REQUIREMENT FOR CONTINUOUS ANTIGENIC STIMULATION IN THE DEVELOPMENT AND DIFFERENTIATION OF ANTIBODY-FORMING CELLS

The Effect of Passive Antibody on the Primary and Secondary Response*

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(Received for publication 13 January 1969)

The levels of both antigen and specific immunoglobulin are considered important regulatory mechanisms which determine the size of the antibody-producing cell compartment. Uhr and Finkelstein (1) demonstrated that the presence of antigen is essential for continuous synthesis of 19S antibody at any stage after its initiation. Recent studies suggest that repeated antigen contact of immunocompetent cells is required for complete differentiation (2–4). The X-Y-Z immune cell maturation scheme of Sercarz and Coons (5) is established on this assumption.

This concept of the continuous role of antigen in the immune response is consistent with the increasing body of evidence demonstrating that antigen does localize and persist in the lymphatic tissue during a primary response (6–9). One site in lymphatic tissue in which antigen has been demonstrated to be localized extracellularly is the germinal center (10–11). Although the functional significance of this antigen deposition is not completely resolved, experimental evidence demonstrates that there may be a causal relationship between the antigen localization and the presence and proliferation of the characteristic, large pyroninophilic cells of the germinal centers (8, 12). These cells by morphologic and functional parameters are considered immunologically competent cells (13–17).

We have further suggested that during the primary immune reaction the persistence of antigen in germinal centers is a dynamic process which is sensitive to and altered by the level of free specific antibody (18). Studies of Sahiar and Schwartz (19) showed that rabbits receiving heterologous passive antibody 1 hr after antigen had a marked decrease in germinal center hyperplasia during the primary response. Thus two parameters reflect antibody-mediated immune suppression, the decrease in the size of the 19S and 7S antibody-producing cell compartments (20) and decreased proliferation of pyroninophilic cells in germinal centers, this being reflected as lack of growth of the centers.

* Research jointly sponsored by the National Cancer Institute, and the United States Atomic Energy Commission under contract with the Union Carbide Corporation.

As defined in the X-Y-Z immune cell maturation scheme, the X cell is the antigen-sensitive cell which, upon stimulation, is converted to sensitized Y cells. Presumably at this stage, the Y cell is not an efficient antibody-producing cell. Triggering of Y cells by antigen results in proliferation and irreversible maturation to antibody-producing Z cells. It follows from this scheme that, during a primary antibody response in which high levels of antigen persist, there should be a depletion of the Y cell compartment because of antigen-driven maturation to Z cells. This condition favors a primary antibody response rather than establishment of secondary antibody-forming potential (memory). Conversely, low levels or short persistence of antigen would preferentially favor Y cell rather than Z cell formation. Experimental evidence supporting both such situations have been reported. Byers and Sercarz (21) have shown that Y cell exhaustion is a consequence of excess antigen. Results of several studies using minimal or suboptimal antigen doses have demonstrated good priming with little or no detectable primary response (22, 3, 23).

In the present study we attempted to induce maximum immune progenitor cell conversion (X→Y) by using high antigen doses. Subsequently, antigen was depleted at various intervals after priming with isologous specific antibody in order to interrupt further immune cell differentiation (Y→Z cell). It was reasoned that this condition would result in depression of the functional antibody-producing cell compartment as measured in the intact animal and subsequently in enhancement of the sensitized immune cell compartment as assayed in the spleen cell transfer system. These data were also correlated with a systematic study of the hyperplasia of spleen germinal centers in an attempt to better categorize the proliferating lymphoid cell compartment of these centers.

Materials and Methods

Animals.—Male $BC3F_1$ (C57B1/6 ♀ × C3H/AN ♂)F_1 specific pathogen free (SPF) mice 10–14 wk of age were used in these experiments. Mice were maintained 10 to the cage and allowed free access to food and water.

Sensitization.—Sheep erythrocytes (SRBC) were obtained sterile in Alsever's solution (Baltimore Biological Company, Baltimore, Md.), washed three times in 10–20 volumes of cold saline, and resuspended in cold saline to give 2.0×10^8 erythrocytes/ml. All antigen injections were intraperitoneal.

Specific Isologous Antiserum.—The antiserum was prepared by using the cell transfer method. Mice were primed with 2×10^8 SRBC and 30 days later spleens were removed, gently teased, and the cells suspended in Hanks' solution. Aliquots of 10^8 nucleated spleen cells were transferred intravenously, along with 10^9 SRBC, to lethally irradiated (850 R X-rays) syngeneic recipients within 6 hr after removal of the donor spleens. 14 days after transfer, recipients were bled for serum which contained 16 $\log_2$ titer units of SRBC hemagglutinin. The serum was pooled, filtered through a Millipore filter, and diluted with saline to achieve 12 $\log_2$ titer units of hemagglutinin. All injections of 1 ml antiserum in experimental animals were intraperitoneal. For control mice, normal $BC3F_1$ serum was used. The serum was pooled and diluted with saline similarly to the antiserum.

Two separate batches of isologous antiserum were prepared for these experiments. For both preparations, preliminary studies were performed to determine the half-life of the antibody in normal $BC3F_1$ mice by injecting 1 ml (12 $\log_2$ titer units) of antiserum and bleeding mice at

intervals up to 4 wk. The results showed that the half-life of hemagglutinating antibody was approximately 12 days. The antiserum was also fractionated on a sucrose gradient and individual fractions were titrated for SRBC hemagglutinin activity. Essentially all activity was found in fractions previously established to contain proteins less than 19S. Therefore the specific antibody is presumed to be primarily 7S globulin.

Serum Hemolysin and Hemagglutinin Determinations.—Individual serum samples were obtained and titrated for SRBC hemagglutinin in disposable plastic plates using a Cooke microtitrator (Cooke Engineering Co., Alexandria, Va.) by a standard 2-fold dilution technique. Veronal buffer (pH 7.2) containing 0.1% gelatin was used as diluent. The plates were read after 2 to 3 hr incubation at room temperature. Hemolysin activity (last well showing definite hemolysis) was then determined in the same plates by adding diluted guinea pig complement preparation (Flow Laboratories, Rockville, Md.) and incubating the plates after resuspending the SRBC with an automatic shaker for 1 hr at 37°C. To determine the relative amount of 2-mercaptoethenol (2-ME)–resistant hemagglutinin or hemolysin, serum was incubated in modified Veronal buffer containing 0.1% gelatin and 0.1 M 2-ME for 1 hr at 37°C. Samples were then titrated for hemagglutinin and hemolysin as described above. The 2-ME–resistant antibody was assumed to represent hemagglutinin or hemolysin, with a sedimentation rate mainly of 7S (24). The difference (total antibody activity −7S antibody activity) is considered to include the heavy, or 19S antibody.

Hemolytic Plaque-Forming Cell (PFC).—The PFC technique was used essentially as originally described (25). An aliquot from the spleen cell suspension was mixed into a 2.0 ml of a 0.7% Bacto-Agar melt in basal Eagle medium at 50°C containing 1 mg of diethylaminoethyl (DEAE) dextran and 5×10^8 freshly washed sheep erythrocytes. Within 30 sec, the mix was poured into a 15 × 100 mm Petri dish containing a base layer of 1.4% Bacto-Agar in basal Eagle medium. After incubation at 37°C for 1 hr, 1.5 ml of guinea pig complement preparation (frozen guinea pig serum preadsorbed with SRBC diluted 1:10) was poured onto the plate. At the conclusion of the second incubation period of 1 hr, direct plaques of hemolysis could be seen in the erythrocyte field.

A similar series of plates made from the same spleen cell preparations were incubated for 1 hr at 37°C after which was added 1.5 cc of a 1:100 preparation of goat anti-mouse gamma globulin serum (Hyland Laboratories, Los Angeles, Calif.). Two lots of goat anti-mouse gamma globulin serum were used in these studies and were determined in our laboratory to be 86 and 90%, respectively, inhibitory to 19S plaque formation. The plates were then further incubated for 1 hr, rinsed with basal Eagle medium and 1.5 cc guinea pig complement preparation was added. After further incubation for 1 hr, indirect plaques were observed. Estimates of number of indirect plaques per donor spleen were adjusted by subtracting the number of direct plaques per donor spleen were adjusted by subtracting the number of direct plaques shown to be not inhibited by the goat anti-mouse gamma globulin. This was done according to the method described by Wortis et al. (26).

Based on studies of Plotz et al. (27), we tentatively considered the direct plaque-forming units to be 19S hemolysin-forming units, or cells (DPFC). Within the limitations described in the above study, we will further consider the indirect plaques to be primarily 7S hemolysin-producing units or cells (IPFC). Generally duplicate plates were made for each sample. All plates were stained with approximately 2% benzidine stain to facilitate scoring of plaques. All plaques were scored at a magnification of approximately 7. The PFC count of duplicate plates multiplied by the dilution factor gave the number of PFC per recipient mouse spleen. No significant variability of donor spleen weights was measured at any of the intervals of the experiment.

Tissue Preparation and Planimetry.—The spleen was removed, weighed, and fixed in Bouin's fluid for histology. Longitudinal spleen sections were taken from the central region and stained

with hematoxylin and eosin. For the determination of surface areas of the white pulp germinal centers in each longitudinal spleen section, the image of the section was microprojected at a magnification of 120. The outline of each germinal center in a longitudinal spleen section was traced on paper and the respective areas were measured with a compensating polar planimeter. One longitudinal spleen section was measured for each of five animals killed at the appropriate intervals. At each interval, the germinal center areas were plotted graphically as the mean germinal center surface area per longitudinal spleen section.

RESULTS

Effect of Isologous Specific Antibody on the DPFC and IPFC Primary Response.—All mice were injected with 2×10^8 sheep erythrocytes at 0 time. At either 1, 2, or 4 days after antigen, groups of mice were injected with 1 ml of diluted isologous specific antiserum. The control mice received diluted normal isologous serum at 1 day after antigen. Mice from appropriate groups were killed at days 2, 3, 4, 5, 6, 10, and 12 after antigen. The results of the DPFC-IPFC assays from these mice, expressed as mean total per donor spleen, are shown in Figs. 1 *a* and *b*.

The kinetics of the normal primary DPFC and IPFC response differ from each other in two major points: (*a*) The DPFC compartment has undergone a 20-fold increase above background by 2 days after antigen while the number of IPFC is still at background at this time. This compartment decreases linearly from day 5 to day 12. (*b*) The number of DPFC decreases rapidly after having peaked between days 4 and 5, while the IPFC compartment which also reached maximum size at 5 days, remains at this level for some time.

Since the results of the antibody-induced suppression of the primary DPFC and IPFC response are essentially in agreement with previous studies, only major points will be discussed. A minimum lag phase of 48 hr occurs before the depressive effect of antibody can be detected in either the DPFC or IPFC response. This is true in all treatment groups. In terms of DPFC the only significant depression of *peak response* was obtained when antibody was injected 24 hr after antigen. In this group compared to controls, there is an approximate 10-fold suppression in peak response. The peak IPFC response is depressed approximately 400-fold in the animals injected with antibody 24 hr after antigen. When antibody was administered 2 and 4 days after antigen, the suppressive effect appears to be primarily in terms of faster disappearance of DPFC and IPFC from the spleen. Since the IPFC response in controls is maintained at high level for some time, the suppressive effect of antibody when administered at 2 and 4 days after antigen is best interpreted as an inhibition of IPFC progenitor cell recruitment.

Effect of Isologous Specific Antibody on the Growth of Germinal Centers During the Primary Response.—The results in terms of mean germinal center (GC) surface area are shown in Fig. 2. In the normal primary response to 2×10^8 SRBC,

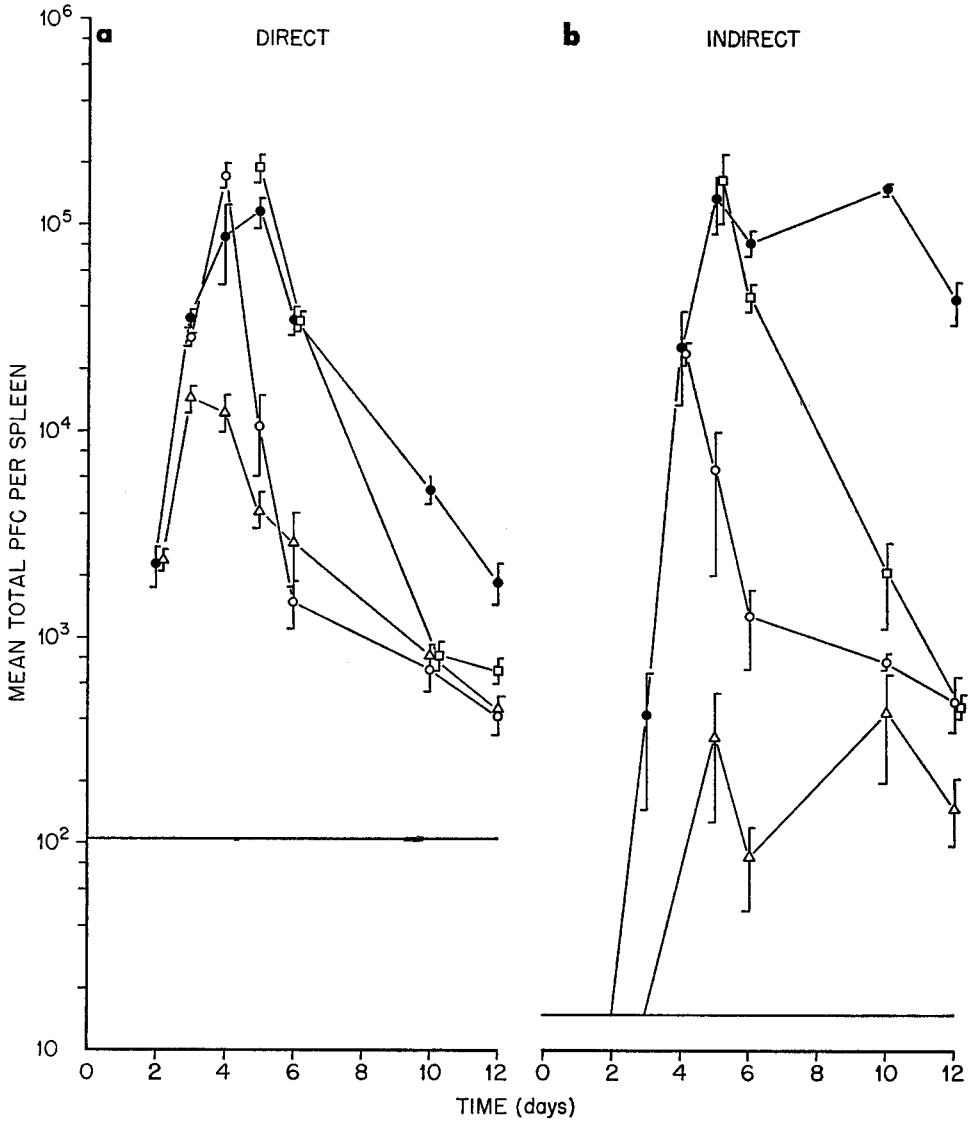

FIG. 1*a*. Mean total direct (IgM) plaque-forming cells per spleen; *b*, adjusted mean total indirect (IgG) plaque-forming cells per spleen. Each point represents the mean of 5 mice, ± 1 standard error of the mean: ●, Mice injected with 2×10^8 SRBC; △, mice injected with 2×10^8 SRBC, plus antibody day 1; ○, mice injected with 2×10^8 SRBC, plus antibody day 2; □, mice injected with 2×10^8 SRBC, plus antibody day 4. Horizontal line represents background PFC.

no sigificant increase in germinal center size occurs until 4 days after antigen stimulation with peak mean surface area being achieved at day 5. After a slight decrease, the germinal center hyperplasia was maintained in these animals to 12 days. In all passively immunized mice, there is a marked suppression of peak

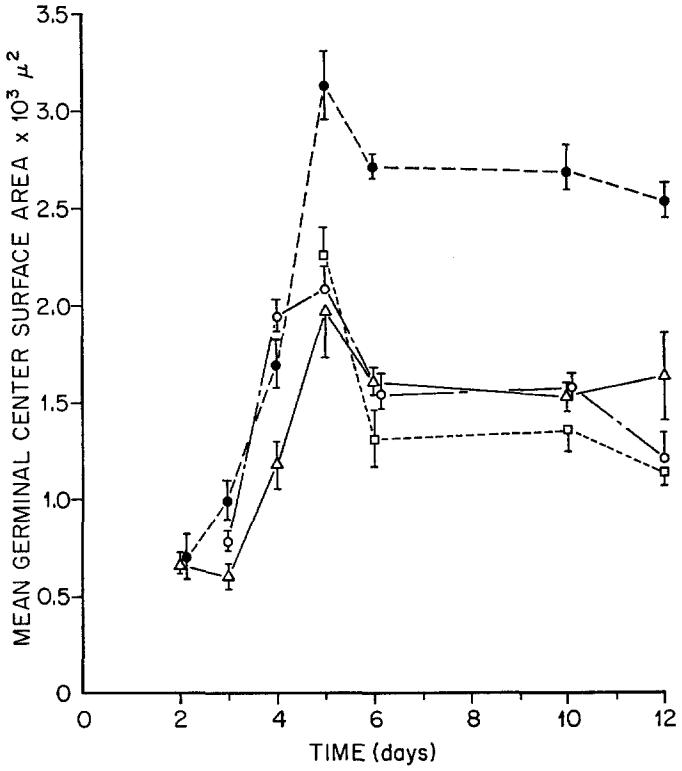

FIG. 2. Mean germinal center surface area $\times 10^3\mu^2$. Each point represents the mean of approximately 100 germinal centers, from a minimum of five longitudinal spleen sections from five mice, $\pm$ 1 standard error of the mean: ●, Mice injected with 2 $\times$ 10^8 SRBC; △, mice injected with 2 $\times$ 10^8 SRBC, plus antibody day 1; ○, mice injected with 2 $\times$ 10^8 SRBC, plus antibody day 2; □, mice injected with 2 $\times$ 10^8 SRBC, plus antibody day 4.

GC size. In animals administered antibody on day 1 and 2 after antigen, there is a minimum 48 hr lag phase before any suppression can be measured in terms of germinal center growth. This is in contrast to the minimum 24 hr lag phase measured in the 4 day passively immunized animals.

In comparing the germinal center growth and increase in number of DPFC and IPFC, during the normal primary response, it can be seen that 2 days after

antigen injection the growth of the DPFC compartment is well underway (20-fold increase above background) while the growth of germinal centers and IPFC cell compartment does not show a significant change above background. When the relative effect of passive antibody on these three parameters is compared, it was found that although the peak GC growth is equally suppressed regardless of the time of administration of antibody, the peak DPFC response is only suppressed when antibody was injected 24 hr later and the peak IPFC response when antibody was given either 24 or 48 hr after antigen stimulation.

Effect of Isologous Specific Antibody on the Secondary Immune Capacity.—Mice injected with 2×10^8 SRBC received one injection of isologous specific antibody either 1 or 4 days later; control mice received SRBC only. 1, 2, and 4 wk after priming, five mice were killed from each group, and the cell transfer method was used to study secondary immune capacity. 25×10^6 nucleated spleen cells with SRBC (1:10) were transferred into 8 to 10 lethally irradiated recipients. Recipient spleens were assayed for DPFC and IPFC at 4, 5, 6, and 7 days after transfer. To estimate the relative degree of priming achieved with the various treatments, equivalent numbers of spleen cells from unprimed animals were also transferred. Further 2×10^8 SRBC primed spleen cells transferred without antigen were also tested. The in vivo culture system is essential to this study because it provides for antigenic triggering of immune progenitor cells without the interference or complications of persisting specific antibody.

Direct Plaque-Forming Cells.—The number of DPFC on day 4 after transfer of nonprimed spleen cells (primary control) is within background range and reaches its peak on day 6 at approximately 2.0×10^4 cells (Fig. 3). When the secondary immune capacity of cells transferred at 1 wk after priming of donor mice are compared to normal unprimed cells, the priming effect is detectable as an elevated DPFC compartment size at 4 days (100-fold increase) reaching its peak at 5 days (4.5×10^4 DPFC). Thus, the peak levels of the primary and secondary response at this time are not different, although it takes nonprimed spleen cells an additional 24 hr to reach peak level.

In comparison to unprimed cells, the secondary antibody-forming potential tested at 2 and 4 wk after priming show only a 2–3-fold increase in compartment size on day 4 after transfer. Both groups reach peak levels between 5 and 6 days after transfer at 1 to 1.5×10^4 DPFC. This is significantly below the primary level as well as the secondary capacity when assayed 1 wk after priming. Thus priming does not result in an increased peak DPFC compartment size. In fact, the peak reached by the DPFC compartment is lower when the transfers are made at 2 and 4 wk after priming.

Specific antibody administered at 1 or 4 days after priming resulted in an increased secondary immune capacity whether tested 1, 2, or 4 wk after primary antigenic stimulation. When tested at 1 wk, the greatest increase in peak response ($\sim$ 3-fold) was obtained in the group receiving antibody 4 days after

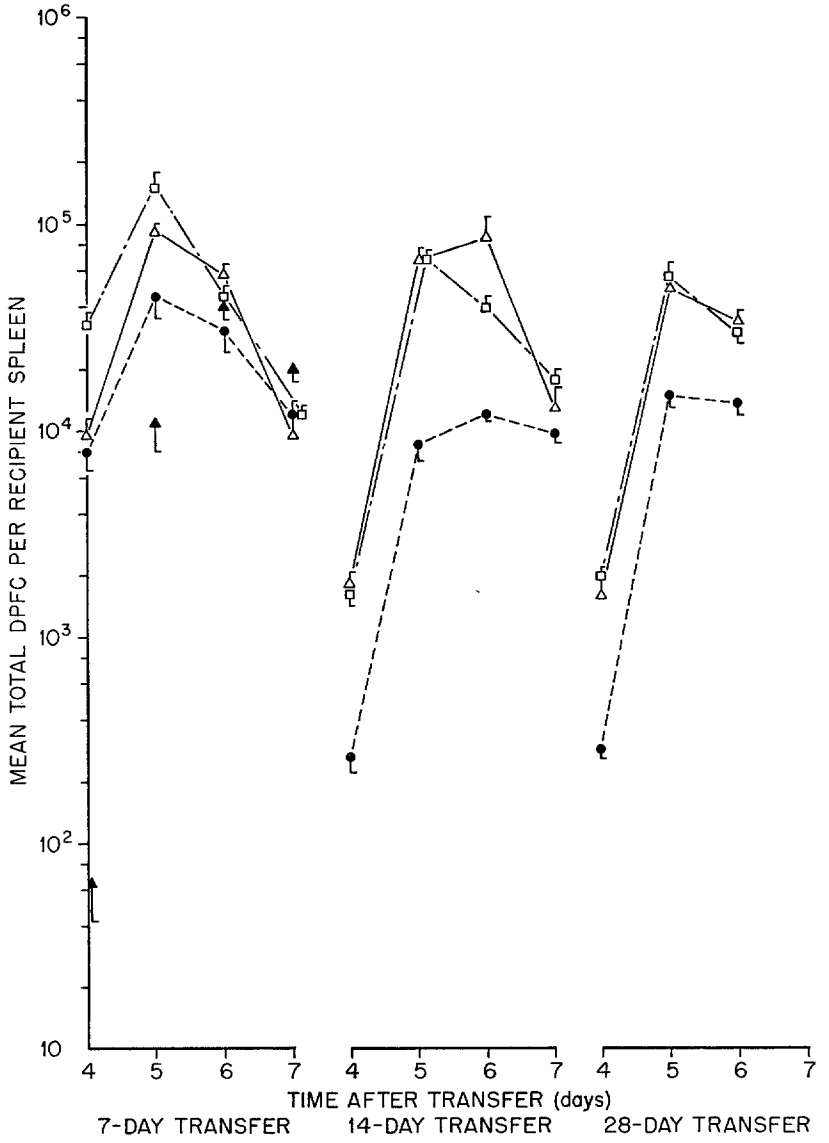

FIG. 3. Mean total direct (IgM) plaque-forming cells per recipient spleen after 7, 14, and 28 day transfers of 25 × 10^6 primed and unprimed spleen cells with antigen. Each point represents the mean of 8 to 10 recipients ± 1 standard error of the mean: ●, donors injected with 2 × 10^8 SRBC; △, donors injected with 2 × 10^8 SRBC, plus antibody day 1; □, donors injected with 2 × 10^8 SRBC, plus antibody day 4; ▲, unprimed donors.

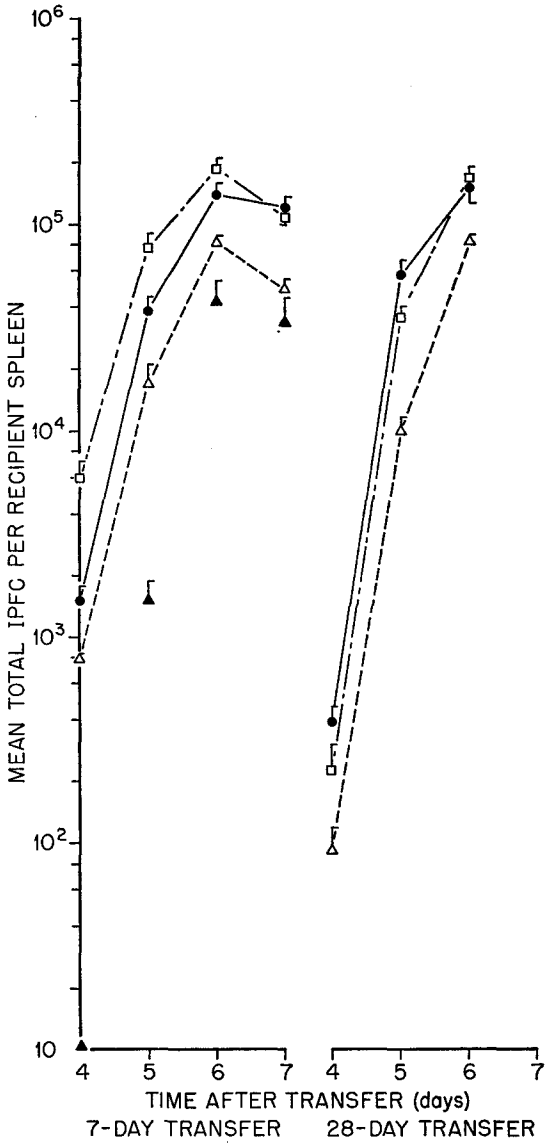

FIG. 4. Adjusted mean total indirect (IgG) plaque-forming cells per recipient spleen after 7 and 28 day transfers of 25×10^6 primed and unprimed spleen cells with antigen. Each point represents the mean of 8 to 10 recipients $\pm$ 1 standard error of the mean: ●, donors injected with 2×10^8 SRBC; △, donors injected with 2×10^8 SRBC, plus antibody day 1; □, donors injected with 2×10^8 SRBC, plus antibody day 4; ▲, unprimed donors.

priming. A 4–7-fold increase was obtained at 2 and 4 wk after priming whether or not antibody was given 1 or 4 days after antigen.

Indirect Plaque-Forming Cells.—In contrast to the DPFC, the IPFC secondary response is always higher than the primary IPFC response whether tested 1, 2, or 4 wk after priming (Fig. 4). This is true for the early response measured on day 4 after transfer as well as for peak of response, which occurs on day 6.

TABLE I

*Cellular and Serologic Assay of Secondary Immune Capacity of Spleen Cells from Passively Immunized (PID) and Nonpassively Immunized Donors (NPID)**

Time after transfer	Treatment	DPFC	IPFC	Total agglutinin‡	2-ME-resistant‡ agglutinin	Total hemolysin‡	2-ME-resistant hemolysin‡
days							
4	PID	2180	12	2.6	0.6	2.6	0
		±327	±8	±0.3	±0.2	±0.2	
	NPID	537	525	3.3	0.3	3.0	0
		±80	±178	±0.3	±0.2	±0.3	
5	PID	55842	8151	7.8	3.0	8.3	2.7
		±4517	±1327	±0.2	±0.4	±0.2	±0.5
	NPID	19521	41812	7.5	6.6	7.0	4.3
		±2756	±5125	±0.2	±0.2	±0.0	±0.3
6	PID	101207	140222	9.0	6.6	10.0	7.1
		±10948	±18096	±0.2	±0.4	±0.0	±0.1
	NPID	26850	201850	10.0	9.7	9.0	7.4
		±3657	±18640	±0.3	±0.3	±0.0	±0.2
7	PID	40671	133321	11.1	8.6	10.7	8.1
		±6735	±14165	±0.2	±0.5	±0.2	±0.2
	NPID	22350	209098	12.2	11.0	10.7	8.1
		±3631	±35971	±0.5	±0.0	±0.2	±0.1

* Each point represents mean of 8 to 10 recipient mice ± one standard error of the mean.
‡ Reciprocal of the $\log_2$ titer.

Antibody given to donor mice 24 hr after priming suppresses the secondary immune capacity when tested 1 and 4 wk after priming. However, when antibody is injected into donor mice 4 days after priming, a slight enhancement of IPFC secondary potential is measured when the transfer assay is performed 1 wk after priming. This enhancement is marked at 4 and 5 days after transfer. When transfer was performed 4 wk after priming, no significant difference was measured between the normal secondary IPFC response compared to the response of donor mice administered specific antibody 4 days after antigen.

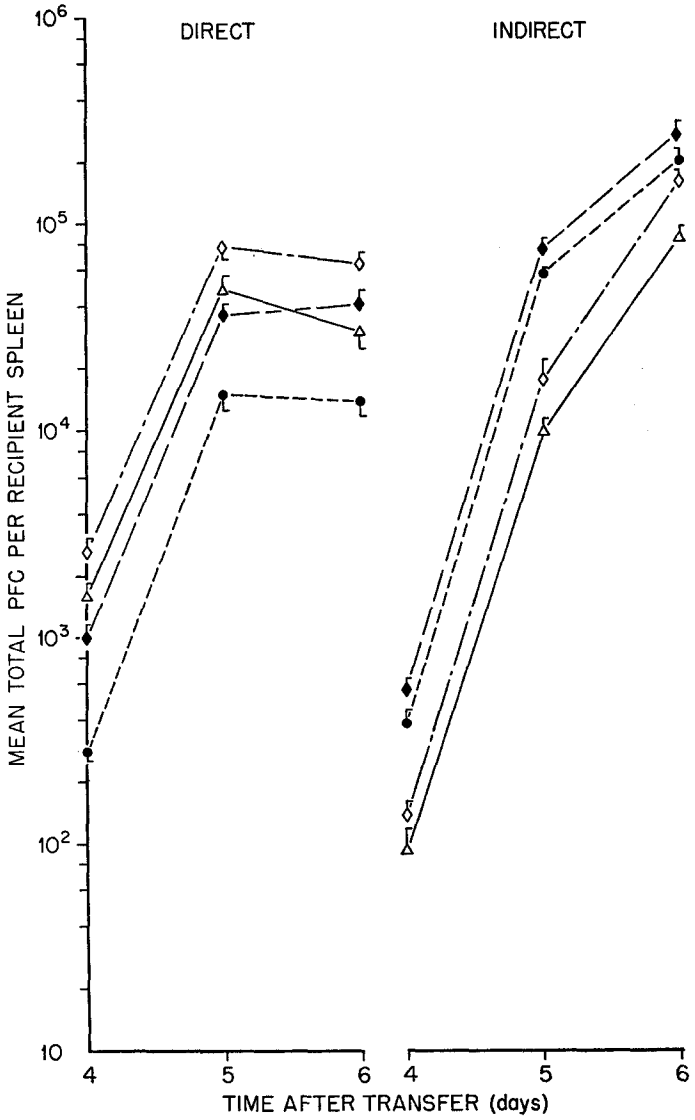

FIG. 5. Mean total direct (IgM) and adjusted indirect (IgG) plaque-forming cells per recipient spleen after 28day transfer of 25 × 10^6 primed and unprimed spleen cells. Each point represents the mean of 8 to 10 recipients, ± 1 standard error of the mean: ●, donors injected with 2 × 10^8 SRBC; △, donors injected with 2 × 10^8 SRBC, plus antibody day 1; ◆, donors injected with 2 × 10^7 SRBC; ◇, donors injected with 2 × 10^7 SRBC, plus antibody day 1.

Hemagglutinin and Hemolysin Response.—A repeat of the 28 day transfer was performed with mice primed with 2×10^8 SRBC and administered diluted specific antibody 1 day after priming. Controls received only antigen. This experiment was performed to correlate the PFC response with the serum hemagglutinin and hemolysin profiles in the recipient mice. The results are shown in Table I. It can also be seen in this experiment that there is an enhanced secondary DPFC and suppressed secondary IPFC level in 24 hr passively immunized mice compared to a normal secondary response.

No significant difference in either total hemagglutinin or hemolysin antibody was measured in recipients receiving 25×10^6 primed spleen cells from passively or nonpassively immunized donors. A marked difference was detected, however, in the amount of 2-ME–sensitive antibody in these animals. At 5, 6, and 7 days after transfer, detectable agglutinin consisted of approximately 80–95% 2-ME–sensitive agglutinin in mice receiving spleen cells from passively immunized donors. Little or no 2-ME–sensitive agglutinin was measured in the control group. This same difference was measured with the serum hemolysin assay.

Effect of Antigen Dose in Secondary Immune Capacity of Passively Immunized Mice.—Mice were primed with either 2×10^7 or 2×10^8 SRBC and 24 hr later were injected with diluted specific antibody. Control mice received only SRBC. 28 days after priming, the secondary immune capacity of their spleen cells was assayed. The DPFC and IPFC measurements at 4, 5, and 6 days after transfer are shown in Fig. 5.

At all time points measured, the DPFC response of spleen cells from donors primed with the lower antigen dose is 2–3-fold higher than that of the spleen cells from donors primed with the higher antigen dose. In both groups there is an enhancement of DPFC response in passively immunized vs. normal primed donors.

In terms of IPFC, a small but consistent increase in response of donors primed with 2×10^7 SRBC existed compared to the response in donors primed with 2×10^8 SRBC. There was at all time points an equivalent and consistent suppression of IPFC from passively immunized donors.

DISCUSSION

The effect of passive antibody on the primary response of intact mice to SRBC in this study is in agreement with previous investigations showing a marked decrease in the direct and indirect hemolysin-producing cells (20). This antibody-mediated suppression of the immune capacity has been mainly viewed as an inhibition of recruitment of the immunocompetent progenitor cells, resulting from antigen-antibody interaction (28–30). Our data indicate that this indeed may be one aspect of the suppression; however, they further suggest that some of the progeny of the antigen-stimulated progenitor cell

(X cell), as a consequence of lack of further antigenic stimulation, are forced into maturation arrest (block in Y → Z transformation). This is suggested by an enhanced Y cell (sensitized cell) compartment during the 19S secondary immune response, as measured in the transfer system.

Hege and Cole (31) showed that in *intact mice* a second injection of sheep erythrocytes between 2 and 10 wk after priming with 4×10^8 SRBC elicits a diminished DPFC response relative to that brought about by the initial injection. Also, in the present *transfer study* comparing the normal DPFC secondary response to the normal primary response, there is little or no priming effect. In fact, when transfer was performed 2 and 4 wk after antigenic stimulation of the donor, the secondary DPFC response obtained with primed cells was lower than that achieved with an equivalent number of unprimed cells. Formation of significant "19S memory" has only been described after priming with antigen doses considerably below ($\sim 10^6$ SRBC) that used in the study of Hege and Cole (31), as well as the present study (32).

The effect of passive antibody when administered as early as 24 hr or as late as 4 days after priming, to produce an enhanced IgM memory, corresponds to the effect obtained with lower antigen dose. It must be assumed that when the antigen is available X cell commitment occurs, which leads to the buildup of the Y cell compartment; however, the unavailability of the antigen as a result of passive antibody after 1 day or 4 days (according to the data of Sercarz and Byers [32], day 1 is the beginning and day 4 close to the peak of 19S memory development) prevents the further differentiation of the Y cells into functional antibody-producing cells (Z cells). Therefore, in comparison to the normal secondary response, the Y cell pool remains larger because of the lack of antigen-stimulated conversion to functional antibody-producing cells. The enhancement of the 19S Y cell compartment also correlates with an increase in 19S hemolysin and hemagglutinin production in the recipients at corresponding times.

Further support for this idea is obtained in our experiments when comparison is made between mice primed with 2×10^8 or 2×10^7 SRBC. The higher "19S memory" in the latter would be attributed to a lack of Y cell exhaustion during the primary response. The enhanced secondary DPFC compartment in the mice primed with 2×10^7 SRBC and passively immunized at 24 hr is compatible with the idea of maturation arrest of sensitized cells as a result of deprivation of the antigen.

An explanation for the lower responsiveness after secondary stimulation in nonpassively immunized animals is that this probably reflects exhaustion during the primary response of both X cells and Y cells (32). This exhaustion through conversion of Y cells to functional antibody-producing cells appears to to take place in the presence of a sufficient level of persisting antigen. Up to now this interpretation has been guarded since earlier studies in intact animals were complicated by the effect of persisting antibody on the induction of the second-

ary response. This complication is eliminated in the present transfer study.

At present it is not clear whether 7S-producing cells represent the later maturation stage of a 19S-synthesizing cell or whether two separate progenitor cell lines are involved. Studies of Nossal et al. (33), and Möller and Wigzell (29), favor the former "Unitarian concept." However, recent studies of Shearer et al. (34) raise the possibility that the two types of antibody-producing cells arise from independent progenitors. The present data are difficult to reconcile with the former (Unitarian) concept for the following reasons: (*a*) the lack of correlation between the degree of antibody-mediated 19S and 7S immune cell suppression during the primary response, the DPFC being much less depressed than IPFC; (*b*) the development of an enhanced 19S-sensitized cell compartment with the depression of the 7S-sensitized cell compartment in 1 day passively immunized mice; (*c*) the development of an enhanced 19S-sensitized cell compartment with little or no effect on the 7S-sensitized cell compartment in 4 day passively immunized mice.

In contrast to lack of "19S memory," a significant "7S memory" was obtained when spleen cells were transferred with antigen 1 or 4 wk after priming of donor mice. This sensitization effect is also detected in mice passively immunized at 1 or 4 days after priming. However, in the 24 hr passively immunized group, the secondary response is significantly depressed. This is in contrast to the enhancement of the sensitized 19S cell compartment measured in this group. In animals passively immunized 4 days after priming, a slight enhancement of 7S sensitization compared to the normal secondary was detected in the 1 wk transfer but was no longer seen at 4 wk.

It is obvious from these data that the development of "19S memory" must differ in some major respects from the development of "7S memory." This is apparent during the normal development of these sensitized cell compartments as well as during their development under the influence of passively administered antibody. In the normal intact primary response, the development of 7S antibody-producing cells lags behind the development of 19S-producing cells by 24–48 hr. It is necessary that a distinction be made between initial recruitment of progenitor cells ($X \rightarrow Y$) and maturation ($Y \rightarrow Z$) both of which are antigen-dependent. When passive antibody is administered 1 day after priming, recruitment that leads to a 19S response is already well established in contrast to recruitment that leads to a 7S response. The consequence of this is an enhanced 19S and a depressed 7S sensitization.

Day 4 after antigenic stimulation is approaching the time of peak development of 19S and 7S response. The former, however, undergoes a subsequent decay while the 7S response remains stable during the intervals studied (12 days). This probably represents a steady state condition such that the $X \rightarrow Y$ and $Y \rightarrow Z$ and disappearance of Z are relatively constant. Also, the life span of the sensitized Y cell in the absence of antigen must be considerably longer

than the life span of most functional antibody-producing cells. This dynamic equilibrium as established in the 7S response occurs at a time in which the 19S antibody response as well as 19S memory formation are decreasing. This fact strongly suggests that under normal conditions either the concentration or the form of the antigen at this time (i.e., 4 days after stimulation) is inadequate to maintain a continued 19S response but is sufficient for a continued 7S response. Thus, 4 day passive immunization has little or no effect in terms of 7S sensitization. This may be interpreted as a simultaneous blockage of further recruitment of 7S sensitized cells ($X \rightarrow Y$) as well as $Y \rightarrow Z$ transformation. This would then maintain the 7S-sensitized cell compartment in 4 day passively immunized mice, at levels comparable to nonpassively immunized mice.

In terms of both the 19S and 7S response, these results seem to indicate that the process of sensitization or priming is the consequence of an accumulation of incompletely differentiated immunocompetent cells. This can be attributed to limited availability of antigen. The secondary response then is a continuation of a primary immune reaction. Our studies also show that 19S memory cells are potentially long lived in the absence of antigen. This has been previously shown for 7S memory cells (35, 36).

Next, we will consider what relations exist between the germinal center growth and the development of 19S and 7S cellular responses. During the primary response, the growth of the direct plaque-forming cell compartment precedes the growth of the germinal centers by 24 hr, while the growth of the indirect plaque-forming cell compartment coincides with the growth of germinal centers. It can also be seen that isologous passive antibody injection results in an inhibition of germinal center growth whether or not antibody is infused 1, 2, or 4 days after priming. In terms of formation of sensitized cells, the marked depression of the 7S memory after passive immunization at 24 hr (in contrast to the enhancement of 19S memory) corresponds to the suppressed growth of germinal centers during the primary response. Thus, if the germinal center is, as has been suggested, a site of the proliferative expansion of immunocompetent cells (17, 12), these data indicate that the germinal center growth is related to the 7S cellular response. The functional significance of persisting antigen in these centers appears to be more closely related to the 7S antibody response and the development of 7S memory.

SUMMARY

The essential role of continuous antigenic stimulation in the development and differentiation of antibody-forming cells as defined in the X-Y-Z immune cell maturation scheme was examined in these studies. Mice were primed with sheep erythrocytes (SRBC) in an attempt to induce maximum immune progenitor cell conversion ($X \rightarrow Y$). Subsequently antigen was depleted at 1 or 4 days after priming with isologous specific antibody in order to interrupt further im-

mune cell differentiation (Y → Z). It was reasoned that this condition would result in depression of the functional antibody-producing cell compartment as measured in the intact mice and subsequently in enhancement of the sensitized (Y cell) compartment as measured in the spleen cell transfer system. These data were also correlated with systematic studies of the hyperplasia of the spleen germinal centers.

The effect of passive antibody on the primary response to SRBC was a marked decrease in direct and indirect hemolysin-producing cells (DPFC and IPFC). However, there was a lack of correlation in the degree of antibody-mediated 19S and 7S immune cell suppression during the primary response, the DPFC being much less depressed than the IPFC. As measured in the transfer system there was an enhanced 19S sensitized cell compartment and a depressed 7S sensitized cell compartment in 1 day passively immunized mice. This was true whether or not transfers were performed 1, 2, or 4 wk after priming. Similarly, there was an enhanced 19S-sensitized cell compartment with little or no effect on the 7S-sensitized cell compartment in 4 day passively immunized mice. These data suggest that progeny of the antigen-stimulated progenitor cells (X cell), as a consequence of lack of further antigenic stimulation, were forced into maturation arrest.

These studies further demonstrate that isologous passive antibody suppresses germinal center growth regardless of whether the antibody is infused 1, 2, or 4 days after priming. In terms of formation of sensitized cells, the marked depression of 7S sensitized cell compartment after passive immunization at 24 hr in contrast to the enhancement of the 19S sensitized cell compartment corresponds to the suppressed growth of germinal centers during the primary response. Thus, if the germinal center is, as suggested, the site of proliferative expansion of immunocompetent cells, these data indicate that the germinal center growth is related to the 7S antibody response and in the formation of "7S memory."

The authors are grateful to Dr. E. H. Perkins for technical advice during the course of these studies.

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