RECEPTORS ON IMMUNOCOMPETENT CELLS

V. Cellular Correlates of the "Maturation" of the Immune Response

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A particularly intriguing facet of the immune response to many antigens is the maturation process; that is, the gradual increase in average binding affinity of serum antibody for the antigen with increasing time after immunization (1). This phenomenon has been interpreted as resulting from a microevolutionary process involving the antigen-driven selection of precursors of antibody-forming cells having receptors of highest affinity for antigen (2). Thus, as the antigen concentration falls, only cells with receptors of sufficiently high affinity to capture antigen would be stimulated to proliferate and/or secrete antibody.

This maturation phenomenon has been analyzed primarily at the level of the affinity of antibody present in the serum and conclusions concerning the cellular events have depended upon inference. However, several studies have indeed established that these affinity changes in the serum reflect changes in the population of antibody-synthesizing cells. Thus, Steiner and Eisen (3) have demonstrated that newly synthesized antibody produced in vitro by lymph node suspensions obtained from recently immunized rabbits is of low affinity, and that antibody produced by cells derived from rabbits immunized long before sacrifice is of high affinity. Similarly, Andersson (4), by analyzing the concentration of bovine serum albumin (BSA)[1] required to inhibit individual plaque-forming cells (PFC), also observed a time-dependent avidity increase on the part of antibody-secreting cells in the mouse. Studies of precursors of antibody-synthesizing cells, the cells upon which the postulated selective forces are believed to act, have not yet been extensively reported. Bullock and Rittenberg (5) have observed that the concentration of trinitrophenyl (TNP)–keyhole limpet hemocyanin required to stimulate secondary responses optimally in vitro diminished with time after immunization, suggesting an increased affinity of an antigen recognition unit. However, it is not clear whether the increase occurred in the precursors of antibody-synthesizing cells or, alternatively, in the "helper" or thymus-derived (T) lymphocyte population. The latter cells, although they do not secrete antibody, are either required for or markedly enhance the activation of precursor cells by antigen.

We have recently described the existence of 2,4-dinitrophenyl (DNP)–specific antigen-binding lymphocytes in the guinea pig (6). The bulk of these cells are not

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[1] *Abbreviations used in this paper:* ABC, antibody-binding cell(s); BSA, bovine serum albumin; CFA, complete Freund's adjuvant; DNP, 2,4-dinitrophenyl; GPA, guinea pig albumin; MEM, minimum essential medium; PFC, plaque-forming cell(s); T, thymus-derived; TNP, trinitrophenyl.

antibody secreting; they bear surface immunoglobulin molecules and their receptors are hapten specific. These cells increase markedly in frequency upon immunization (7), and, under certain conditions of tolerance, they are diminished in number (8). Removal of these cells from a population reduces the ability of that cell population to transfer DNP-specific antibody responsiveness to an irradiated syngeneic recipient (6). Because of the large amount of surface immunoglobulin which these cells bear, their presence in the bone marrow, absence from the thymus, and the high degree of hapten specificity of their receptors, they appear to be lymphocytes of the bone marrow–derived series and to include the antibody-forming cell precursors.

Our previous studies demonstrated that the concentration of ϵ-DNP–L-lysine required to block DNP-guinea pig albumin–^{125}I (DNP-GPA) from binding to antigen-binding cells (ABC) diminished with time after immunization (7) and that the equilibrium constant of DNP–ligand–cell interaction increased with time after immunization (9). This established an increase in the affinity of cell surface receptors possessed by DNP-specific ABC.

In this paper we analyze the avidity of the DNP-specific receptors on ABC, of the anti-DNP antibody secreted by individual PFC, and of serum anti-DNP antibody at various times in the course of the immune response. We show that average avidity for hapten of the antigen-binding units in these three compartments increases at generally similar rates, providing strong evidence for the cellular basis of "maturation" and indicating that the selectional process operates on the precursors of antibody-forming cells. Moreover, the nature of the changes in the PFC population provides insight into the cellular dynamics of the maturation process.

Materials and Methods

Animals and Immunization.—Adult strain 13 guinea pigs were obtained from the Division of Research Services of the National Institutes of Health. All animals were immunized by injection, into the four footpads, of an emulsion of 50 μg of DNP-GPA in complete Freund's adjuvant (CFA) (Difco Laboratories, Detroit, Mich.). At various times after primary immunization, a few animals received injections in the footpads of 0.4 ml of an emulsion of incomplete Freund's adjuvant alone or of incomplete adjuvant containing 50 μg of DNP-GPA. Guinea pig albumin was prepared from guinea pig serum by the method of Schwert (10) and was conjugated with 1-fluoro-2,4-dinitrobenzene (Eastman Organic Chemicals, Rochester, N. Y.). The DNP-GPA contained an average of 23 dinitrophenyl groups per molecule.

Preparation of Cell Suspensions.—At timed intervals after immunization, animals were exsanguinated and draining lymph nodes were removed. Cell suspensions were prepared by teasing the tissues in minimum essential medium (MEM, Grand Island Biological Co., Rockville, Md.) supplemented with 10% fetal bovine serum (Industrial Biological Laboratories, Rockville, Md.). The suspensions were passed through gauze pads to remove aggregates; the cells which passed through were washed three times.

Detection of ABC and Determination of their Relative Avidity for Hapten.—DNP-GPA–^{125}I, prepared by the chloramine-T method (11), had a specific activity of 56 μCi/μg. Details of the ABC assay have been presented elsewhere (6). Lymph node cells (20–40 $\times$ 10^6) were suspended in 0.2 ml of cold MEM supplemented with 10% fetal bovine serum and sodium azide (1 mg/ml). In addition, ϵ-DNP–L-lysine was included in the medium at varying con-

centrations. Generally, each cell population was suspended in final concentrations of 1×10^{-2}, 10^{-4}, 10^{-6}, and 10^{-8} M DNP-lysine, although cells obtained long after immunization were exposed to 1×10^{-10} M in addition. Immediately after the cells were suspended in the hapten solution, 40 ng of DNP-GPA-^{125}I was added. After 30 min incubation in an ice bath, the cell suspension was diluted to 1 ml with fresh medium and layered over 3 ml of fetal bovine serum in a 15 ml plastic tube. The cells were then sedimented through the serum by centrifugation at 1000 rpm for 10 min. The washing sequence was repeated three times. The total quantity of antigen adsorbed to cells was measured by the total radioactivity of the cell pellet. Portions of the cell suspension were smeared on gelatin-coated slides, air dried, and fixed in 1% glutaraldehyde. Radioautographs were prepared using NTB-2 Nuclear Track Emulsion (Eastman Kodak Co., Rochester, N. Y.) and an exposure time of 1 day. The cells were stained with methyl green–pyronin.

An ABC was defined as any intact lymphoid cell which had 5 grains or more directly over it or on its margins. When the frequency of ABC was low, the total number of cells scanned was estimated using a 10×10 mm ocular grid at 540 magnification. The average number of cells per grid was initially determined and the total number of cells evaluated was estimated by scanning given numbers of grid fields. In those cases where the frequency of ABC was higher, cells were individually scored for silver grains.

The relative avidity for hapten of ABC was determined as follows. The frequency of DNP-GPA-^{125}I binding cells when exposed to antigen in medium containing various concentrations of free hapten was compared to the frequency obtained in the absence of hapten. The per cent inhibition by hapten was plotted as a function of the log of hapten concentration. The concentration of DNP-lysine which caused 50% inhibition (I_{50}) of the frequency of DNP-GPA-^{125}I binding cells was considered a measure of avidity for hapten of the cell receptors. This is based on the fact that antigen-binding receptors of high affinity may be saturated by low hapten concentrations whereas low affinity receptors require high hapten concentration for saturation. It was also possible to determine the inhibition of antigen binding by hapten by measuring the total amount of antigen bound to the cell pellet before radioautography. This method was feasible only when the frequency of ABC reached about 1% or more. While this method of determining hapten avidity was accurate and simple, only results from the direct evaluation of antigen binding by radioautography will be presented in this paper.

Detection of Anti-DNP Facilitated PFC and the Determination of the Relative Avidity for Hapten of the Secreted Antibody.—Anti-DNP antibody-secreting cells were measured using a modification of the hemolytic plaque technique. Sheep erythrocytes were conjugated with 2,4,6-trinitrobenzenesulfonic acid (12), and rabbit polyvalent anti–guinea pig immunoglobulin antiserum was used for facilitation of hemolysis. Only facilitated plaques were measured, since direct PFC were totally inhibited by this antiserum. A method for determining the relative avidity for hapten of the PFC, based on the method of Andersson (4), was used. Varying concentrations of free DNP-lysine ranging from 1×10^{-3} to 1×10^{-9} M were included in the agar. Antibody of high affinity would be bound by low concentrations of free hapten and prevented from lysing TNP-sheep erythrocytes. Conversely, antibody of low affinity would require high concentrations of free hapten in order to be blocked from forming plaques. Therefore, the distribution of PFC of various relative avidities for hapten could be determined.

For comparison, the murine plasmacytoma, MOPC-315, was tested for the hapten inhibition of PFC formation. MOPC-315 cells produce a homogeneous IgA protein with antibody specificity for TNP groups (13, 14), and therefore should provide a reasonable standard for the method. MOPC-315 (a gift from Dr. Michael Potter) was maintained in the ascites form by serial transfer in BALB/c mice. Hemolysis in gel was facilitated by rabbit anti-MOPC-315 antiserum.

Analysis of Serum Antibody.—The average association constant (K_o) of each serum was

determined by the modified Farr test as described by Stupp et al. (15). The antibody concentration was determined by extrapolation of the value of hapten bound to infinite hapten concentration.

RESULTS

Change in Frequency of ABC, PFC, and Concentration of Antibody after Immunization.—The change in frequency of DNP-GPA ABC and anti-DNP
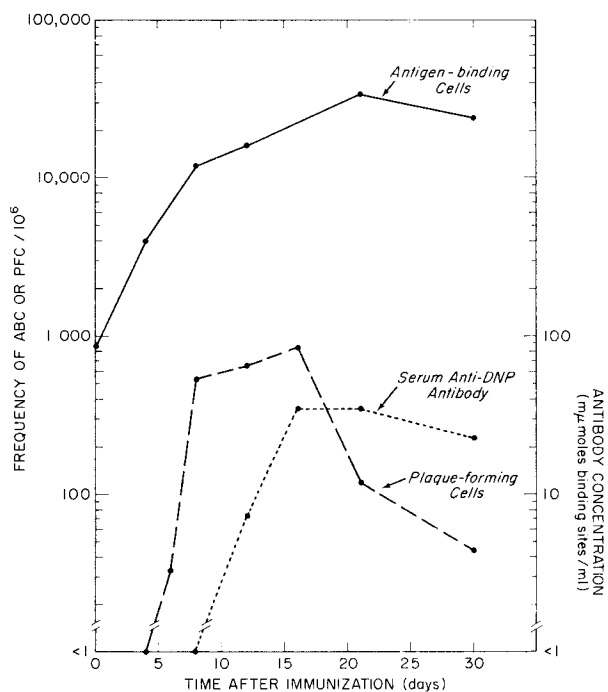

Fig. 1. Change in frequency of DNP-GPA–^{125}I ABC and anti-DNP PFC and in concentration of serum anti-DNP antibody in guinea pigs after immunization with 50 μg of DNP-GPA in CFA. Each point is the average value obtained from two animals.

PFC in draining lymph nodes and the increase in serum anti-DNP antibody is shown in Fig. 1. Each point represents an average of two animals. The change in frequency of ABC in lymph nodes is similar to that shown previously for the frequency of these cells in the peripheral blood (7). A rapid increase in the frequency of ABC occurs before the appearance of either anti-DNP PFC or serum antibody. Indirect PFC appear about 6 days after immunization, reach peak levels between days 8 and 16, and then rapidly decrease in frequency. While some ABC may secrete antibody, the fact that the frequency of ABC is always at least 20-fold greater than that of PFC indicates that the great majority of ABC are not secreting significant amounts of antibody. De-

TABLE I

Change in Avidity for Hapten of DNP-GPA-^{125}I ABC after Immunization

Time after immunization	Frequency of ABC (per 10^3 lymphocytes)	Log I_{50} DNP-lysine
days		
0	0.85	−3.00
	0.80	−3.05
	0.21	−2.46
		Mean −2.84
8	11.9	−3.25
	10.2	−3.15
	19.4	−3.70
		Mean −3.37
12	15.0	−5.47
	17.6	−5.31
	49.9	−5.40
		Mean −5.39
21	32.7	−7.30
	24.6	−6.15
	36.9	−6.52
		Mean −6.62
29	36.9	−9.00
	13.3	−8.22
		Mean −8.61
30	16.0	−8.70
	32.3	−8.44
		Mean −8.57
171 (No secondary challenge)	2.0	−9.00
171 (7 days after secondary challenge)	7.8	−8.30

tectable antibody appears in the serum several days after the appearance of PFC and reaches a peak value later than the PFC peak. Moreover, serum antibody concentration remains relatively constant during the period in which PFC frequency declines. Direct PFC (not shown in Fig. 1) appear on day 4, reach a peak on day 8 (70 PFC/10^6 cells), and then rapidly decline in frequency.

Change in Avidity for Hapten of ABC.—The DNP-GPA ABC present in guinea pigs before immunization were found, as before (6), to require high concentrations of hapten to block DNP-GPA-^{125}I binding. The cells tested in the present study required between 0.9 and 3.5 $\times$ 10^{-3} M DNP-lysine to block half the ABC from binding radiolabeled antigen. As shown in Table I, the I_{50} value for DNP-lysine inhibition of antigen binding changes dramatically after immunization. After an initial lag period of 8 days the avidity of the cellular receptors changes rapidly and steadily until, by day 29 or 30, the I_{50} is approximately six orders of magnitude lower than the concentration required before
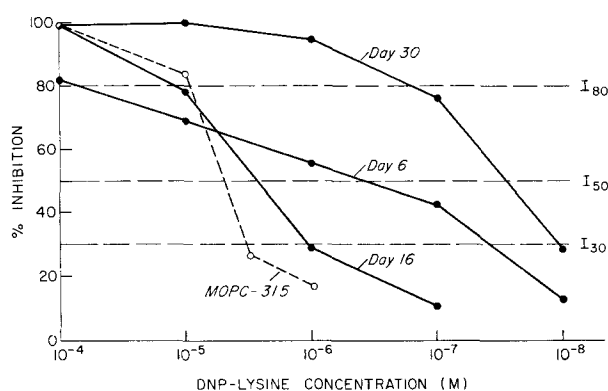

Fig. 2. Profiles of inhibition by ϵ-DNP-L-lysine of anti-DNP PFC. Anti-DNP PFC in lymph node cells from guinea pigs immunized 6, 16, and 30 days previously with 50 μg of DNP-GPA in CFA and MOPC-315 myeloma cells were inhibited by various concentrations of ϵ-DNP-L-lysine incorporated into the agar-TNP-erythrocyte suspension. The I_{30}, I_{50}, and I_{80} inhibitory concentrations were calculated.

immunization. Two animals were examined 6 months after they had been immunized. One animal received incomplete adjuvant and 50 μg of DNP-GPA in the footpads 7 days before sacrifice; the other received only adjuvant at this time. The animal that received only adjuvant had low levels of ABC whereas the animal that had been challenged had a four-fold higher frequency of ABC. In both animals, the avidity of the ABC was very high, comparable to that seen at 30 days after primary immunization.

Change in Avidity for Hapten of PFC.—Antigen-binding lymphoid cells, which appear to contain the antibody-forming cell precursors, undergo marked population changes which result in the apparent replacement of cells with low avidity receptors with cells having high avidity receptors. If the ABC are indeed the precursors of the antibody-secreting cells, similar changes in avidity of mature antibody-secreting cells should be seen also.

The observation by Pasanen and Mäkelä (16) that free hapten could compete with hapten-erythrocyte complexes for antibody in the hemolytic plaque assay provided a means of determining the relative avidity for hapten of anti-

bodies being actively secreted by single cells. We utilized this technique to determine the avidity for hapten of anti-DNP PFC using DNP-lysine as the inhibitor and TNP-sheep erythrocytes as the indicator ligand.

The changes in PFC characteristics may be presented in terms of cumulative per cent inhibition at any ligand concentration. The inhibition profiles of PFC from three representative lymph node populations are shown in Fig. 2 where the per cent of inhibition is plotted *versus* the hapten concentration. Included here is the inhibition profile of plaques formed by MOPC-315, a murine myeloma tumor which produces an IgA protein with TNP-binding capacity. The earliest PFC (day 6) have inhibition curves with very shallow slopes when compared to either day 16, day 30, or MOPC-315 PFC. This undoubtedly represents a larger heterogeneity of avidities in the early PFC. On the other hand, while similar in the degree of heterogeneity, day 16 and day 30 PFC are quite different in the concentration of hapten required for a given degree of inhibition. Therefore, to compare the inhibition profiles of PFC from various times, two parameters are measured. The concentration of hapten necessary to inhibit 50% of the PFC (I_{50}) will be used to indicate average avidity. The reciprocal of the I_{50} of MOPC-315 PFC (1.9×10^5) is fairly similar to its K_o for ϵ-DNP-L-lysine at $37°C$ (1.0×10^6). This relationship was noted previously by Yamada et al. (14). The ratio of the concentration of hapten which gives 80% inhibition (I_{80}) to that which gives 30% inhibition (I_{30}) will be used to measure the degree of heterogeneity of avidities. A low value for this ratio would indicate limited heterogeneity, whereas a high value would indicate a high degree of heterogeneity. The values for MOPC-315 and day 6 PFC are 2.4 and 2120, respectively, indicating that this ratio does, indeed, reflect the degree of heterogeneity.

Fig. 3 demonstrates the changes seen in the inhibition profiles of PFC during the first 30 days after immunization. From 6 to 12 days there occurs a striking decrease in the degree of heterogeneity of PFC as well as a fall in the average avidity. From 12 to 30 days, the degree of heterogeneity remains constant while the average avidity steadily increases.

The avidity distribution of populations of PFC may better be determined on the basis of the difference in the number of plaques inhibited by any two ligand concentrations. Thus, the highest avidity PFC are those inhibited by 10^{-7} M ϵ-DNP-L-lysine (the difference in PFC number with no inhibitor and that with 10^{-7} M ligand); in the next highest avidity group are those inhibited by 10^{-6} M ϵ-DNP-L-lysine but not by 10^{-7} M. By determining the incremental number of PFC which are inhibited by 10-fold changes in ligand concentration from 10^{-7} to 10^{-4} M, the avidity distribution of the PFC was analyzed. The absolute numbers of PFC inhibited by various concentration ranges of DNP-lysine is shown in Fig. 4. It is seen that the earliest PFC (day 6) are distributed among a wide range of avidities and thus are highly heterogeneous as was evident from the I_{80}/I_{30} ratio. The rapid increase in frequency of PFC seen

between days 6 and 8 is an increase in PFC of all avidities. Days 12 and 16, however, are characterized by striking changes in the distribution of avidities, in spite of the fact that only modest changes occur in the total number of PFC. PFC of both high and low avidities decrease while those of intermediate avidity continue to increase. The rapid loss in total PFC which occurs after day 16 is seen to involve primarily a loss in PFC of lower avidity and a relative preser-
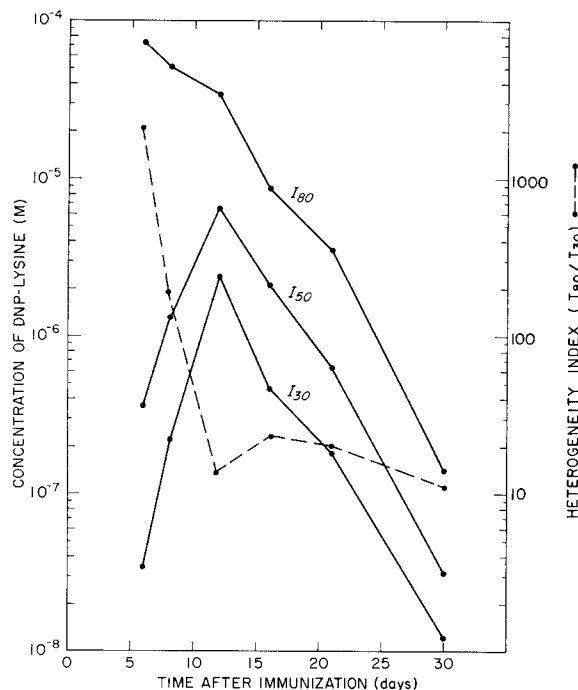

FIG. 3. Change in inhibitory concentrations of DNP-lysine for anti-DNP PFC. The I_{30}, I_{50}, and I_{80} inhibitory concentrations of DNP-lysine (see Fig. 2) at various times after immunization are plotted. The dashed line denotes the heterogeneity index (I_{80}/I_{30}) at each time period. Each point is the average value obtained from two animals.

vation of those of higher avidity. It is clear that the highest number, in absolute terms, of high avidity PFC has been achieved by day 8. The analysis, as in Fig. 4, of the avidity distribution of PFC demonstrates that "maturation" in this system is primarily due to a relatively stable level of high avidity PFC in the face of a marked diminution in number of low avidity PFC rather than to a sequential appearance of PFC of increasing avidity.

It is well known that antibody produced during a secondary response is of high avidity and generally does not proceed through a period of maturation comparable to that seen during the primary response. What is less clear, however, by considering serum antibody alone, is whether secondary challenge

results in the synthesis of any low avidity antibody. Fig. 5 presents the avidity profiles of late primary (30 days after initial immunization) and early secondary PFC. It can be seen that, while not identical, the two profiles are very similar. Clearly, the antibody produced 7 days after a secondary immunization resembles in distribution of PFC the fully mature response seen during a late primary response rather than that of an early primary response; nonethe-
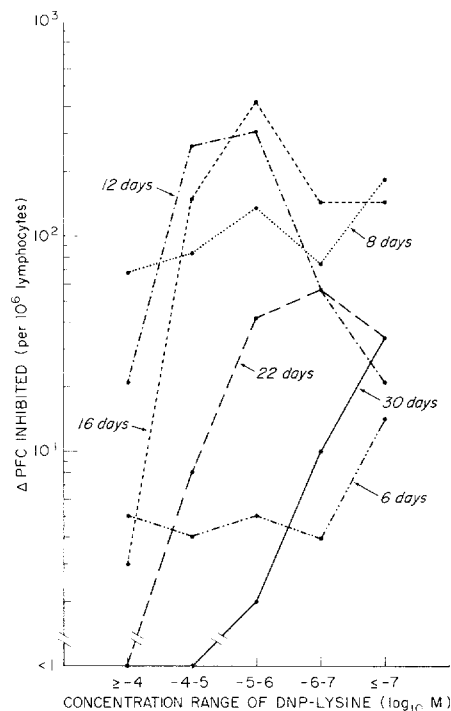

Fig. 4. Frequency of PFC in various avidity subgroups during primary response. Anti-DNP PFC of various avidities were determined (see Results) at various times after immunization.

less, a small number of low avidity PFC are present in the cells of the boosted animal which are absent from the cells of the "late primary" animal.

Change in Affinity for Hapten of Serum Anti-DNP Antibody.—Classically, maturation of the immune response has been measured by the change in affinity of serum antibody for antigen. Table II summarizes the binding characteristics of the serum antibody from the guinea pigs studied here. No antibody was detected in the serum on days 6 or 8 when tested by the Farr assay. By day 16, however, the total antibody concentration was quite high, and remained so for at least the next 30 days. However, the average association constant gradually increased from 2×10^6 on day 12 to 50-100 $\times$ 10^6 on day 30. This 25-50-

fold change is comparable to the changes seen by other investigators (1, 2). While ABC and PFC have reached maximum avidities by day 30, it can be seen that further changes in the serum antibody occur after this time. 6 months after immunization, the affinity is 6.2×10^8 and with secondary challenge reaches 3.2×10^9. Both the Sips index of heterogeneity of serum antibody
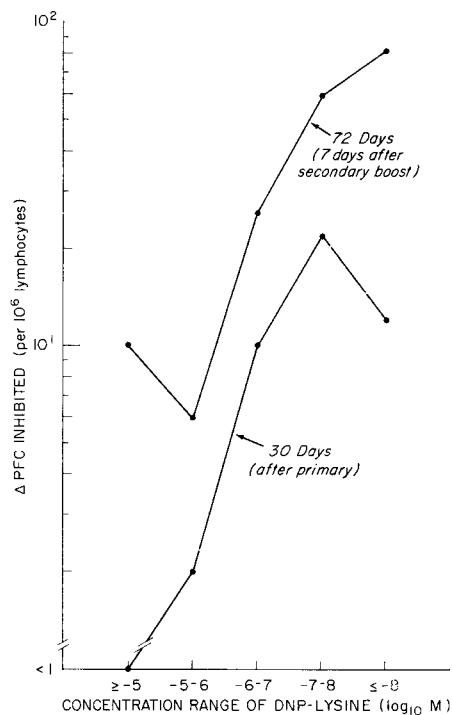

FIG. 5. Comparison of late primary and early secondary anti-DNP PFC. The avidity profiles of anti-DNP PFC from animals immunized 30 days earlier with 50 μg of DNP-GPA in CFA were compared to those from an animal immunized 72 days earlier with 50 μg of DNP-GPA in CFA and boosted 7 days before assay with 50 μg of DNP-GPA in incomplete Freund's adjuvant.

and the PFC heterogeneity index (I_{80}/I_{30}) are relatively stable over this period except that both reveal a greater degree of heterogeneity in the PFC and antibody of the boosted animal.

Comparison of Rates of Change of Avidity for Hapten of ABC, PFC, and Serum Antibody.—We have demonstrated here that maturation occurs at the level of the ABC as well as the PFC and serum antibody molecules. The relative changes in avidity for hapten of ABC, PFC, and serum antibodies are shown in Fig. 6. Except for the early portion of the primary response (before day 12), the rates of change of ABC and PFC are nearly identical. We have

shown that the early portion of the PFC response is complex and not easily characterized by an "average" value. Furthermore, the rate of change of the serum antibody affinity is similar, although somewhat less dramatic, than the cellular changes. This is not unexpected because of the differences in half-lives of the cells compared to the serum antibody.

TABLE II

Change in Antibody Characteristics with Time after Immunization

Time after immunization	Antibody concentration	Average association constant log K_o	Sips index of heterogeneity a^*	PFC heterogeneity index (I_{80}/I_{30})
days	*mµmole binding sites/ml*			
6	—‡	—	—	2120
8	—	—	—	58
				321
12	7.4	6.29	0.80	14
16	28.8	6.55	0.65	11
21	40.0	6.43	0.80	35
	51.2	6.21	0.85	17
	17.5	7.33	0.75	22
30	28.5	7.70	0.80	12
	17.0	8.01	0.85	10
45	50.0	7.39	0.80	N.D.§
	47.0	7.41	0.80	N.D.
171	1.7	8.80	0.80	—
(No secondary challenge)				
171	10.0	9.51	0.60	180
(7 days after secondary challenge)				
MOPC-315	—	6.56‖	1.03‖	2.4

* Sips indices were calculated from the expression

$$\log r/(n - r) = a \ \log K_o + a \log c$$

where r = ratio of bound ligand molecules to total antibody molecules, n = valence of antibody, c = free ligand concentration.

‡ Insufficient material for analysis.

§ N.D. = not done.

‖ Stupp et al. (15).

DISCUSSION

It is widely held that the production of humoral antibody after immunization results from the antigen-mediated stimulation, proliferation, and maturation of antibody-forming cell precursors. While numerous studies have demonstrated that various classes of immunocompetent cells, such as antigen-binding cells and plaque-forming cells, increase in number after immunization, the possible interrelationships of such subclasses have been conjectural. Indeed,

the identification of antibody-forming cell precursors, a class of cells which is central to selectional theories of immunity, has only recently been accomplished (17).

Our study demonstrates a very large change in the apparent avidity of the receptors of ABC during the course of the immune response. Thus, within the first 30 days a six order of magnitude diminution in the concentration of univalent ligand required to inhibit binding of DNP-GPA–^{125}I occurs. After day 12, proportional changes occur in the avidity of the cell surface receptors of ABC and in that of the antibody produced by mature antibody-secreting cells.
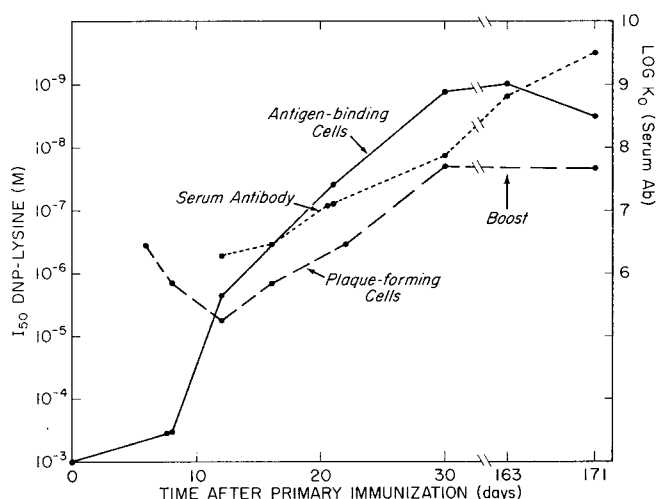

Fig. 6. Change in avidity for DNP-lysine of ABC, PFC, and serum anti-DNP antibody with time after immunization. The I_{50} values for DNP-lysine inhibition of ABC and PFC, and the log K_o of serum anti-DNP antibody are plotted *versus* time after primary immunization with 50 µg of DNP-GPA in CFA. At 163 days, animals received 50 µg of DNP-GPA in incomplete Freund's adjuvant in the footpads.

This finding provides indirect evidence for a common ancestry of these two subclasses of lymphoid cells.

Assuming then, that PFC are descendants of ABC, several conclusions are forthcoming from the present study. First, high avidity PFC are present when the indirect PFC are first detected. This strongly suggests that high avidity precursor cells exist before immunization, even though their frequency may preclude detection by direct means. In turn, this implies that somatic mutational events after antigen stimulation are not crucial to the generation of high avidity receptors and antibody molecules. Second, maturation of serum antibody results from the gradual loss of low and middle avidity PFC and the persistence of high avidity PFC. This process among PFC is paralleled by the gradual increase of the receptor avidity of ABC. It is most likely that the cellu-

lar basis of maturation lies in a competition for antigen among the ABC. Thus, as antigen becomes limiting, only receptors of high avidity can trap antigen and thereby an affinity-dependent stimulation and proliferation occurs. In addition, the appearance of serum antibody undoubtedly contributes to the selectional process in that free antibody can compete with cell receptors for available antigen and further diminish the ability of ABC bearing low avidity receptors to bind antigen.

At this point, it may be appropriate to examine the methods for measuring relative avidity of ABC and PFC. Both methods involve the inhibition of antibody reactions with multivalent antigens by monovalent ligands. Since ABC receptors can "cooporate" to bind single DNP-GPA molecules by binding more than one DNP group (9), hapten must occupy most of the receptors for a large fraction of the time to inhibit DNP-GPA–^{125}I from binding. It is not surprising that large molar excesses of hapten are needed to block ABC receptors of low affinity from binding DNP-GPA. It is probable that the valence effects are less important when receptor affinity is very high, i.e., when monovalent hapten is held very tightly. This is suggested by the fact that the concentration of hapten needed to inhibit half the ABC from binding detectable DNP-GPA–^{125}I approaches at 30 days the molar concentration of DNP-GPA–^{125}I.

Possible complications to the interpretation of the inhibition of antigen-binding data would be introduced if the density of cell receptors changed during cellular proliferation, since cooperative binding of multivalent antigens might be possible only when the receptors have certain spatial relations. It might be suggested that the changes seen reflect changes in receptor density rather than changes in affinity. This is very unlikely, however, since the maximum amount of antigen bound per ABC does not change appreciably with time (9). Furthermore, if the increasing capacity of ϵ-DNP-L-lysine to inhibit DNP-GPA–^{125}I binding reflected decreased cooperative interaction rather than increased affinity for ϵ-DNP-L-lysine, the avidity for DNP-GPA should diminish with time. However, we have demonstrated (9) that marked increases in the avidity for DNP-GPA occur, thus rendering this alternate explanation untenable.

The inhibition of PFC by hapten is somewhat different from the inhibition of ABC. The introduction of continuously changing concentration gradients as antibody diffuses from the PFC to be met by free hapten is far more complex than the situation with ABC. In spite of these differences, it appears that the two assays do indeed measure avidity changes in comparable ways, if for no other reason than the fact that the rates of change of the two populations are similar.

It should be emphasized that the analysis of antibody avidity by the plaque method provides a level of insight not possible with the usual methods for measuring serum antibody affinity. It is evident from this study that significant events occur before serum antibody becomes detectable and before affinity measurements are possible. In addition, by expressing the PFC data in terms of

avidity subclasses, the avidity profile of the antibody produced at any time after immunization is easily obtained.

From the affinity distribution analysis, it is clear that the cellular dynamics of maturation are primarily a maintenance of a relatively stable level of high affinity PFC while the frequency of low affinity PFC diminishes strikingly. Moreover, the maintenance of the high affinity PFC pool appears to involve proliferation among the PFC or their precursors. Preliminary studies[2] have, indeed, shown that at times when rapid changes in distribution of PFC are occurring, administration of tritiated thymidine to the animal results in the preferential labeling of high affinity PFC. A detailed evaluation of the selectional process as it operates on ABC will require the study of proliferation rates of ABC bearing receptors of varying avidity.

Finally, the identification of ABC as precursors of PFC is still based on indirect evidence. A definitive demonstration of this point will require a direct proof that a suitably labeled ABC transforms into a PFC. This may be approachable through the study of anamnestic antibody responses, either in vivo or in vitro, utilizing animals with relatively large numbers of high avidity ABC and few, if any, PFC.

SUMMARY

During the course of the immune response to dinitrophenylated guinea pig albumin (DNP-GPA), a striking and parallel increase in avidity for ϵ-DNP–L-lysine occurs in the receptors on antigen-binding lymphocytes, antibody secreted by individual plaque-forming cells, and serum antibody molecules. A detailed analysis of the avidity distribution of antibody produced by plaque-forming cells indicates that this "immunologic maturation" is primarily due to a preservation of the high avidity subpopulation and a striking loss in the low avidity population rather than to sequential appearance of these cells.

Moreover, the demonstration of the increased avidity of receptors of antigen-binding lymphocytes, which appear to be precursors of antibody-synthesizing cells, strongly suggests that the antigen-driven selectional process operates primarily on this cell type.

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