | 1.1        | 42.0       | 0.8        | 33.0       | 0.6        |
| Anti-γ1      | 33           | 0.33       | 53.0       | 57.0       | 2.6        | 61.0       | 3.5        | 51.0       | 1.5        | 47.0       | 1.1        | 42.0       | 0.8        | 38.0       | 0.7        |
| Anti-γ2      | 37           | 0.37       | 43.0       | 46.0       | 2.5        | 50.0       | 2.9        | 57.0       | 1.7        | 66.0       | 1.5        | 47.0       | 0.9        | 44.0       | 0.8        |
| Anti-γA      | 102          | 1.02       | 100.0      | 5.7        | 95.0       | 4.4        | 90.0       | 5.1        | 106.0      | 3.1        | 103.0      | 2.4        | 92.0       | 1.8        | 93.0       | 1.7        |
| γ2           | —            | 1.00       | 4.4        | 5.6        | 7.8        | 8.9        | 16.1       | 2.5        | 2.5        |             |            |            |            |            |            |
| Anti-μ       | 12           | 0.12       | 18.0       | 20.0       | 1.1        | 38.0       | 3.0        | 78.0       | 7.0        | 75.0       | 12.1       | 60.0       | 1.5        | 52.0       | 1.3        |
| Anti-γ1      | 30           | 0.30       | 54.0       | 53.0       | 3.0        | 57.0       | 4.5        | 47.0       | 4.2        | 47.0       | 7.6        | 35.0       | 0.9        | 31.0       | 0.8        |
| Anti-γ2      | 41           | 0.41       | 43.0       | 49.0       | 2.7        | 50.0       | 3.9        | 55.0       | 4.8        | 59.0       | 9.5        | 46.0       | 1.2        | 38.0       | 1.0        |
| Anti-γA      | 97           | 0.97       | 96.0       | 4.2        | 87.0       | 4.9        | 99.0       | 7.7        | 89.0       | 7.9        | 103.0      | 16.9       | 96.0       | 2.4        | 90.0       | 2.3        |
| γA           | —            | 1.00       | 8.8        | 10.0       | 16.3       | 6.9        | 6.3        | 4.4        | 3.6        |             |            |            |            |            |            |
| Anti-μ       | 15           | 0.15       | 12.0       | 15.0       | 1.5        | 17.0       | 2.8        | 17.0       | 1.2        | 25.0       | 1.6        | 20.0       | 0.9        | 20.0       | 0.7        |
| Anti-γ1      | 97           | 0.97       | 105.0      | 9.2        | 95.0       | 9.5        | 95.0       | 15.5       | 92.0       | 6.4        | 106.0      | 6.7        | 110.0      | 4.8        | 101.0      | 3.6        |
| Anti-γ2      | 95           | 0.95       | 87.0       | 7.7        | 95.0       | 9.5        | 93.0       | 15.2       | 97.0       | 6.7        | 106.0      | 6.7        | 102.0      | 4.5        | 100.0      | 3.6        |
| Anti-γA      | 22           | 0.22       | 34.0       | 3.0        | 40.0       | 4.0        | 55.0       | 9.0        | 49.0       | 3.4        | 47.0       | 3.0        | 45.0       | 2.0        | 42.0       | 1.5        |

* %* = per cent control response (PFC/10⁶ antiglobulin-treated culture ÷ PFC/10⁶ untreated culture) × 100.

† R.M. = relative magnitude of immunized spleen cell response to unimmunized spleen cell response. In antiglobulin-treated cultures, relative magnitude = (relative magnitude untreated culture) ÷ (per cent control response/100).
interval after immunization, the Ig class-specific responses in cultures after addition of each of the Ig class-specific antiglobulins as the per cent of the corresponding Ig class-specific response in cultures without addition of antiglobulin (control response). Also shown for each interval after immunization is the magnitude of each Ig class-specific response relative to the magnitude of that response in cultures from normal mice. Relative magnitude in antiglobulin-treated cultures is the relative magnitude of a particular response in untreated cultures multiplied by per cent control/100 for that antiglobulin at that interval after immunization.

RESULTS

Ig Class-Specific PFC Responses in Cultures of Spleen Cells from Immunized Mice.—Mice were immunized intraperitoneally with $10^8$ SRBC 3, 5, 7, 10, 14, 21, and 28 days before initiation of spleen cell cultures; 5-day Ig class-specific PFC responses are presented in Fig. 1. Maximum γM PFC responses were obtained in cultures from 3-day immunized mice; these responses were approximately 100 times greater in magnitude than γM responses in cultures from normal mice (Table I). The magnitudes of γM PFC responses in cultures established at longer intervals after immunization were progressively less and at 28 days after immunization γM responses were not significantly different from those in cultures from normal mice.

In cultures from mice 3–7 days after immunization, γ1 PFC responses were 4–6 times greater than responses in this Ig class in cultures from normal mice.
IMMUNE RESPONSES IN VITRO. V

(Fig. 1 and Table 1). Cultures initiated 7 days or more after immunization had progressively smaller $\gamma_1$ PFC responses, but at 28 days after immunization these were still approximately twice the $\gamma_1$ responses observed in cultures of normal spleen cells. The magnitude of responses in the $\gamma_2$ class steadily increased with time after immunization to a maximum at 14 days which was about 16 times greater than the $\gamma_2$ responses in cultures from normal mice; the $\gamma_2$ responses in cultures initiated 28 days after priming were 2.5 times as great as $\gamma_2$ responses in cultures of normal spleen cells. $\gamma_A$ PFC responses also increased with maximum responses observed in cultures from mice 7 days after immunization. Like $\gamma_1$ and $\gamma_2$ PFC responses, $\gamma_A$ responses had not yet returned to response levels of normal cultures by 28 days after immunization (Fig. 1 and Table 1).

Effect of Goat Anti-$\mu$ on Ig Class-Specific PFC Responses in Cultures of Spleen Cells from Immunized Mice.—High concentrations of anti-$\mu$ (50 $\mu$l of 1:25 dilution) significantly suppressed $\gamma_M$ PFC responses in cultures from normal and immunized animals (Fig. 2). A moderate, but significant decrease in suppression of $\gamma_M$ responses was observed in cultures from mice 5 and 7 days after immunization. Similarly, $\gamma_A$ PFC responses were effectively suppressed by anti-$\mu$ in normal and immunized cultures; however, a moderate decrease in suppression of $\gamma_A$ PFC responses was observed in cultures from mice 14 days after immunization. Specificity controls for anti-$\mu$ have been presented previously (24). The
moderate, but probably significant changes in suppression of \( \gamma \)M and \( \gamma \)A responses by anti-\( \mu \) in cultures from immunized mice were relatively slight when contrasted with decreases in suppressive ability of anti-\( \mu \) on \( \gamma_1 \) and \( \gamma_2 \) responses. Anti-\( \mu \) effectively suppressed \( \gamma_1 \) and \( \gamma_2 \) responses in cultures from normal animals (24) (Fig. 2 and Table I). However, with time after immunization, the capacity of anti-\( \mu \) to suppress \( \gamma_1 \) and \( \gamma_2 \) PFC responses steadily decreased. In cultures from mice 10 days after immunization to which anti-\( \mu \) was added, \( \gamma_1 \) responses were essentially normal and \( \gamma_2 \) responses were suppressed only about 25%. Anti-\( \mu \) had relatively less suppressive effect on \( \gamma_1 \) than on \( \gamma_2 \) responses in cultures from mice during the first 10 days after immunization. In cultures from mice 14 or more days after immunization, anti-\( \mu \) had relatively less suppressive effect on \( \gamma_2 \) than on \( \gamma_1 \) responses, but in both cases, the per cent suppression by anti-\( \mu \) increased from the minimum observed between days 10–14 after immunization. Increasing the amount of anti-\( \mu \) added to 50 \( \mu \)l of a 1:10 dilution had no significant effect on the results. Thus after immunization, populations of precursor cells were generated whose subsequent \( \gamma_1 \) and \( \gamma_2 \) responses in culture were not as susceptible to suppression by anti-\( \mu \) as responses of precursor cells from virgin mice, when data are analyzed in terms of per cent control responses as in Fig. 2.

To determine if this change in susceptibility to suppression of \( \gamma_1 \) and \( \gamma_2 \) PFC responses by anti-\( \mu \) was restricted to responses against the immunizing antigen or was applicable to all antigens, cultures from mice immunized 10 days previously with SRBC were stimulated with SRBC or HRBC with or without anti-\( \mu \) (Fig. 3). The effects of anti-\( \mu \) on \( \gamma \)M, \( \gamma_1 \), \( \gamma_2 \), and \( \gamma \)A responses in cultures stimulated with SRBC were essentially the same as those presented in Fig. 2; \( \gamma \)M and \( \gamma \)A responses were suppressed; \( \gamma_1 \) and \( \gamma_2 \) responses were not suppressed. In contrast, all Ig class-specific responses to HRBC in cultures of the same spleen cells were suppressed by anti-\( \mu \). Thus the change in susceptibility to suppression by anti-\( \mu \) after immunization is specific for the antigen used for primary immunization.

Effect of Antibody to \( \gamma_1 \), and \( \gamma_2 \), and \( \gamma \)A Ig Classes on Ig Class-Specific PFC Responses in Cultures from Immunized Mice.—\( \gamma \)M PFC responses were essentially normal in SRBC-stimulated cultures from mice at any interval after immunization studied to which anti-\( \gamma_1 \), anti-\( \gamma_2 \) or anti-\( \gamma \)A (50 \( \mu \)l of a 1:10 dilution) were added (Table I). Anti-\( \gamma_1 \) and anti-\( \gamma_2 \) had suppressive activity against both \( \gamma_1 \) and \( \gamma_2 \) PFC responses in cultures from normal mice (24) and in cultures from immunized mice. The capacity of anti-\( \gamma_1 \) to suppress \( \gamma_1 \) and \( \gamma_2 \) PFC responses decreased with time to a low in cultures from mice 5 and 7 days after immunization; thereafter, the capacity of anti-\( \gamma_1 \) to suppress \( \gamma_1 \) and \( \gamma_2 \) responses again increased, and by 28 days after immunization had reached the level of suppression in cultures of normal spleen cells. Similarly, the suppressive capacity of anti-\( \gamma_2 \) on \( \gamma_1 \) and \( \gamma_2 \) responses gradually decreased with time to a low in cultures from mice 14 days after immunization; by day 28 after immunization, the level
of suppression had returned to that in cultures from normal mice. It must be noted that despite the fact that susceptibility to suppression by all antiglobulins decreased with time up to 10 to 14 days after immunization, this decrease was relatively small in cases of suppression of $\gamma_1$ and $\gamma_2$ responses by anti-$\gamma_1$ or anti-$\gamma_2$, but was large in the case of suppression of $\gamma_1$ and $\gamma_2$ responses by anti-$\mu$.

Neither anti-$\gamma_1$ nor anti-$\gamma_2$ had a significant effect on $\gamma A$ PFC responses. Anti-$\gamma A$ had no significant effect on $\gamma M$, $\gamma_1$, or $\gamma_2$ PFC responses; $\gamma A$ responses were suppressed in cultures from normal and immunized mice; suppression of this response also decreased slightly with time to a low in cultures from mice 7–10 days after immunization (Table I).

**DISCUSSION**

The experiments described above demonstrate that immunization has profound effects on the reactivity and properties of precursors of antibody-forming cells committed to respond after a second exposure to the specific antigen. These effects are manifested in spleen cell cultures from immunized mice in three distinct ways: 

(a) increased magnitude of PFC responses in all Ig classes with maximum responsiveness for each Ig class at characteristic intervals after immunization; 

(b) decreased suppression of Ig class-specific responses by constant amounts of specific antiglobulin at the time of maximum responsiveness in that Ig class after immunization; and 

(c) decreased suppression by anti-$\mu$ of $\gamma_1$ and
γ2 PFC responses at the time of maximum responsiveness in these Ig classes after immunization compared to suppression by anti-μ of these responses in normal cultures. It should be emphasized that these results were obtained after a single immunization with 10 × 10⁸ SRBC, a dose specifically designed to cause maximum changes in the reactivity of the precursor cells after one exposure to antigen.

The increased magnitude of PFC responses in all Ig classes in cultures from immunized mice is one reflection of the expansion of the clones of precursor cells specific for SRBC antigens after immunization, regardless of their Ig receptor class. The rapid increase in γM precursors after immunization to a maximum at 3 days and its steady decline thereafter is similar to the phenomenon of short-term immunologic memory for γM responses (32, 41–43) and has also been observed previously in this culture system (38, 44, 45). Of more interest, however, is the differential enlargement of γ1, γ2, and γA precursor cell pools. The precursor cells committed to γ1 antibody synthesis increase rapidly in number and exhibit maximum reactivity from 5 to 7 days after immunization. The γ2 precursor cells increase in number more slowly, reaching maximum reactivity at about 14 days after immunization. Maximum reactivity among those cells giving rise to γA PFC is at about 7 days after immunization.

Another manifestation of the altered reactivity of precursors of antibody-forming cells after immunization is the decreased suppressive activity of a constant amount of any specific antiglobulin on that Ig class-specific response. In γM responses this is evident in cultures from mice 7 days after immunization. In the cases of γ1, γ2, and γA responses, this phenomenon is most pronounced at that time after immunization of maximum responsiveness in that particular Ig class. The antiglobulin might be neutralized by antibody of that Ig class secreted into the culture fluids or the cells might not be as susceptible to suppression by antiglobulin due to rapid turnover of surface immunoglobulin. Another possibility is that the amount of antiglobulin added to the cultures is insufficient to saturate antigen receptors on the increased numbers of precursor cells with γG or γA receptors and thereby prevent antigenic stimulation.

The most striking effect of immunization on the properties and characteristics of precursor cells is the apparent change in the Ig class of surface receptors. In normal spleen cell cultures, anti-μ suppressed responses in all Ig classes probably by saturating antigen receptors with μ-chain determinants on precursor cells and thereby preventing antigenic stimulation (24). This implies that either separate precursor cells with γM receptors and committed to γM synthesis must first be stimulated for stimulation of precursor cells with γG or γA receptors, or that precursor cells capable of synthesizing and secreting γG or γA antibody have γM surface receptors (24, 46). In cultures from immunized mice, γ1 and γ2 responses were progressively less susceptible to suppression by anti-μ as the interval after immunization approach 10 days. At this time, anti-μ effectively suppressed γM and γA responses but had little effect on either γ1 or
\( \gamma_2 \) responses. Later after immunization the suppressive effect of anti-\( \mu \) on \( \gamma_1 \) and \( \gamma_2 \) responses gradually returned. This change in susceptibility to suppression by anti-\( \mu \) was limited to cells responsive to the immunizing antigen and was not observed in control cultures in which the same cells were stimulated with an antigen other than that used for primary immunization.

Immunization, then, appears to generate a shift in the Ig class of cell surface receptors from \( \gamma \)M to the \( \gamma_1 \) or \( \gamma_2 \) Ig class, a shift from precursor cells susceptible to anti-\( \gamma \) to precursor cells relatively independent of suppression by anti-\( \mu \). The responses of these cells, however, are still suppressed by anti-\( \gamma_1 \) and anti-\( \gamma_2 \). This shift in surface receptor Ig class parallels other changes in precursor cell reactivity. Maximum responsiveness for \( \gamma_1 \) responses is reached in the first 7 days after immunization. The increase in responsiveness parallels the decrease in suppressive capacity of anti-\( \gamma_1 \) on the \( \gamma_1 \) response. The loss of the suppressive effect of anti-\( \mu \) on \( \gamma_1 \) responses is most pronounced during the first 10 days after immunization. Maximum responsiveness for \( \gamma_2 \) responses is not reached until about 14 days after immunization, the time when the maximum decrease in suppressive effect of anti-\( \gamma_2 \) on \( \gamma_2 \) responses is noted; the loss of suppressive effect of anti-\( \mu \) on \( \gamma_2 \) responses is maximum at 10-14 days after immunization. Thereafter, anti-\( \mu \) has less suppressive effect on \( \gamma_2 \) than on \( \gamma_1 \) responses.

Despite the changes observed in the relative susceptibility of precursors of \( \gamma_1 \) and \( \gamma_2 \) antibody-producing cells to suppression by anti-\( \mu \) after immunization (when considered as per cent control response), it may not be concluded that the absolute size of the pool of precursor cells with \( \gamma \)M receptors, but capable of developing \( \gamma_1 \) or \( \gamma_2 \) responses, decreases after immunization. For example, 10 days after immunization, the \( \gamma_2 \) response is 8.9 times greater than the \( \gamma_2 \) response in cultures from unimmunized mice, and suppression of this response by anti-\( \mu \), expressed as per cent control response, is least (Table I). Nonetheless, the difference between the number of \( \gamma_2 \) PFC in cultures to which anti-\( \mu \) was added (1270) and the number of \( \gamma_2 \) PFC in cultures to which no antiglobulin was added (1630) is twice as large (1630 - 1270 = 360) as the \( \gamma_2 \) response obtained in cultures of spleen cells from normal mice (180). In other words, the number of \( \gamma_2 \) PFC that were suppressed by virtue of adding anti-\( \mu \) to these cultures is twice as large as the usual \( \gamma_2 \) response in cultures from normal mice. This suggests that the number of precursors with \( \gamma \)M receptors which eventually synthesize \( \gamma_2 \) antibody also increases after immunization. Analysis of the actual precursor cell pools is incomplete; however, comparisons of magnitudes of Ig class-specific responses in cultures with and without antiglobulins at various intervals after immunization relative to magnitudes of responses in cultures of normal mice gives some notion of the sizes of precursor cell pools. The Ig class-specific responses in cultures from normal mice are assigned an arbitrary value of one; relative magnitude values greater than one suggest the presence of a precursor cell pool for that Ig class larger than that found in normal animals. Inspection of Table I reveals that the response in any Ig class in
cultures from immunized mice treated with anti-μ is rarely less than one when expressed in terms of relative magnitudes (relative magnitude control — relative magnitude anti-μ treated). This suggests that the number of precursors with γM receptors, but capable of γ1, γ2, or γA antibody synthesis, is increased at most intervals after immunization. The one notable exception is the γ1 response in cultures from mice at 10 days after immunization; at this time, anti-μ has virtually no effect. This observation also demonstrates that normal γG responses can be obtained in cultures in which γM responses are markedly suppressed by anti-μ. This strongly suggests that stimulation of separate precursor cells with γM receptors and committed to γM synthesis is not a necessary and integral step in stimulation of precursor cells with γG receptors, as had been proposed as a possible explanation for suppression of primary responses in all Ig classes by anti-μ (24).

The gradual reappearance of suppression of γ1 and γ2 responses by anti-μ may be due to loss of cells with γ1 or γ2 receptors from the spleen and their replacement with new precursor cells capable of γ1 or γ2 synthesis but possessing γM receptors. Another possibility, however, is that precursor cells with γ1 or γ2 receptors generated as a result of immunization lose these receptors and replace them with γM receptors; that is, the cells may return to the condition with respect to Ig receptors which existed before immunization.

SUMMARY

Suppression of Ig class-specific PFC responses by class-specific antibody to mouse immunoglobulin was studied in cultures of spleen cells from immunized mice. In contrast to cultures from normal mice where anti-μ suppressed responses in all Ig classes, anti-μ had progressively less suppressive effect on γ1 and γ2 responses in cultures from immunized mice with time after immunization. This was most pronounced at 10 days after immunization when anti-μ suppressed γM and γA responses, but had no or slight effect on γ1 or γ2 responses which were still suppressed with anti-γ1 and anti-γ2. These changes in precursor cell susceptibility to anti-μ were antigen specific.

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