

SYNTHESIS OF TWO CLASSES OF ANTIBODY, γ M AND γ G OR
 γ M AND γ A, BY IDENTICAL CELLS

Amplification of the Antibody Response to Pneumococcal Polysaccharide
Type III

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The problem of immunoglobulin class restriction of precursors of antibody-forming cells has long been an object of intensive research and discussion with respect to whether the restriction in the synthesis of different classes of antibodies occurs before antigenic stimulation (the ontogenic sequence) or as a result of antigen-induced maturation (antigen-driven sequence; for review, see 1-3). The following observations support the first alternative:

(a) The ratio of γ M/ γ G antibodies can be shifted by varying the hapten density of the immunogen. Antigen with a high epitope density preferentially binds to γ M receptors, resulting in preferential synthesis of γ M antibodies. On the other hand, an antigen of low epitope density has a tendency to bind to higher affinity γ G receptors, thereby stimulating the synthesis of γ G receptors, with the synthesis of γ G antibodies (4).

(b) Anti- μ treatment of germfree mice interferes with the development of μ as well as γ and α receptor-bearing B lymphocytes in addition to secretion of these classes of antibodies (5, 6).

(c) The numbers and class distribution of B lymphocytes in mouse spleen are identical in germfree and conventional animals of the same strain. (R. Asofsky and M. B. Hylton, unpublished data).

The second alternative is supported by the following experimental evidence:

(a) After immunization with most antigens, γ M plaque-forming cells (PFC)¹ appear before γ G and γ A PFC (7, 8).

(b) Varying numbers of specific antibody-secreting cells which secrete two classes of immunoglobulin have been detected (9-12).

(c) Blockage of receptors with anti- γ M before stimulation in vitro resulted in the inhibition of γ M as well as γ G and γ A PFC responses to sheep erythrocytes in the unprimed spleen cell population, whereas after priming there was a gradual loss of suppression of γ G1 (γ 1) and γ G2 (γ 2) PFC by anti- γ M. γ A PFC were always inhibited with anti- μ (13, 14).

(d) Germ-free C3H mice in comparison with mice conventionally reared secreted only γ M immunoglobulins (15).

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¹ Abbreviations used in this paper: PFC, plaque-forming cells; SSS-III, type III pneumococcal polysaccharide.

In the present study, evidence is presented to indicate that antigen-driven expression of H-chain classes occurs during the antibody response to SSS-III (type III pneumococcal polysaccharide), an antigen that is helper T-cell independent (16, 17). When two different methods were used for the detection of class-specific $\gamma 1$, $\gamma 2$, and γA PFC, we found that a large percentage (>50) of γM PFC are also detected as $\gamma 1$, $\gamma 2$, or γA PFC and that those PFC populations most likely "switch" to exclusively γG - or γA -secreting PFC. This response was enlarged by nonspecific T-cell stimulation provided by the allogeneic effect (18, 19).

Materials and Methods

Animals. BALB/c AnN and CBF₁ (BALB/c AnN \times C57 BL/6N) mice, 4–9 wk of age of both sexes, were obtained from the Small Animal Section of the Division of Research Resources, National Institutes of Health.

Antigen, Immunization, and Allogeneic Stimulation. The immunologic properties of the type III pneumococcal polysaccharide (SSS-III) used and the method by which it was prepared have been described (20–23). CBF₁ mice were given a single intraperitoneal injection of an optimally immunogenic dose (0.5 μ g) of SSS-III in 0.5 ml saline with or without known numbers of parental (BALB/c) lymphoid cells. BALB/c lymphoid cell suspensions from various sources were prepared as described elsewhere;² they were given intravenously at the time of immunization. The magnitude of plaque-forming cell (PFC) responses was assessed at different times after immunization (see text).

Detection of Splenic Antibody-Forming Cells. Direct γM PFC, specific for SSS-III, were determined by the technique of localized hemolysis in gel (20). Sheep erythrocytes coated with SSS-III by the chromium chloride method were used as indicator cells (24). $\gamma 1$, $\gamma 2$, and γA PFC were determined by two different methods:

THE SEQUENTIAL METHOD: Assay slides were first incubated for 2 h at 37°C and then developed for γM PFC as described above. Then, a set of two slides was incubated at 37°C for 1 h with monospecific rabbit anti- $\gamma 1$, $\gamma 2$, or γA mouse immunoglobulins, diluted to optimal concentrations in guinea pig complement. Another set of two slides was incubated a second time with complement only for 1 h; this served as a control for late-appearing γM PFC. PFC counts were made on both sets of slides, and all additional PFC detected on slides treated with antiserum were considered to be PFC having the same class specificity as the facilitating antiserum used for their detection. Details concerning the preparation and specificity of these reagents, as well as their use as facilitating antisera for the detection of class-specific PFC, have been described (25, 26).

GOAT ANTI- γM BLOCKING METHOD: γM PFC were inhibited initially by incorporating goat anti- γM in a final concentration of 1:1,500 in the assay mixture. Slides were first incubated for 2 h and then developed for an additional 2 h with the facilitating antisera diluted in complement for class-specific PFC or with complement only for the detection of residual or unblocked γM PFC. The difference between unblocked (1–10 PFC/slide) and developed PFC was recorded as class-specific PFC.

The class specificity of $\gamma 1$, $\gamma 2$, and γA PFC detected with the anti- γM blocking method was affirmed by previously described plaque-inhibition tests in which different amounts of class-specific myeloma proteins were used (25). The specificity of the myeloma proteins used, as well as the method by which they were prepared, has been described (25). The class specificity of PFC detected by the sequential method has been affirmed (26) in a similar way.

Results

Initial experiments indicated that if the sequential method was used, few γG or γA PFC were detected. By contrast, if the blocking method was used, many additional

² Pasanen, V. J., R. Asofsky, and P. J. Baker. The allogeneic effect on the antibody response to pneumococcal polysaccharide type III in mice. Comparison of different sources of allogeneic lymphoid cells. Manuscript in preparation.

TABLE I
Numbers of Class-Specific PFC Detected by the γ M Blocking and Sequential Method for CBF₁ Mice Immunized with 0.5 μ g SSS-III and Given Different Amounts of Allogeneic (BALB/c) Spleen Cells

No. allogeneic cells given \ddagger	Type of class-specific PFC	PFC/spleen*		Percent γ M PFC making γ_1 , γ_2 , or γ A antibody \S
		Method used		
		γ M blocking (A)	Sequential (B)	
0	γ M	13,000		
	γ_1	3,200	<200	25
	γ_2	7,100	<200	55
	γ A	3,100	2,870	2
4×10^7	γ M	427,000		
	γ_1	132,000	23,600	25
	γ_2	462,000	109,600	83
	γ A	56,000	16,300	9
8×10^7	γ M	628,000		
	γ_1	318,000	120,000	32
	γ_2	602,000	52,000	88
	γ A	86,000	180,000	0

* PFC counts were made 5 d after immunization with 0.5 μ g of SSS-III; pooled spleen cell suspensions from three similarly treated mice were used for each determination.

\ddagger Allogeneic spleen cells were given at the time of immunization with SSS-III.

\S $\frac{A - B}{\text{No. of } \gamma\text{M PFC}}$

PFC were seen. Administration of allogeneic cells from parental spleen or lymph node to F₁ hybrids substantially raised the numbers of PFC seen by direct counting and the added PFC seen with the blocking method.

Frequency and Number of γ M PFC Making Antibody of the γ_1 , γ_2 , or γ A Class (Double-Class Producers). Inasmuch as the aforementioned results were derived from separate experiments, these issues were examined in the following study in which the same spleen cell suspensions from mice given SSS-III, with or without allogeneic cells, were assayed for class-specific PFC by both facilitating procedures.

CBF₁ mice were given 0.5 μ g of SSS-III, with or without 4 or 8×10^7 allogeneic spleen cells. 5 d later, pooled spleen cell suspensions from three similarly treated mice were prepared and each was assayed, in triplicate, for class-specific PFC by both the γ M blocking and the sequential procedure; the results obtained are summarized in Table I. If one assumes that (a) all PFC detected by the sequential method (column B, Table I) represent cells making γ_1 , γ_2 , or γ A antibody, in the absence of γ M antibody synthesis (single-class producers), and that (b) the additional PFC detected by the γ M blocking procedure (column A, Table I) represent γ M PFC also making antibody of the γ_1 , γ_2 , or γ A class (double-class producers), the following equation can be used to provide an estimate of the frequency (percentage) of PFC making antibody, specific for SSS-III, of at least two immunoglobulin classes (double-class producers):

$$\frac{(A - B)}{\text{No. of } \gamma\text{M PFC detected}} \times 100 = \text{double-class producers.}$$

Accordingly, the results obtained indicate that 25, 55, and 2% of γ M PFC detected

TABLE II
Appearance of Class-Specific PFC in CBF₁ Mice at Various Times after Immunization with 0.5 µg SSS-III and the Administration of Allogeneic (BALB/c) Spleen Cells

Day after immunization	No. of experiments	Type of class-specific PFC	PFC/spleen*		
			No. of allogeneic spleen cells given		
			0	4 × 10 ⁷	8 × 10 ⁷
3	2	γM	2.577 ± 0.177‡ (380)	2.845 ± 0.000 (700)	3.487 ± 0.186 (3,065)
		γ ₁	<100	<200	<200
		γ ₂	<100	<200	<200
		γA	<100	<200	<200
4	1	γM	8,500	46,750	109,000
		γ ₁	100	1,000	1,000
		γ ₂	580	2,500	21,500
		γA	350	2,500	1,500
5	5	γM	4.035 ± 0.130 (10,800)	5.275 ± 0.086 (188,000)	—
		γ ₁	3.736 ± 0.194 (5,450)	5.228 ± 0.219 (169,000)	—
		γ ₂	3.678 ± 0.243 (4,770)	5.187 ± 0.030 (154,000)	—
		γA	3.288 ± 0.199 (1,940)	4.635 ± 0.035 (43,200)	—
6	3	γM	3.815 ± 0.202 (6,500)	5.008 ± 0.040 (102,000)	4.903 ± 0.037 (80,000)
		γ ₁	3.737 ± 0.193 (5,450)	4.879 ± 0.037 (75,700)	4.377 ± 0.194 (23,800)
		γ ₂	3.636 ± 0.432 (4,300)	4.916 ± 0.037 (82,300)	4.453 ± 0.211 (28,400)
		γA	3.415 ± 0.716 (2,600)	4.991 ± 0.016 (98,000)	4.760 ± 0.124 (57,600)
7	3	γM	3.364 ± 0.418 (2,310)	4.084 ± 0.058 (12,100)	4.219 ± 0.074 (16,500)
		γ ₁	2.100 ± 0.100 (125)	2.758 ± 0.058 (570)	3.032 ± 0.202 (1,100)
		γ ₂	2.766 ± 0.090 (580)	3.094 ± 0.437 (1,240)	3.407 ± 0.356 (2,550)
		γA	2.244 ± 0.123 (180)	4.313 ± 0.488 (13,500)	4.635 ± 0.141 (43,200)

Results obtained with the γM blocking method.

* Pooled spleen cell suspensions from three mice immunized with 0.5 µg SSS-III were used to obtain the values for each experiment.

‡ Log₁₀ ± SE PFC/spleen for two to five experiments; geometric means are given in parentheses.

are also making antibody of the γ₁, γ₂, and γA classes, respectively; the administration of allogeneic cells leads to a substantial increase in the numbers, and to a slight increase in the frequency (25–32%), of γM-γ₁ double-class producers.

About 55% of γM PFC appear to be making antibody of the γ₂ class. The administration of allogeneic spleen cells results not only in an increase in the number of γM-γ₂ double-class producers, but also a substantial increase in their frequency (55 vs. 83–88%). In contrast, the frequency of γM-γA double-class producers is quite low,

TABLE III
Class-Specific PFC Response to SSS-III 7 d after Immunization (0.5 μ g) with and without Allogeneic Spleen Cell Transfer

Type of class-specific PFC	PFC/spleen*			
	No. of allogeneic cells given			% doubles
	0	4×10^7	8×10^7	
γ M	$3.364 \pm 0.418\ddagger$ (2,310)	4.084 ± 0.058 (12,121)	4.219 ± 0.074 (16,538)	
γ 1	2.448 ± 0.224 (280)	3.390 ± 0.483 (2,454)	3.041 ± 0.342 (1,100)	0
γ 2	2.381 ± 0.381 (242)	2.800 ± 0.536 (634)	3.288 ± 0.319 (1,939)	3.7
γ A	2.688 ± 0.354 (486)	4.115 ± 0.359 (13,034)	4.494 ± 0.112 (31,211)	72

Results obtained with the sequential method.

* Pooled spleen cell suspensions from three similarly treated mice were used for each determination.

\ddagger $\text{Log}_{10} \pm$ SE PFC/spleen for three experiments; geometric means are given in parentheses.

2–9% with or without 4×10^7 allogeneic spleen cells. This latter increase nevertheless was statistically significant ($P < 0.05$). With 8×10^7 allogeneic spleen cells, more γ A PFC were detected with the sequential than with the γ M blocking method. These data show that (a) with the strong allogeneic stimulation on day 5 all γ A antibodies were secreted independently of γ M antibodies, and (b) our γ M blocking serum also interfered to some extent with the development of other classes of PFC, because more γ G PFC were detected on day 7 (Tables II and III) with the sequential method than with the γ M blocking method. This implies that our figures underestimate rather than overestimate the double-class producers. The same tendency is apparent when the day 7 data are compared (Tables II and III).

These data provide no information concerning whether some of the PFC detected may be making antibody of three different classes (triple-class producers, γ M, γ 1, γ 2). In this context, it should be noted that some of the values (percentages) found for the numbers of double-class producers exceed 100%.

Kinetics for the Appearance of Class-Specific PFC, with and without Allogeneic Stimulation. Groups of CBF₁ mice were immunized with 0.5 μ g SSS-III and given 0, 4×10^7 , or 8×10^7 allogeneic (BALB/c) spleen cells. The numbers of γ M, γ 1, γ A PFC produced were determined 3–7 d after immunization. In these experiments for days 3, 4, and 7, both the sequential and γ M blocking methods were used; for days 5 and 6 only the γ M blocking method was used. The results obtained with the γ M blocking method are summarized in Table II and Fig. 1. The 7th d sequential method data are given in Table III. Days 3 and 4 sequential method data are not given in separate tables because no γ 1, γ 2, or γ A PFC were detected. We later confirmed these results in an independent series of similar experiments to detect double-class staining with fluorescinated antiimmunoglobulins.³

Small numbers of γ M PFC (380 PFC/spleen) were found 3 d after immunization;

³ Morse, H. C., V. J. Pasanen, and A. Lawton. Unpublished observations.

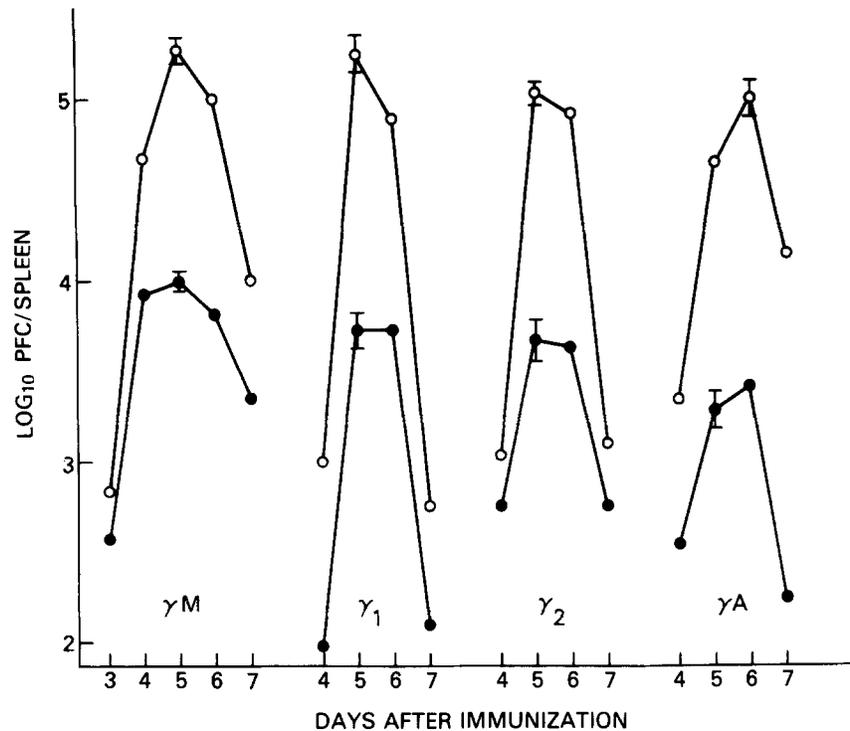


FIG. 1. Appearance of class-specific PFC and CBF₁ mice from 3 to 7 d after immunization with 0.5 μ g SSS-III with or without induction of allogeneic effect by 4×10^7 BALB/c spleen cells. Numbers of γ_1 , γ_2 , and γ_A PFC were obtained by the anti- γ_M blocking method. ●, SSS-III, ○, SSS-III 4×10^7 BALB/c spleen cells.

γ_M PFC increased in number until maximal values (10,800 PFC/spleen) were obtained 5 d after immunization. The response was 2- and 10-fold greater 3 d after immunization for mice given 4×10^7 and 8×10^7 allogeneic cells, respectively. At day 5 after immunization there was an 18-fold increase in the γ_M PFC response of mice given 4×10^7 allogeneic cells. Fewer γ_M PFC were detected 6–7 d after immunization; however, on day 7, the magnitude of the γ_M PFC response of mice given allogeneic cells was six to eight times greater than for mice not given allogeneic cells.

Similar temporal relationships were also observed in the case of γ_1 and γ_2 PFC, although these types of class-specific PFC were not detected until day 4 by the γ_M blocking procedure (Table II). On day 4 the detectability by the sequential method was <100 PFC for the control group and <200 for the groups given allogeneic spleen cells. It also should be noted that in those F₁ mice not given allogeneic cells no γ_1 or γ_2 PFC could usually be detected by the sequential method on day 5 (unpublished observation). This suggests that all γ_1 and γ_2 PFC detected on day 4 and day 5 were also detected as γ_M PFC. Maximal numbers of γ_1 and γ_2 PFC were found on days 5–6 with and without allogeneic stimulation. In the case of mice given allogeneic cells, there was a 30- to 32-fold increase in the magnitude of the γ_1 and γ_2 PFC response. Thereafter, γ_1 and γ_2 PFC declined at a rate more rapid than that observed for γ_M PFC. In general, all γ_1 and γ_2 PFC found on day 7 were detectable by the

γ M blocking as well as the sequential method. The synthesis of γ 1 and γ 2 antibodies appeared to be independent from that of γ M antibody. Again, in most instances, the PFC numbers with the sequential method exceeded those with the γ M blocking method.

γ A PFC were first detected by the γ M blocking method on day 4 (not the sequential method). At this time, the allogeneic stimulation resulted in a seven fold increase in the magnitude of the γ A PFC response. Larger numbers of γ A PFC were detected by both the γ M blocking and the sequential methods also in the control groups on day 5 (Tables I and II). Administration of 4×10^7 allogeneic spleen cells resulted in a 22-fold increase in the magnitude of the γ A PFC response. Maximal numbers of γ A PFC were found on day 6, with or without allogeneic stimulation. For mice not given allogeneic cells, few γ A PFC were detected on day 7 (180 PFC/spleen); the numbers found were considerably larger (13,500–43,000 PFC/spleen) in the case of mice given the allogeneic spleen cells.

Discussion

In this work we present data that show that in response to SSS-III, antibody-producing PFC proceed along the differentiation path from γ M to γ A or from γ M to γ G. At the peak of the immune response, $\cong 80\%$ of γ M secreting PFC are also secreting other classes (double-class secretion). These results strongly support the hypothesis that antigen-driven events regulate the expression of H-chain classes in B-cell differentiation. Two different procedures were used for the detection of γ 1, γ 2, and γ A PFC: the anti- γ M blocking method and the sequential development method. In the CBF₁ mice used in these studies only the anti- γ M blocking method detected γ 1, γ 2, and γ A PFC on the 4th to 5th d in mice not given the allogeneic cells; but on the 7th d, equal numbers of such PFC were detected by both methods (Tables II and III). The sequential method detected only γ 1, γ 2, and γ A PFC which appeared in addition to already formed γ M PFC, i.e., they secreted those antibodies independently of γ M antibodies. PFC detectable with the anti- γ M blocking method but not with the sequential method (the early PFC) secreted γ 1, γ 2, or γ A along with γ M antibodies. It is highly unlikely that the "unblocking" of some anti- γ M reagents used to inhibit γ M PFC (8) is responsible for our results. The class specificity of these reagents has previously been demonstrated by several investigators working with different antigens (25, 26). The SSS-III specificity of the developed PFC has been previously confirmed (26) and was done by us as well (see Methods).

Our experiments comparing the γ M blocking method and the sequential method indicate that SSS-III is highly efficient in inducing large numbers of double-class producers early in the immune response in CBF₁ mice. Because this difference was seen regularly in CBF₁ mice, but not in the inbred BALB/c parent strain, it is probable that genetic factors contributed by the C57BL/6 parent are permissive in the expression of "doubles"; or that BALB/c mice possess a recessive gene that restricts such expression. Pierce et al. (25), using sheep erythrocytes as antigen, could detect no difference in numbers of PFC at day 5 when the previous methods were compared (25). The failure to detect a difference is most likely because of the relatively low numbers of double-class producers induced in response to sheep erythrocytes (1.5%) as shown by Nossal et al. (10).

From the kinetic data it is clear that in response to SSS-III, antibody classes secreted

by PFC change with time. On day 3, PFC exclusively secrete γ M, on days 4–5, individual PFC secrete both γ M and either γ 1, γ 2, or γ A, and from day 7 on each PFC secretes only one antibody class, either γ M, γ 1, γ 2, or γ A. These results support the idea that there is an antigen-driven switch from the γ M class of antibody to other classes with an intervening period when individual PFC can secrete more than one class. It is unlikely that the exclusively γ G- and γ A-secreting PFC detected on the 7th d were independently stimulated by the antigen at the end phase of the immune response, because all classes of PFC were declining to background values at that time. There is also the slight possibility that a few independent γ G secretors might have remained undetected at the earlier phases because of the inherent difficulty of counting additional numbers of PFC compared with γ M PFC. As our techniques are able to detect as few as 250 γ G PFC/spleen (Tables I–III), the number of single-class producers, if at all present on days 3–4, must be extremely low. In addition, no single γ G or γ A class producers are found on days 3–4 even when stimulated with the allogeneic cells giving a response, on day 7, 8- to 10-fold higher than the nonstimulated. This implies that the number of PFC independently secreting γ G or γ A on days 3–4 would have to be even lower than 250/spleen.

Our results show that γ A PFC arose independently from γ M-secreting PFC and not through the sequence (γ M \rightarrow γ G \rightarrow γ A) because independent γ A-secreting PFC were detected on day 5 in mice immunized with SSS-III alone, which usually did not show independent γ G PFC (Table I). Also, Pierce et al. (13) could inhibit the induction of γ A PFC response with anti- γ M, but not with anti- γ G. Barthold et al. (26), with BALB/c mice, found SSS-III-independent γ G and γ A secretors earlier than we did with CBF₁ hybrid mice. At present, we have no explanation for this difference.

A major objection to the antigen-driven class-differentiation theory has been the question of how such rarely reported double-class producing cells could account for the major differentiation pathway from γ M to γ G (10). The low numbers of double-class producers found by other investigators could be the result of: (a) technical difficulty in detecting them, and (b) asynchronous appearance of double-class producers which occurs during the immune response to most antigens. The frequency of double-class producers to different antigens in previous studies varied from 1.5 to 14% (9–12), a range substantially broadened by our findings. Factors that may influence the class spectrum of antibodies produced by a single cell at a given time could be the immune status of experimental animals (previous antigen exposure) as well as the specific antigen structure (epitope density, carrier effect) both of which have differential stimulatory effects on B and T cells.

The concept of “switch”, although appearing often in the literature, has been vague because it is difficult to study. If its existence were accepted at all, T-cell participation was usually implicated (15, 27–33). Our results show that B-cell stimulation with SSS-III alone (a T-helper cell independent antigen) that minimally stimulates T-cell proliferation (29) induces the “switch” from γ M to γ A to γ G. In support of this statement, athymic nude mice give normal primary γ M, γ G, and γ A PFC responses to SSS-III (26), and apparently undergo switches like those found in normal mice.

The allogeneic effect has been shown to affect the B-cell function either by increasing the number of responding clones, the size of a clone, or the maturation of triggered cells (32, 33). Our findings with the allogeneic stimulation support and

extend these findings: (a) Depending on the intensity of allogeneic stimulation (cell source and dose), the numbers of γ M, γ 1, γ 2, and γ A PFC were greatly increased. The proportional increment of γ G and γ A PFC was slightly higher than that of γ M PFC. (b) The increase in the numbers of all classes of PFC occurred between days 3 and 5 after immunization. (c) Rather strong allogeneic stimulation resulted in the earlier appearance of exclusively γ G- and γ A-secreting PFC on the 5th d than appeared with immunization alone. (d) The effect of γ A PFC was most marked and prolonged (Tables I and III).

SSS-III may be unique in its ability to stimulate a synchronous primary immune response, permitting visualization of a large cohort of simultaneously differentiating cells. We feel this is an unusually valuable tool for dissecting the various stages in the antigen-driven differentiation of B cells. Our hypothesis is that even though the response of B precursor cells to other antigens is less synchronous, they still most likely follow the same pathway of differentiation.

Summary

Class-specific plaque-forming cell (PFC) (γ M, γ 1, γ 2, and γ A) responses to type III pneumococcal polysaccharide (SSS-III) were studied in BALB/c \times C57BL/6F₁ (CBF₁) mice with and without induction of an allogeneic effect. γ 1, γ 2, and γ A PFC were detected in two ways: (a) With the sequential development of the assay slides, first for direct (γ M) PFC followed by incubation with class-specific antiimmunoglobulin and complement for the development of additional γ 1, γ 2, and γ A PFC (γ M-independent γ 1, γ 2, and γ A PFC); and (b) by blocking γ M PFC with goat anti- γ M and simultaneously developing γ 1, γ 2, and γ A PFC (total γ 1-, γ 2-, and γ A-secreting PFC).

The results showed that whereas γ M PFC arose on the 3rd d after immunization, γ 1-, γ 2-, and γ A-secreting PFC arose on the 4th to 5th d after immunization. They appeared in association with γ M-secreting PFC because they were detected with the γ M blocking method but not with the sequential method. By the 7th d most γ 1, γ 2, and γ A PFC were detected by the sequential method as well, indicating that those antibodies were secreted independently of cells secreting γ M. When the numbers of double-class-secreting PFC were evaluated on the 5th d, the following results were obtained: 83% of γ M PFC were secreting either γ 1 (25%), γ 2 (55%), or γ A (2%). We interpret these data as evidence for an antigen-driven class differentiation from γ M to γ A and from γ M to γ G in the majority of anti-SSS-III-secreting clones without T-cell help.

When an allogeneic effect was provided by inoculation of parental BALB/c spleen cells together with antigen, the numbers of all classes of PFC were increased. Furthermore, the frequency of γ M- γ G (108%) or γ M- γ A (9%) double-class secretors was increased, and γ M-independent γ G and γ A secretors were detected earlier, indicating an overall maturation-promoting effect. In addition, prolonged appearance of γ A PFC was dependent on the allogeneic effect.

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