CHARACTERISTICS OF IMMUNOLOGICAL MEMORY IN MICE

I. Separate Early Generation of Cells Mediating IgM and IgG Memory to Sheep Erythrocytes*

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A secondary (2°)[1] or anamnestic antibody response differs from the primary (1°) response, and may be identified by some or all of the following characteristics: a reduced or absent lag period before the appearance of antibody-forming cells (AFC); an earlier and higher peak of AFC and circulating antibody (often of higher affinity); an early bias towards the synthesis of 7S antibodies (1).

Using these criteria, it has been demonstrated that priming for a 2° response to sheep erythrocytes (SRBC) in mice occurs during a 4–5 day period after immunization (2, 3). For other antigens, the development of memory may be a longer process (1, 4) and the long-term persistence of antigen may be an important requirement, as it may also be for the maintenance of memory (5). At a cellular level, preparation for a 2° response includes the generation of primed IgM and IgG AFC precursors (2, 6), together with a helper T-lymphocyte population (7). The X-Y-Z scheme of immune cell maturation (8, 9) was devised to account for the relationships between unprimed lymphocytes, memory cells, and AFC. However, this scheme was formulated before the separate but cooperative roles of T and B lymphocytes in humoral responses became apparent. There is also the further problem of whether IgG-secreting cells in the 1° or 2° response are derived from unique precursors, or from a line which gives rise to IgM-secreting cells (10). Despite more recent attempts to interpret the X-Y-Z scheme in terms of IgM- and IgG-secreting populations (2), the relationships which primed B and T cells bear to each other and to unstimulated cells are largely unknown.

The following experiments were designed to study the kinetics of the generation of primed IgM and IgG precursors, and of the helper T-cell poulation, by first halting the development of 1° responses in mice to SRBC at various times. The degree of priming in separate cell lines which had occurred before the 1° re-

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[1] *Abbreviations used in this paper:* 1°, primary; 2° secondary; AFC, antibody-forming cells; CP, cyclophosphamide monohydrate; PFC, plaque-forming cells; I-UdR, 5-iodo-2′-deoxyuridine; F-UdR, 5-fluorodeoxyuridine; HBSS, Hank's balanced salt solution.

sponses were blocked was then assessed by measuring the response to challenge 12 wk later. In some experiments, groups of mice were injected before challenge with primed B or T lymphocytes, and this allowed us to distinguish between the times of B- and T-cell priming after initial exposure to antigen.

Materials and Methods

Animals.—2–4-mo old mice of the inbred strain C3H, bred and maintained in the Department of Zoology, University of Edinburgh, were used throughout this study.

Antigen.—SRBC were obtained in sterile Alsever's solution (Tissue Culture Services Ltd., Slough, England), and were washed four times in isotonic saline before use.

Immunization.—5×10^5 or 5×10^8 SRBC were injected intraperitoneally (i.p.) in saline. Preliminary experiments demonstrated that the former dose was the minimum which could prime for a 2° response, while the latter was arbitrarily chosen as a high priming dose. After 12 wk, mice were re-immunized i.p. with 5×10^8 SRBC.

Measurement of DNA Synthesis.—DNA synthesis was measured by the uptake of [^{125}I]5-iodo-2′-deoxyuridine ([^{125}I]UdR; Radiochemical Centre, Amersham, England) in mice which had been injected 1 h previously with 5×10^{-8} mol of 5-fluorodeoxyuridine (F-UdR; Roche Products Ltd., London) in 0.2 ml saline i.p. (11, 12). [^{125}I]UdR was adjusted immediately before use to an activity of 5 μCi/ml and a concentration of 1 μg/ml by addition of unlabeled I-UdR. Mice were injected with 0.2 ml of this solution i.p., and 2 h later were killed so that the lymphoid tissues to be assayed could be removed for fixation and washing in 70% ethanol (13, 14). Tissues were counted for 2 min in a Packard Autogamma spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.), and the uptake of [^{125}I]UdR into each expressed as a proportion of the injected activity, according to the formula: uptake = $\log_{10}$ (sample cpm $\times$ 10^6/injected cpm).

Hemolytic Plaque Assay.—A modification (15) of the hemolytic plaque assay of Jerne et al. (16) was employed. Agarose (L'Industrie Biologique Francaise, Gennevilliers, France) was made up in the phosphate-buffered medium of Mishell and Dutton (17) for both top and bottom layers, and IgG PFC were detected by the inclusion of a rabbit antimouse immunoglobulin antiserum in the top layer. The developing constant of this antiserum was 1.70 and the inhibition constant 0.88 at the dilution used. Results were expressed as $\log_{10}$ PFC/spleen, and groups were compared by means of the Student's t test.

Immunosuppression.—Anti-SRBC antiserum was raised in C3H mice by two i.p. injections of 5×10^8 SRBC given 3 wk apart. The mice were bled out 7 days after the second injection and the serum so obtained was found to have a $\log_2$ 2-mercaptoethanol resistant hemagglutination titer of 15. 0.1 ml of this pooled preparation was injected intravenously (i.v.). Cyclophosphamide monohydrate (CP; Koch-Light Laboratories Ltd., Colnbrook, England) was administered i.p. at a dose of 150 mg/kg body weight.

Irradiation.—Whole-body X irradiation was administered with a Westinghouse machine (Westinghouse Electric Corp., Pittsbourgh, Pa.) under the following conditions: 230 kV; 15 mA; 0.5 mm Cu, and 1.0 mm Al filtration; 66 R/min. Mice were restrained in polystyrene boxes 75 cm from the source and exposed to a total of 900 R.

Anti-θ Serum.—An anti-θ C3H serum (18) was prepared in AKR mice by four weekly injections of 10^7 CBA thymus cells with 2×10^9 *Bordetella pertussis* organisms in a total volume of 0.2 ml i.p. The activity of the serum was confirmed by incubation of 10^6 C3H spleen or lymph node cells in 0.1 ml Hank's balanced salt solution (HBSS) at 37°C for 45 min, with addition of 0.1 ml of a 1:8 dilution of the antiserum and 0.05 ml guinea pig complement. The complement, anti-θ serum, and normal AKR serum for control tubes were previously absorbed twice with C3H erythrocytes. Viable counts in nigrosin after incubations showed that the anti-θ serum had killed 40% of the spleen cells and 75% of the lymph node cells, while the mortality

in normal AKR serum and complement was 5% and 11% respectively for the two cell suspensions.

For the preparation of primed B cells, 10^8 spleen cells from mice which had been immunized 8 days previously with 5×10^8 SRBC were suspended in 5 ml HBSS. They were then incubated at 37°C for 1 h with 0.2 ml anti-θ serum and 0.5 ml complement. The viable cell yield after incubation was 50–55% of the original cell count, while in control tubes with normal AKR serum it was 75–89%.

Primed T Lymphocytes.—Mice which had received 900 R whole-body X irradiation were injected iv with 10^8 syngeneic thymocytes. 1 day later, these animals were immunized with an i.p. injection of 10^8 SRBC and after a further 6 days, spleen cells (82% θ-bearing cells, by cytotoxicity) were havested.

RESULTS

Immunosuppression of the 1° Response to SRBC.—For this study, mice were primed with either 5×10^8 or 5×10^{15} SRBC injected i.p. Fig. 1 illustrates the development of 1° IgM and IgG PFC responses following immunization with these doses of antigen, and shows that for both groups the IgM response peaked on day 5. Both responses were dose-dependent. Groups of mice which had received a single i.p. injection of 5×10^8 SRBC were also treated with CP and anti-SRBC antibody from day 0–5 after antigen, and their spleens assayed for DNA synthesis and the development of PFC (Tables I and II). Administration of CP and antibody from 0–2 days after antigen reduced the day 3 DNA synthesis to background levels, and the day 6 PFC response to less than 100 IgM and virtually no IgG PFC per spleen. The PFC response was also completely suppressed when these agents were given 3 days after antigen, while if injected on day 4 or 5, they reduced the IgM response 10-fold and the IgG response 35-fold.

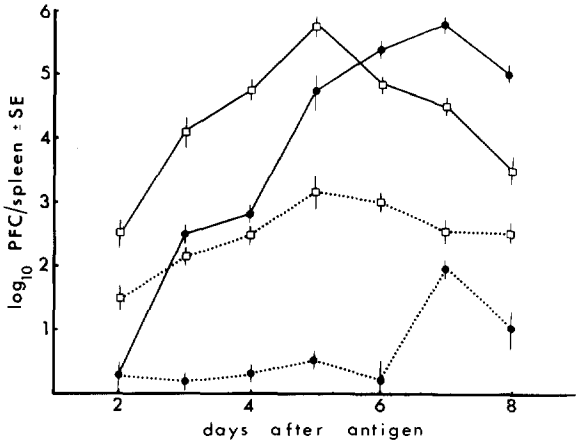

FIG. 1. The primary PFC response to high and low doses of SRBC in the spleens of C3H mice. □—□, IgM, 5×10^8 SRBC; ●—●, IgG, 5×10^8 SRBC; □···□, IgM, 5×10^5 SRBC; ●···●, IgG, 5×10^5 SRBC.

TABLE I

The Effect of Cyclophosphamide Monohydrate and Specific Antibody on the Day 3 Splenic DNA-Synthetic Response to 5 × 10^8 SRBC i.p.

Time of treatment with CP and antibody	DNA-synthetic response	
	Log_{10} I-UdR uptake ± SE	Antilog
(day after antigen)		
0	4.22 ± 0.05	16,600
1	4.24 ± 0.06	17,380
2	4.21 ± 0.05	16,220
Unsuppressed control	4.75 ± 0.03	56,230
Background*	4.40 ± 0.02	25,120

Each result is the mean from observations on eight mice.

* Background is the I-UdR uptake in normal unimmunized spleens.

TABLE II

The Effect of Cyclophosphamide Monohydrate and Specific Antibody on the Day 6 Splenic PFC Response to 5 × 10^8 SRBC i.p.

Time of treatment with CP and antibody	Mean log_{10} PFC/spleen ± SE	
	IgM	IgG
(day after antigen)		
0	1.45 ± 0.20	0.10 ± 0.03
1	1.81 ± 0.17	0.09 ± 0.01
2	1.30 ± 0.11	0.20 ± 0.06
3	1.52 ± 0.09	0.17 ± 0.05
4	3.04 ± 0.20	3.52 ± 0.13
5	3.25 ± 0.14	3.68 ± 0.09
Unsuppressed control	4.10 ± 0.10	5.13 ± 0.08
CP, antibody, no SRBC	0.81 ± 0.31	0.31 ± 0.23
No CP, antibody, SRBC	0.63 ± 0.23	0.23 ± 0.11

Each result is the mean from observations on six mice.

Experimental Scheme to Assess the Kinetics of Memory Generation.—Mice were injected with 5 × 10^8 or 5 × 10^5 SRBC i.p. in 0.3 ml saline. On days 0 (30 min), 1, 2, 3, 4, 5, 7, 10, or 15 after antigen, groups of these animals were treated with CP and anti-SRBC antibody. These agents were chosen to partially remove antigenic stimulus (2, 19), and to kill, or halt division of, activated T and B lymphocytes (20, 21). Control groups received either antigen (control 2° response) or immunosuppressants (control 1° response) alone. 12 wk after priming, all animals were challenged with 5 × 10^8 SRBC and the degree of priming which had occurred was measured by the size of their 2° IgM and IgG PFC responses 3 days later. The 12-wk interval was chosen to allow decay of 1° antibody to levels at which it would not affect the course of a 2° response (22). All results represent the pooled data for 10–15 mice.

Memory Generation Following 5 × 10^8 SRBC i.p.—Figs. 2 and 3 show the 2°

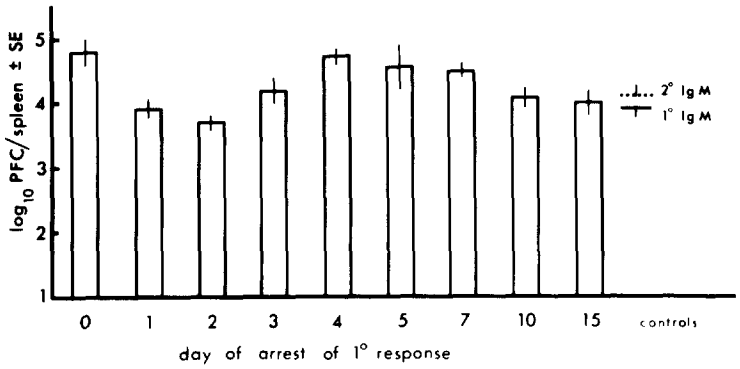

FIG. 2. Generation of IgM memory after priming with 5×10^8 SRBC: IgM PFC in the spleens of mice 3 days after challenge with 5×10^8 SRBC.

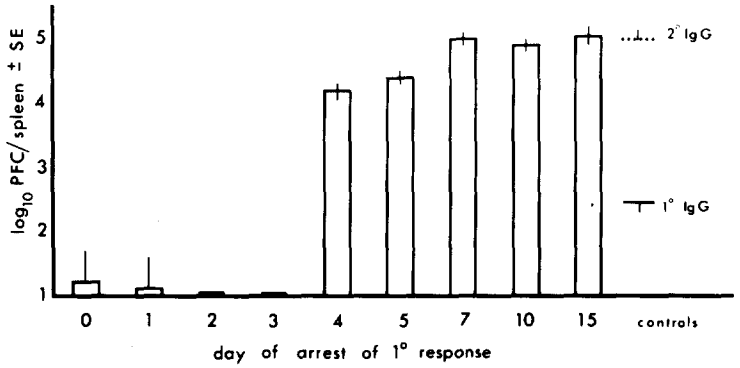

FIG. 3. Generation of IgG memory after priming with 5×10^8 SRBC: IgG PFC in the spleens of mice 3 days after challenge with 5×10^8 SRBC.

responses in mice primed with 5×10^8 SRBC, and illustrate the kinetics of IgM and IgG memory generation. It can be seen (Fig. 2) that an enhanced 2° IgM PFC response ($P < 0.01$) was observed in animals to which immunosuppressants were administered 30 min (day 0) after priming antigen, or after 4 or 7 days ($P < 0.01$ and < 0.05 respectively). For the group suppressed on day 0 this represented a fivefold increase in PFC over the control 2° response. The 2° IgM response was normal following blockade of the 1° response on days 1 or 3, but was significantly lowered in those animals whose 1° response had been blocked 2 days after antigen, ($P < 0.05$). Longer intervals of 10 or 15 days between priming and blockade resulted in 2° IgM responses which did not differ from the control level.

These results suggested that IgM priming occurred very rapidly after administration of antigen, but that primed cells then became involved in the development of the 1° response. This was reflected in the decrease in IgM memory

to normal or subnormal levels when the 1° response was blocked on days 1 or 2. By days 3–4, IgM memory cells were less actively involved in the 1° response and were thus no longer subject to assassination by CP. The capacity to express memory then decreased over the next 11 days, an observation which is further considered in the discussion.

In contrast to these observations, no IgG memory was expressed if the 1° response was halted on days 0–3 after priming (Fig. 3). When it was halted on day 4 a definite but significantly reduced 2° response was seen ($P < 0.05$). The capacity to produce a 2° IgG PFC response was therefore not fully developed until 7 days after priming. This might be explained either by a failure of IgG B-memory cells to develop until 4 days after a 1° injection of SRBC, or by a delay in the development of helper T-memory cells until day 4, or by a combination of the two.

Memory Generation Following 5 × 10⁵ SRBC i.p.—Figs. 4 and 5 illustrate the kinetics of the generation of IgM and IgG memory following priming with 5 × 10^5 SRBC. It was found that the capacity to give an enhanced 2° IgM PFC response on challenge arose as rapidly after 5 × 10^5 as after 5 × 10^8 SRBC (Fig. 4), but that there was no subsequent reduction of the response in mice in which the 1° response was halted on days 1 or 2 after antigen. As the splenic 1° IgM response to 5 × 10^5 SRBC was substantially less than that produced by the higher antigenic dose (Fig. 1), it seems probable that the lower dose was insufficient to recruit many primed cells into the 1° antibody response. Blockade of the 1° response between 5 and 15 days after priming did not result in a significantly enhanced response after challenge. The ability to give an enhanced 2° IgM response was thus lost during the latter stages of the 1° response to both antigen doses.

In mice primed with 5 × 10^5 SRBC, no expression of IgG memory was found when the 1° response was halted on days 0–3, but the 2° IgG response was fully developed in all other groups (Fig. 5). In this respect, the development of memory followed a very similar pattern to that seen after priming with 5 ×

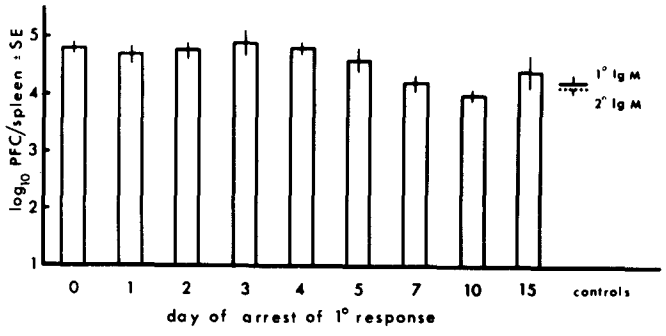

FIG. 4. Generation of IgM memory after priming with 5 × 10^5 SRBC: IgM PFC in the spleens of mice 3 days after challenge with 5 × 10^8 SRBC.

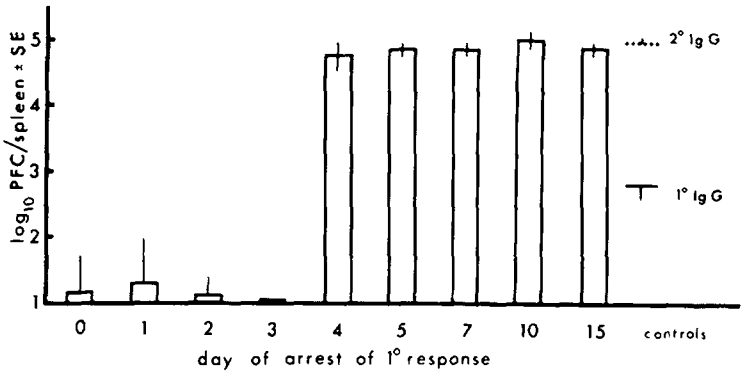

FIG. 5. Generation of IgG memory after priming with 5×10^5 SRBC: IgG PFC in the spleens of mice 3 days after challenge with 5×10^8 SRBC.

10^8 SRBC, and again suggested that at least one of the primed populations required for the production of 2° IgG PFC had not developed before day 4 after priming. This resulted, in both experiments, in 2° IgG responses which were lower than the normal day 3 levels of 1° IgG PFC.

Priming of B and T lymphocytes by SRBC.—Experiments were carried out to establish whether IgG B-cell memory or helper T-cell memory (7) developed before day 4 of a 1° response to SRBC (see following sections). Since these experiments involved the transfer of primed lymphocytes, preliminary assays of SRBC-primed T cells and primed anti-θ-treated spleen cells (B cells) in syngeneic X-irradiated recipients were made, in order to compare their activities with those of normal cells.

The various combinations of cells assayed are recorded in Table III, together with the results. Five times more normal thymocytes than primed T cells were injected into recipient animals in order to reduce the difference in the numbers of potential helper cells in each population (23). 18 h after the cell transfers, all animals were injected i.p. with 10^7 SRBC and 4 days later the PFC response in each spleen was assayed.

It was found that primed T and B lymphocytes together (group 1) gave higher IgM ($P < 0.01$) and IgG ($P < 0.001$) responses than did normal cells (group 8), and that the IgG response in group 1 was also higher than those in groups which combined normal and primed cells (groups 4 and 6; $P < 0.01$ and < 0.001 respectively). Small responses were seen in recipients of primed or normal B cells only, while primed T cells alone or normal thymocytes alone gave very few PFC. These results thus confirmed the presence of primed T lymphocytes after exposure to SRBC, and IgG B-memory cells in the anti-θ-treated spleens of SRBC-injected mice.

IgG B-Memory Cell Generation Following 5×10^5 SRBC i.p.—Groups of mice were injected with 5×10^5 SRBC i.p. At intervals of 0 (30 min), 1, 2, 3, 4, or 7 days after antigen, the developing 1° response was blocked by administration of

CP and anti-SRBC antiserum. Control groups received 5×10^5 SRBC or CP and antibody alone. After 12 wk, 10^7 T cells, primed 6 days previously with SRBC, were injected i.v. into half of those mice whose 1° responses had been arrested on days 0–3, and into half of each control group. They were also injected into a further control groups of X-irradiated normal mice. After 18 h all animals were challenged i.p. with 5×10^8 SRBC, and 3 days later their IgM and IgG PFC responses were assayed (Figs. 6, 7).

The presence of primed T cells had no effect on the expression of IgM memory in either the experimental or control groups (Fig. 6; compare Fig. 4). In

TABLE III

Immunological Activity of SRBC-Primed T and B Lymphocytes in Syngeneic X-Irradiated Recipients

Group	Lymphocytes injected[1]	Mean $\log_{10}$ day 4 PFC/spleen ± SE	
		IgM	IgG
1	10^7 T* cells + 5×10^7 B* cells	2.79 ± 0.12	3.49 ± 0.23
2	10^7 T* cells	1.06 ± 0.21	1.04 ± 0.10
3	5×10^7 B* cells	2.32 ± 0.09	0
4	10^7 T* cells + 5×10^7 B cells	2.61 ± 0.12	2.39 ± 0.11
5	5×10^7 B cells	1.72 ± 0.03	0
6	5×10^7 T cells + 5×10^7 B* cells	2.06 ± 0.08	2.42 ± 0.07
7	5×10^7 T cells	1.02 ± 0.36	0
8	5×10^7 T cells + 5×10^7 B cells	2.12 ± 0.05	1.72 ± 0.08
9	No cells injected	0.20 ± 0.15	0.25 ± 0.21

Each result is the mean of observations on six mice.

[1] Lymphocytes were as follows: T*, primed thymus cells harvested from the spleens of irradiated recipients; B*, anti-θ-treated primed spleen cells from normal mice; T, normal thymus cells; B, anti-θ-treated normal spleen cells.

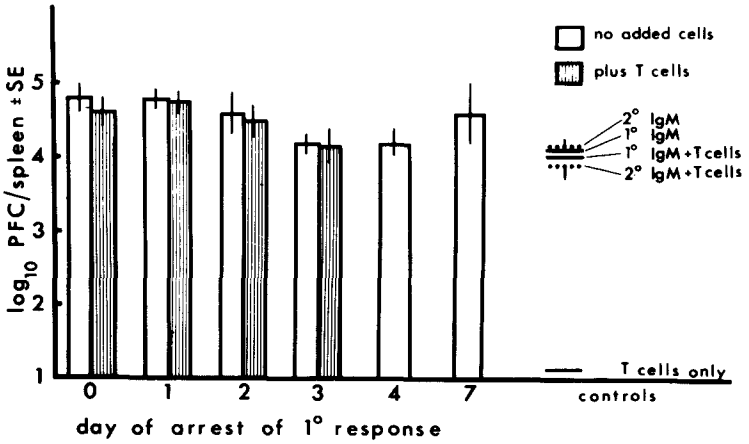

FIG. 6. Effect of primed T cells on the secondary IgM response in mice primed with 5×10^5 SRBC. T cells were injected 18 h before challenge.

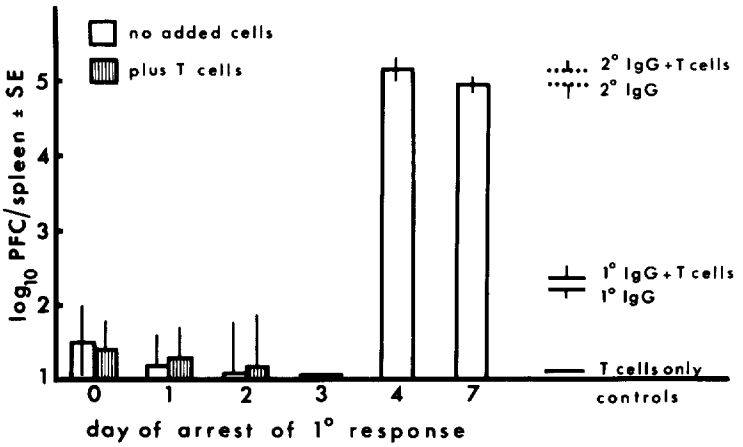

FIG. 7. Effect of primed T cells on the secondary IgG response in mice primed with 5×10^5 SRBC. T cells were injected 18 h before challenge.

addition, primed T cells were unable to improve the expression of IgG memory in those groups where blockade with CP and antibody resulted in a deficient 2° response. (Fig. 7). These findings suggested that IgG B-memory cells had not developed before day 4 of the 1° response, and that, in these experiments, the low levels of IgG PFC precursor cells was a limiting factor in the expression of a 2° IgG response.

Helper T-Memory Cell Generation Following 5 × 10^5 SRBC i.p.—The same immunization and suppression regimes were used as in the previous section. After 12 wk, 2×10^7 SRBC primed anti-θ-treated spleen cells (B cells) were injected into half of the mice whose 1° response had been blocked on days 0–3 half of the control groups, and a group of X-irradiated normal mice. 18 h later the mice were challenged as before and the PFC in the spleens were assayed after a further 3 days (Figs. 8, 9).

The presence of SRBC-primed B cells did not alter the expression of IgM memory in any group, although they did produce a substantial number of IgM PFC in the X-irradiated controls (Fig. 8). On the other hand, primed B cells had a pronounced effect on the expression of IgG memory in mice whose 1° response had been halted on day 3 (Fig. 9), raising the number of PFC to almost control levels. There was no effect on control 1° and 2° IgG responses, and the number of IgG PFC in the X-irradiated controls was very small. The restoration of the 2° IgG response in mice whose 1° response had been blocked on day 3 suggested that a helper T-memory cell population had developed by day 3, and was therefore present in advance of the appearance of IgG B-memory cells.

DISCUSSION

Sercarz and Coons (8) postulated a scheme for immune cell maturation following antigenic stimulation, according to which, antigen-sensitive cells (X

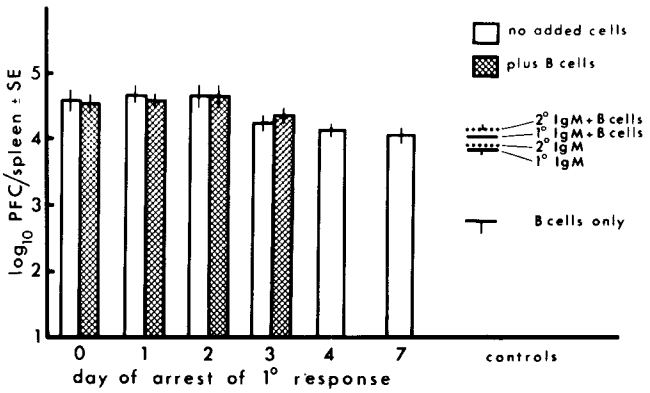

FIG. 8. Effect of primed B cells on the secondary IgM response in mice primed with 5×10^5 SRBC. B cells were injected 18 h before challenge

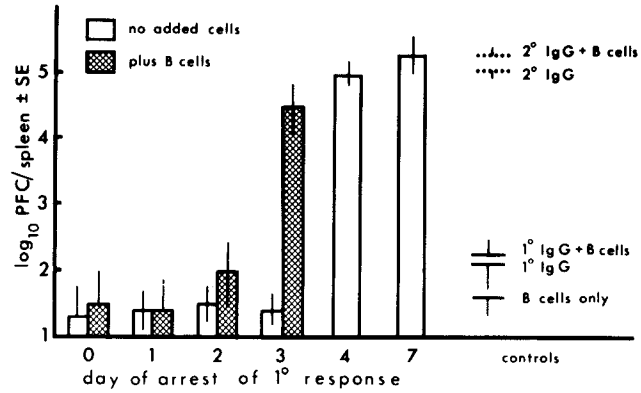

FIG. 9. Effect of primed B cells on the secondary IgG response in mice primed with 5×10^5 SRBC. B cells were injected 18 h before challenge.

cells) are first converted to sensitized Y cells. The Y cell, however, is not an efficient antibody-producing cell, but acts as a progenitor cell or memory cell that is qualitatively distinct from the antigen-sensitive cell. Further stimulation of Y cells results in proliferation which expands the Y-cell compartment before irreversible maturation to antibody secreting Z cells. The relevance of this scheme in a three cell system (IgM and IgG B cells and helper T cells) to memory generation following i.p. injection of SRBC was investigated in the present study.

1° and 12-wk 2° responses to 5×10^8 SRBC resulted in equal numbers of IgM PFC, but when the 1° response was arrested by injection of CP and antibody only 30 min after antigen, significant increases in the numbers of 2° IgM PFC were seen. It therefore appeared that IgM Y cells arose within a very short time of the exposure of IgM X cells to SRBC (8, 2, 24). This step occurred without

cell proliferation, and at a rate which was independent of the priming dose. However, the amount of available antigen did influence subsequent proliferation in the IgM Y-cell compartment before maturation to 1° Z cells, and thus controlled the size of the 1° PFC response.

The IgM Y cells were potentially long-lived lymphocytes, but since they did not take part in the normal 12 wk 2° response, it follows that in unsuppressed mice they were exhausted during the 1° response itself or during the interval before challenge. The Y-cell population was numerically stable however, during the first 4 days after priming. For instance, administration of CP and antibody on days 1, 2, or 3 after priming with 5×10^8 SRBC, resulted in a normal or slightly depressed 2° IgM response which indicated the complete loss of IgM Y cells in these animals. This presumably meant that IgM Y cells were dividing during this period, but not so as to increase the total population, since maximum priming was achieved during the first 24 h and was not surpassed at 4 days when this proliferative phase ceased (Fig. 2). Therefore, between days 1 and 3 of a normal 1° response to SRBC, the Y population "cycles," maintaining its own size, while at the same time producing IgM Z cells or PFC by cell division. This process stops in advance of the peak IgM PFC response, suggesting that further proliferation takes place in the differentiating Z population before maximum numbers of PFC are produced.

In contrast to the situation after priming with 5×10^8 SRBC, a decrease in IgM Y cells between days 1 and 3 was not seen following blockade of mice primed with the lower antigen dose. 5×10^5 SRBC resulted in a much smaller 1° IgM PFC response, so it seems likely that there was insufficient antigen in these animals to provoke extensive Y-cell division, possibly as a result of administered antibody. The IgM Y population was therefore maintained in size up to day 4. Reduction of available antigen by passive antibody would also account for the stability of the IgM-memory population in mice immunized with 5×10^8 SRBC and suppressed on day 0.

In mice which were suppressed between days 5 and 15 after priming with either dose of SRBC, there was a gradual decline to normal levels in the capacity to give a 2° IgM response at 12 wk. This may have reflected the normal run down of the IgM Y population (25), a situation which could have been detected by earlier challenge and assay of the 2° response. Nevertheless, the data do suggest that the decline was dependent upon antigen stimulation, proliferation or both of these factors, and was thus sensitive to the effects of CP plus antibody. Although the possibility cannot be excluded, it seems unlikely that Y cells were maturing to IgM Z cells at this time, as that process was associated with an earlier period of the response. The question then arises as to whether they were switched to IgG Y cells (10). If the IgG Y-cell compartment is normally balanced by gain from IgM Y cells and loss to IgG Z cells, and if both these events are susceptible to CP plus antibody, then the level of IgG memory

in mice suppressed towards the end of the 1° response should be equal to that in normal-primed animals. Our findings were in agreement with this prediction.

The rapid rise and early decline in IgM memory observed in these experiments were also suggested by the previous studies of Wigzell (25) and Sercarz and Byers (9). However, these results stand in contrast to those of Cunningham (26), who found with an adoptive cell transfer assay that IgM memory was maintained for several months after immunization of mice with 5×10^6 SRBC. It has been shown in the present study that IgM Y cells are potentially long-lived, but this property is usually only realized after priming with very low doses of antigen (24, 27). On the other hand, Cunningham (26) was unable to demonstrate IgG antibodies in his recipients of primed cells, so the apparent transcience of IgM memory in normal animals may be related to suppression of IgM synthesising (Z) cells by IgG antibody feedback (19). This suggestion is not supported by some of our own data, for instance the presence of both IgM and IgG memory in mice primed with 5×10^5 SRBC and suppressed at day 4.

One further point about the 2° IgM response is its apparent independence of any contribution by primed T cells. Transfer experiments confirmed that primed T lymphocytes were absent in mice suppressed 30 min after antigen, yet these same animals developed excellent 2° IgM responses. The relative T-cell independence of IgM responses is well known (27), but it seems from these results as if IgM Y cells can maintain themselves and differentiate to Z cells entirely on their own, unless they require the cooperation of some unidentified cell.

In contrast, the expression of IgG anti-SRBC memory requires cooperation between IgG Y cells and SRBC-primed helper T cells (7). The present studies suggested that proliferation leading to functional primed T cells was essentially complete by 3 days after antigen, and proliferation of the IgG Y-cell line by 1 day later. In view of the relative T-cell dependence of IgG synthesis (28, 29), it is possible that T cells are also involved in the development of IgG Y cells, controlling the X to Y step of an IgG progenitor cell or the differentiation of another cell type to an IgG X or Y cell. Generation of IgG Y cells was completed by 4 days after injection of SRBC, but since it was not accompanied by a decrease in IgM memory, it is unlikely that IgG Y cells developed directly from nondividing IgM Y cells. Thus, IgG Y cells must have originated from proliferating IgM Y cells or from a population independent of those involved in the 1° IgM response. In view of the evidence in favour of a T cell-mediated switch from IgM to IgG synthesis in individual B cells (10, 28, 29), the first alternative is the more appealing.

It was apparent that the generation of helper T cells required cell proliferation. This conclusion may be drawn from the observation that anti-SRBC antiserum does not prevent T-cell activation (30), yet administration of CP and specific antibody on days 0–3 of a 1° response prevented the development of IgG memory. These experiments did not reveal, however, whether continued cell division was necessary for 2° IgG responsiveness, or whether a single pro-

liferative phase on days 2–3 for T cells and 3–4 for B cells was sufficient (31, 32). It also remains possible that only T-cell proliferation was required and that simple activation of the B-cell population by primed T lymphocytes resulted in the capacity to give a 2° IgG response. Because injection of CP and antibody after T-cell memory had developed (day 3) still suppressed the preparation for a 2° IgG response, it appears that B-cell activation for IgG synthesis required some further stimulus by antigen, either directly or by way of the help of activated T lymphocytes.

The IgG B-cell population may also follow an X-Y-Z scheme of maturation, although the exact nature of the X cell remains unknown. The view is supported by observations on the relative kinetics of IgG Y-cell generation after 5×10^8 or 5×10^5 SRBC i.p. After priming with the lower dose, maximum priming for a 2° response had occurred by 4 days, while after the higher dose it took 7 days. The higher antigen dose may have driven a greater number of IgG Y cells to Z cells, a possibility supported by observations on the 1° response which showed that IgG PFC appeared contemporaneously with IgG Y cells at 4 days. This would account for the delay in the accumulation of maximum Y-cell numbers after the higher dose.

After generation of the IgG Y-cell compartment, this cell population was apparently maintained at full potential during the later stages of the normal 1° response, in contrast to IgM Y cells at that time. The data tend to imply that the IgG Y population was static, although it remains possible that some cells continued to become involved in the 1° response. In that case they may have been replaced by a cycling of the IgG Y-cell population too slow to be noticeably susceptible to CP, or by switching of IgM to IgG Y cells as previously discussed.

The early development of memory reported here agrees with the observations of other workers who have studied the 2° humoral or cellular responses to erythrocyte antigens (2, 9, 25, 26). In contrast to these results is the length of time taken for memory to develop to some protein antigens (1, 4). It remains to be determined what properties of an antigen dictate the period over which it will continue to stimulate memory cells. The development of IgM and IgG memory to SRBC occurred at different times, and generation of IgG Y cells in particular may be linked to the presence of primed T cells. Our results tend to emphasize the close relationship between 1° and 2° IgM and IgG responses which is implied by the X-Y-Z scheme, and by the observations of Pernis and his colleagues (10). They also demonstrate that the generation of Y cells is an integral part of the 1° immune response to SRBC, and that it is a matter of chance and the availability of antigen whether further differentiation to the Z-cell stage follows immediately, or after some future exposure to antigen.

SUMMARY

The kinetics of the generation of primed IgM and IgG antibody-forming cell precursors, and of helper T-cell populations, were analyzed in mice whose pri-

mary responses to high and low doses of SRBC were arrested at intervals by the immunosuppressive agents cyclophosphamide monohydrate and specific antibody. The extent to which immunological memory was established in these animals before blockade of the primary response was assessed by the hemolytic plaque assay following challenge 12 wk after priming. The presence of IgG B-memory cells and T-memory cells in suppressed mice was further investigated by the transfer into these animals of syngeneic SRBC-stimulated thymocytes or anti-θ-treated spleen cells.

It was found that the progenitors of secondary IgM-synthesizing cells were primed almost immediately after injection of antigen, and that early blockade of the primary response resulted in a raised IgM response after challenge. On the other hand, priming for a secondary IgG response took at least 4 days, and was dose-dependent, although helper T populations for a secondary IgG response appeared 3 days after antigen injection. It appeared that both IgM and IgG memory cells may be considered as Y cells in terms of the X-Y-Z scheme of lymphocyte activation, but that the two populations are generated at different times after exposure to antigen. The size of either Y-cell population at any given time is dependent upon the amount of antigen available to provoke differentiation to antibody-forming Z cells, and the IgM Y-cell population in particular is likely to be depleted during the course of a normal 1° response. When IgM Y cells were maintained for long periods as a result of immunosuppression, their secondary antibody response was independent of the primed T cells necessary for a secondary IgG response.

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