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Studies on the Control of Antibody Synthesis

VII. CHANGE IN AFFINITY OF DIRECT AND INDIRECT PLAQUE-FORMING CELLS WITH TIME AFTER IMMUNIZATION IN THE MOUSE: LOSS OF HIGH AFFINITY PLAQUES LATE AFTER IMMUNIZATION

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Summary. The change in avidity of anti-hapten antibody with time after immunization was studied in mice at the level of the antibody-forming cell. A progressive increase in avidity was seen in both direct and indirect plaque-forming cells. Late (38 days) after immunization with a large dose of antigen there was a preferential loss of high avidity plaque-forming cells and the average avidity decreased. High avidity memory cells were still present since boosting resulted in the prompt appearance of very high avidity plaque-forming cells. There was a strong positive correlation between the avidity of the direct and the indirect plaque-forming cells present in the same spleen. A pattern of change in avidity and heterogeneity of avidity similar to that observed with intact animals was seen in lethally irradiated mice reconstituted with normal spleen and thymus cells.

INTRODUCTION

It has been shown that the normal immune response is characterized by a marked degree of heterogeneity of antibody with respect to its affinity for the antigenic determinant (Karush, 1962; Eisen and Siskind, 1964). In addition, it is known that there is a progressive increase in average affinity with time after immunization (Eisen and Siskind, 1964; Siskind, Dunn and Walker, 1968; Goidl, Paul, Siskind and Benacerraf, 1968; Paul, Yoshida and Benacerraf, 1970; Werblin, Kim, Quagliata and Siskind, 1973; Kim and Siskind, 1974). A number of workers (Andersson, 1970, 1972; Miller and Segre, 1972; Davie and Paul, 1972; Huchet and Feldmann, 1973; Möller, Bullock and Mäkelä, 1973; Claflin, Merchant and Inman, 1973) have demonstrated a similar increase in the avidity of the antibody produced by plaque-forming cells (PFC). While there is a general agreement regarding the increase in affinity of IgG antibodies and of indirect PFC, conflicting data have been presented regarding the possibility of an increase in affinity of direct PFC (i.e. IgM antibodies) (Voss and Eisen, 1968; Doria, Schiaffini, Garavini and Mancini, 1972; Claflin and Merchant, 1972; Wu and Cinader, 1972, 1973; Huchet and Feldmann, 1973; Möller, Bullock and Mäkelä, 1973; Roszman, 1974; Smith, Hammarstrom and

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Möller, 1974). Presumably the brief duration of the IgM response under most circumstances makes selection for high affinity IgM synthesis more difficult to observe.

In the present report a formal study of the effect of antigen dose and time after immunization on antibody avidity was carried out at the PFC level in the mouse. In addition, a comparable study was carried out in lethally irradiated mice which were reconstituted with syngeneic lymphoid tissue. A definite increase in the avidity of direct PFC was demonstrated. Late after immunization with a large dose of antigen a decrease in average avidity was observed in both direct and indirect PFC. Although high avidity PFC tended to be preferentially lost late after immunization, high affinity memory cells remained since boosting resulted in the prompt appearance of a large population of high avidity PFC. The changes in avidity and heterogeneity of avidity were similar in the cell transfer recipient to that seen in the intact animal suggesting that transfer systems can be reliably used to study the control of antibody affinity.

MATERIALS AND METHODS

Preparations of antigens and haptens

Bovine gamma-globulin (BGG) five times recrystallized, ovalbumin (Ova) and poly-L-lysine (molecular weight 30,000–70,000) were obtained from Miles-Yeda Ltd (Kankakee, Illinois).

Dinitrophenylated (DNP) protein conjugates were prepared by the reaction of 1-fluoro-2,4-dinitrobenzene (DNFB) (Eastman Organic Chemicals, Rochester, New York) with protein under alkaline conditions (Eisen, Belman and Carsten, 1953). The derivatized proteins were purified by extensive dialysis; their concentrations were determined by drying a known volume to constant weight at 95°; and their degrees of derivatization were estimated from their 360 nm absorbancy.

DNP-ε-amino-*n*-caproic acid was prepared by the reaction of DNFB with ε-amino-*n*-caproic acid (EACA) (Sigma Chemical Company, St Louis, Missouri) under basic conditions. The product was purified by repeated crystallization from hot water. Details of the procedures for preparation and characterization of this hapten were presented previously (Werblin *et al.*, 1973).

Animals

Young adult, male, LAF1 mice (18–20 g) obtained from Jackson Laboratories (Bar Harbor, Maine) were used throughout except as thymus cell donors when weanlings (approximately 3 weeks of age) were employed.

Immunization

Mice were immunized by the intraperitoneal injection of 50 or 500 μg of DNP_{50}–BGG (subscripts refer to the number of hapten groups per molecule of protein) emulsified in Freund's complete adjuvant (FCA) containing 1 mg/ml of *Mycobacterium butyricum.*

Cell transfer studies

Recipients were lethally irradiated (800 rad) using a caesium 137 source (Gammator M Radiation Machinery Corporation, Parsippany, New Jersey) 2–4 hours before cell transfer. Mice were reconstituted with 1×10^8 thymus cells and 5×10^7 pooled, adult,

spleen cells injected intravenously, and were immunized 1 day after cell transfer. Cell suspensions were prepared by teasing in Hanks's basic salt solution (BSS) (GIBCO, Grand Island, New York) containing 35 mg/ml of Na_2HCO_3 and 0·02 mg/ml of sodium heparin sulphate) spleens or thymuses obtained after killing donor mice by cervical dislocation. The cell suspensions were filtered through a thin layer of cotton gauze, washed once with BSS and resuspended in BSS for injection.

Preparation of DNP–Ova-coated sheep red blood cells

DNP–Ova·SRBC were prepared by a modification of the method of Jandl and Simmons (1957). Four millilitres of 0·017 M $CrCl_3$ in saline and 10 ml of 7·5 mg/ml DNP_8–Ova in saline were added to 2 ml of packed SRBC which had been previously washed extensively with 0·15 M NaCl. The mixture was held at room temperature for 5 minutes with occasional mixing. The cells were then washed twice with 0·15 M NaCl and twice with phosphate-buffered saline (PBS) (0·15 M NaCl, 0·01 M potassium phosphate buffer, pH 7·4) and made up as a 1 per cent suspension in PBS for use.

Assay of plaque-forming cells (PFC)

PFC were assayed by the monolayer method of Kennedy and Axelrad (1971) using DNP_8–Ova coated SRBC as target cells. Lyophilized guinea-pig serum (Grand Island Biological Company, Grand Island, New York) dissolved in water and diluted 1:10 in PBS was used as a source of complement. Spleens obtained after killing mice by cervical dislocation followed by exsanguination from the axillary artery, were gently teased in BSS. The cell suspension was filtered through a thin layer of cotton gauze, washed once and resuspended in a known volume of BSS for assay. Generally, each plate contained cells from a thirtieth of a spleen in a total volume of 2·0 ml of complement. The plates were incubated at 37° for 1 hour and plaques were counted using a dissecting microscope. Indirect PFC were developed by the addition of 0·1 ml of a 1:10 dilution of rabbit anti-mouse globulin antiserum to the incubation mixture. This dilution of developing antiserum had previously been found to result in maximum development of indirect PFC while inhibiting approximately 75 per cent of direct PFC to SRBC.

Measurement of avidity of PFC

The avidity of direct or indirect anti-DNP–PFC was assayed by inhibition of plaque formation by various concentrations of DNP–EACA (Andersson, 1970; Yamada, Yamada and Hollander, 1970). Nine concentrations of hapten were used ranging in half-log units from 1×10^{-9} to 1×10^{-5} M. The concentrations of hapten used did not have any non-specific inhibitory effect on plaque formation as even 1×10^{-5} M DNP–EACA did not effect the number of anti-SRBC PFC produced by spleen cells from SRBC-immunized mice. This method for measurement of avidity is based upon the fact that plaque formation by a high affinity antibody-producing cell is inhibited by low concentrations of hapten while inhibition of plaque formation by low affinity antibody-producing cells requires high concentrations of hapten. From the pattern of inhibition of plaque formation with increasing hapten concentrations the distribution of PFC with respect to avidity can be computed. Average avidity is calculated as the reciprocal of the free hapten concentration required for 50 per cent inhibition of the number of plaques. A mathematical analysis justifying the use of hapten inhibition of plaque formation as an assay of affinity has been carried out recently by DeLisi and Goldstein (1974).

RESULTS

EFFECT OF TIME AFTER IMMUNIZATION AND ANTIGEN DOSE ON AVIDITY OF PFC

The response of mice to 50 μg of DNP_{50}–BGG in FCA is indicated in Table 1. The magnitude of both direct and indirect anti-DNP–PFC is maximum at 7–9 days after antigen injection. The average avidity of the indirect PFC increases progressively until 39 days after immunization. Boosting results in a marked increase in the number of indirect PFC perhaps associated with a very slight increase in average avidity. The number of direct PFC is insufficient to obtain avidity measurements except at 7 days after immunization when the response appears to be of low avidity. There is no increase in the number of direct PFC following boosting.

TABLE 1

EFFECT OF TIME AFTER IMMUNIZATION ON THE NUMBER AND AVIDITY OF THE ANTI-DNP–PFC OF MICE IMMUNIZED WITH 50 μg DNP_{50}–BGG*

Days after immunization	Direct PFC		Indirect PFC	
	Number/spleen	Avidity† ($\times 10^{-5}$)	Number/spleen	Avidity† ($\times 10^{-5}$)
3	423 (5)	n.m.‡	143 (5)	n.m.‡
5	383 (5)	n.m.‡	128 (5)	n.m.‡
7	3212 (6)	2·72 (6)	7215 (6)	5·03 (6)
9	1520 (5)	6·42 (5)	13,230 (5)	7·15 (5)
13	308 (5)	n.m.‡	6130 (5)	361 (5)
19	218 (5)	n.m.	2292 (5)	151 (5)
39	178 (5)	n.m.	1005 (5)	1110 (4)
5 (2°)‡	190 (5)	n.m.	80,702 (5)	41,600 (5)

n.m. = Not measurable because of an insufficient number of plaques.

* Mice were injected intraperitoneally with antigen in FCA., killed at the time indicated and their spleens assayed for anti-DNP–PFC. Data are presented as the geometric mean of the number of animals studied.

† Geometric mean of the reciprocal of the concentration of hapten required for 50 per cent inhibition of the number of PFC.

‡ Assayed 5 days after boosting with 0·5 mg of DNP_{50}–BGG intraperitoneally 38 days after priming.

The distribution of avidities of indirect PFC as a function of time after immunization is illustrated in Fig. 1. The progressive shift towards high avidity PFC is apparent. However, low avidity PFC definitely persists throughout the immune response.

The PFC response of mice immunized with 500 μg of DNP_{50}–BGG in FCA is presented in Table 2. The magnitudes of both the direct and indirect PFC responses peak about day 8 and tend to be greater than in mice immunized with 50 μg of antigen. A definite increase in average avidity of the indirect PFC is apparent from 5 to 20 days after immunization. However, in contrast to animals immunized with 50 μg of DNP–BGG, a distinct fall in average avidity occurs between 20 and 37 days after immunization. Boosting at 38 days after primary immunization results in a marked secondary response of very high avidity. An increase in average avidity of direct PFC is also detected from day 5 to day 20 after immunization. A definite increase in the number of direct PFC, associated with a marked increase in average avidity, occurs following boosting.

The changes in the distribution of avidities of PFC in animals immunized with 500 μg of antigen are illustrated in Figs 2 and 3. A relative shift in the distribution of PFC towards higher avidity subpopulations with time after immunization is apparent in both direct and indirect PFC. There is a relative decrease in high avidity subpopulations of

indirect PFC late (37 days) after immunization. Following boosting, both direct and indirect PFC exhibit highly heterogeneous populations which are predominantly of high avidity.

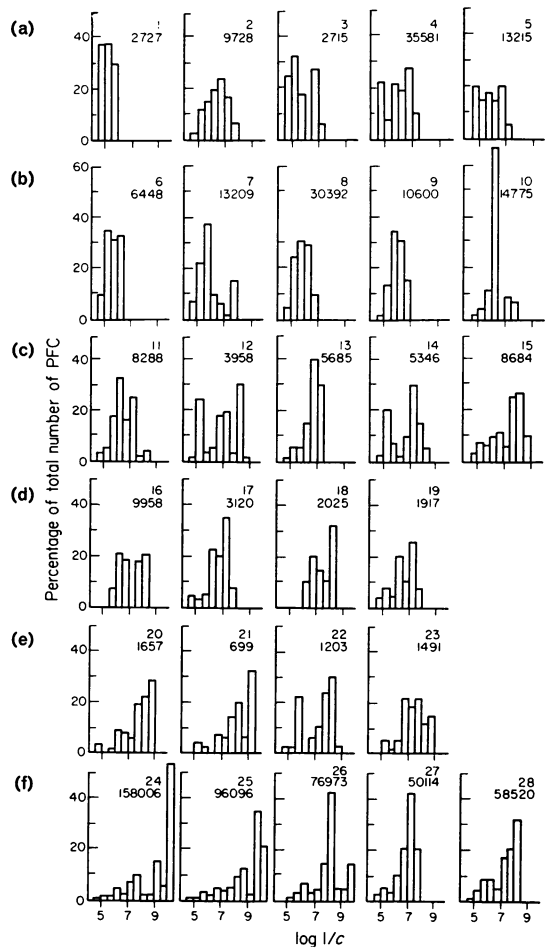

FIG. 1. Each histogram illustrates the distribution of indirect PFC with respect to avidity in the spleen of an individual mouse immunized with 50 μg of DNP–BGG in FCA. Each row presents data on animals killed at a different time after immunization. The time of killing after immunization was: (a) 7 days; (b) 9 days; (c) 13 days; (d) 19 days; (e) 39 days; (f) 5 days after boosting on day 38 with 500 μg of DNP–BGG. The abscissa represents the log of the inverse of the free hapten concentration used in the plaque inhibition assay. The ordinate represents the percentage of the total population of PFC present in each subpopulation. The animal identification number (top) and the total number of PFC per spleen are shown in the right upper corner of each histogram. Avidity increases to the right.

AVIDITY OF THE ANTI-DNP RESPONSE BY LETHALLY IRRADIATED MICE RECONSTITUTED WITH SYNGENEIC SPLEEN CELLS

Since cell transfer studies are commonly employed in contemporary immunological research it was deemed desirable to determine if an increase in avidity comparable to that seen in normal mice also occurred in the somewhat artificial situation of a lethally irradiated animal reconstituted with normal, syngeneic spleen and thymus cells. The data

on animals immunized with 50 μg of DNP_{50}–BGG are presented in Table 3 and Fig. 4. The data for animals immunized with 500 μg of antigen are shown in Table 4 and Figs 5 and 6.

In comparison with the response of intact animals the magnitude of the response of reconstituted mice is delayed. In addition, there is a tendency for direct PFC to persist longer after antigen injection in the reconstituted as compared with the intact animals.

TABLE 2

EFFECT OF TIME AFTER IMMUNIZATION ON THE NUMBER AND AVIDITY OF THE ANTI-DNP–PFC OF MICE IMMUNIZED WITH 500 μg DNP_{50}–BGG*

Days after immunization	Direct PFC		Indirect PFC	
	Number/spleen	Avidity† ($\times 10^{-5}$)	Number/spleen	Avidity† ($\times 10^{-5}$)
3	370 (6)	n.m.	296 (6)	n.m.
5	2685 (5)	2.50 (5)	3146 (5)	4.78 (5)
9	4083 (5)	5.06 (5)	166,004 (5)	10.9 (5)
13	661 (5)	11.0 (3)	35,666 (5)	78.6 (5)
20	296 (5)	54·0 (1)	16,774 (5)	345 (5)
37	898 (5)	16.2 (5)	6389 (5)	39·1 (5)
5 (2°)‡	2271 (5)	4300 (1)	44,793 (5)	1700 (5)

n.m. = Not measurable because of an insufficient number of plaques.

* Mice were injected intraperitoneally with antigen in FCA, killed at the time indicated and their spleens assayed for anti-DNP–PFC. Data are presented as the geometric mean of the number of animals studied.

† Geometric mean of the reciprocal of the concentration of hapten required for 50 per cent inhibition of the number of PFC.

‡ Assayed 5 days after boosting with 0·5 mg of DNP_{50}–BGG intraperitoneally 38 days after priming.

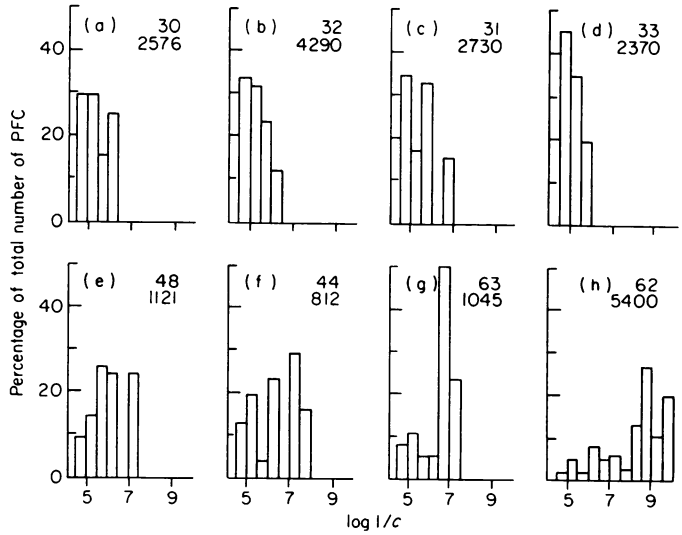

FIG. 2. Each histogram illustrates the distribution of direct PFC with respect to avidity in the spleen of an individual mouse immunized with 500 μg of DNP–BGG in FCA. (a)–(d) present data for animals killed at 5 days after immunization, (e) and (f) for animals killed at 13 (numbers 48 and 44) and (g) 20 (number 63) days after immunization, and (h) 5 days after boosting on day 38 with 500 μg of DNP–BGG (number 62). Avidity increases to the right. The abscissa represents the log of the inverse of the free hapten concentration used in the plaque inhibition assay. The ordinate represents the percentage of the total population of PFC present in each subpopulation. Animal identification number (top) and the total number of PFC per spleen are shown in the right upper corner of each histogram.

Despite these differences, the pattern of change in average avidity and in the distribution of avidities is essentially the same in intact and reconstituted animals. An increase in the relative amounts of high avidity PFC subpopulations, and an increase in average avidity is

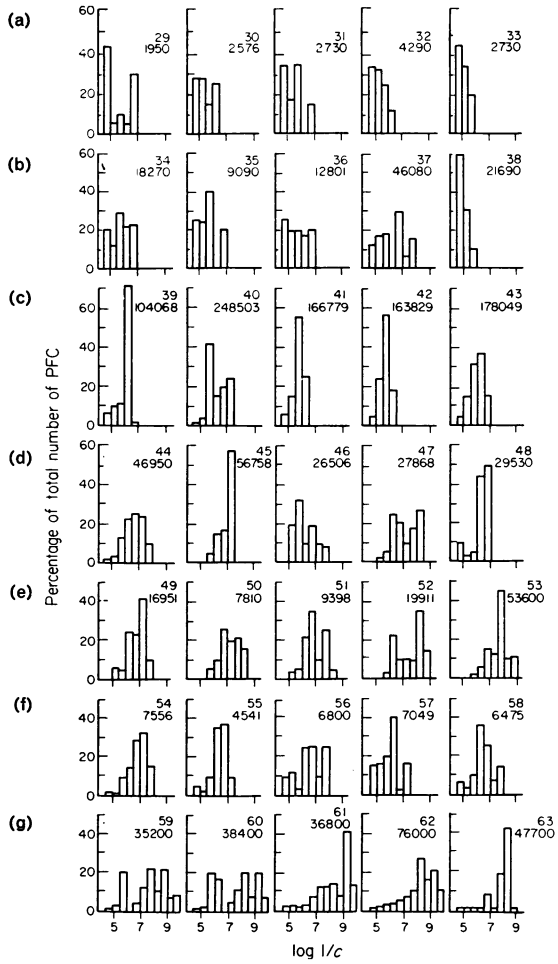

FIG. 3. Each histogram illustrates the distribution of indirect PFC with respect to avidity in the spleen of an individual mouse immunized with 500 μg of DNP–BGG in FCA. Each row presents data on animals killed at a different time after immunization. The time of killing after immunization was: (a) 5 days; (b) 7 days; (c) 9 days; (d) 13 days; (e) 20 days; (f) 37 days; (g) 5 days after boosting on day 38 with 500 μg of DNP–BGG. Avidity increases to the right. The abscissa represents the log of the inverse of the free hapten concentration used in the plaque inhibition assay. The ordinate represents the percentage of the total population of PFC present in each subpopulation. Animal identification number (top) and the total number of PFC per spleen are shown in the right upper corner of each histogram.

seen with time after immunization. A tendency for a decrease in avidity at 38 days after immunization with 500 μg of DNP_{50}–BGG is also seen in the transfer system. The increase in avidity of direct PFC is even clearer in the transfer system than it was in the intact animals.* The pattern of change in avidity of direct PFC is comparable to the changes in

* Direct PFC for two representative animals, killed at day 20 post-immunization, were more than 90 per cent inhibitable by rabbit anti-mouse μ chain. (Kindly provided by Dr R. Asofsky, National Institutes of Health, Bethesda, Maryland.)

TABLE 3

EFFECT OF TIME AFTER IMMUNIZATION ON THE NUMBER AND AVIDITY OF THE ANTI-DNP–PFC PRODUCED BY LETHALLY IRRADIATED MICE RECONSTITUTED WITH SYNGENEIC ADULT SPLEEN AND THYMUS CELLS AND IMMUNIZED WITH 50 μg DNP_{50}–BGG*

Days after immunization	Direct PFC		Indirect PFC	
	Number/spleen	Avidity† ($\times 10^{-5}$)	Number/spleen	Avidity† ($\times 10^{-5}$)
9	477 (4)	2·10 (2)	547 (4)	7·69 (1)
14	2274 (4)	11·8 (4)	9937 (4)	184 (4)
20	1533 (4)	17·0 (1)	15,804 (4)	112 (4)
39	171 (4)	n.m.	920 (4)	80 (1)
5 (2°)‡	3051 (5)	1600 (5)	63,781 (5)	574 (5)

n.m. = Not measurable because of an insufficient number of plaques.

* Mice were injected intraperitoneally with antigen in FCA, killed at the time indicated and their spleens assayed for anti-DNP–PFC. Data are presented as the geometric mean of the number of animals studied.

† Geometric mean of the reciprocal of the concentration of hapten required for 50 per cent inhibition of the number of PFC.

‡ Assayed 5 days after boosting with 0·5 mg DNP_{50}–BGG intraperitoneally 38 days after priming.

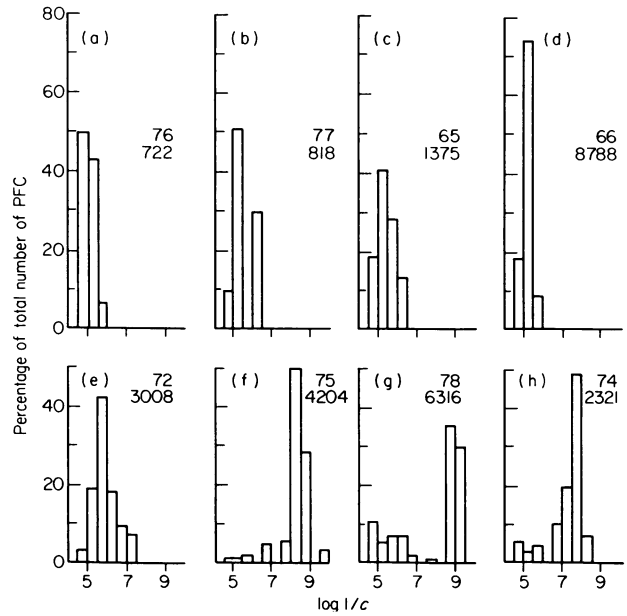

FIG. 4. Each histogram illustrates the distribution of direct PFC with respect to avidity in the spleen of an individual mouse. The animals had been lethally irradiated and reconstituted with normal, adult, syngeneic spleen and thymus cells 1 day before immunization with 50 μg DNP–BGG in FCA. The data in (a)–(d) refer to animals killed 9 (a) and (b) or 14 (c) and (d) days after immunization. (e)–(h) Animals killed 5 days after boosting on day 38 with 500 μg of DNP–BGG. Avidity increases to the right. The abscissa represents the log of the inverse of the free hapten concentration used in the plaque inhibition assay. The ordinate represents the percentage of the total population of PFC present in each subpopulation. Animal identification number (top) and the total number of PFC per spleen are shown in the right upper corner of each histogram.

avidity of indirect PFC. Following boosting there is an increase in both the number and avidity of direct PFC. The distributions of avidities illustrated in Figs 4, 5 and 6 show a progressive increase in high avidity subpopulations until day 20; a relative decrease in the high avidity subpopulations at day 38 in animals immunized with 500 μg of DNP_{50}–BGG; and a marked increase in high avidity subpopulations following boosting. The low avidity population of PFC persists throughout the immune response so that the heterogeneity of avidity tends to increase with time after immunization.

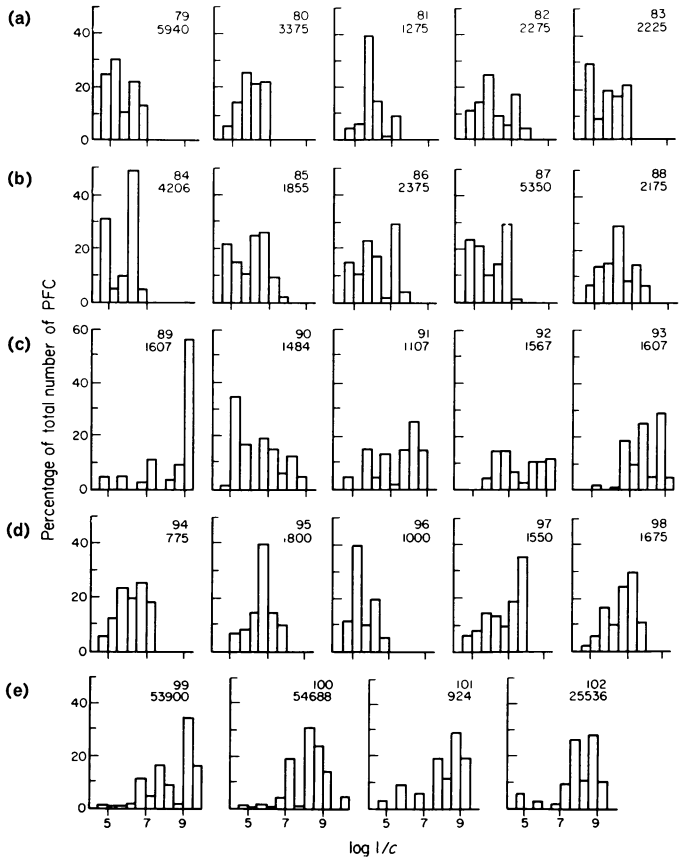

FIG. 5. Each histogram illustrates the distribution of direct PFC with respect to avidity in the spleen of an individual mouse. The animals had been lethally irradiated and reconstituted with normal, adult, syngeneic spleen and thymus cells 1 day before immunization with 500 μg of DNP–BGG in FCA. Each row presents data on animals killed at a different time after immunization. The time of killing after immunization was: (a) 9 days; (b) 13 days; (c) 20 days; (d) 38 days. (e) Animals killed 5 days after boosting on day 38 with 500 μg of DNP–BGG. Avidity increases to the right. The abscissa represents the log of the inverse of the free hapten concentration used in the plaque inhibition assay. The ordinate represents the percentage of the total population of PFC present in each subpopulation. Animal identification number (top) and the total number of PFC per spleen are shown in the right upper corner of each histogram.

COMPARISON OF AVIDITIES OF DIRECT AND INDIRECT PFC

During the course of this work the avidity of direct and indirect PFC in the same spleen was frequently measured. Sixty-five such pairs of data were examined statistically to

TABLE 4

EFFECT OF TIME AFTER IMMUNIZATION ON THE NUMBER AND AVIDITY OF THE ANTI-DNP–PFC PRODUCED BY LETHALLY IRRADIATED MICE RECONSTITUTED WITH SYNGENEIC ADULT SPLEEN AND THYMUS CELLS AND IMMUNIZED WITH 500 μg OF $DNP_{-50}BGG$*

Days after immunization	Direct PFC		Indirect PFC	
	Number/spleen	Avidity† ($\times 10^{-5}$)	Number/spleen	Avidity† ($\times 10^{-5}$)
9	2645 (5)	7.21 (5)	3281 (5)	29·3 (5)
13	2937 (5)	10·9 (5)	10,425 (5)	87·0 (5)
20	1803 (6)	2460 (5)	13,356 (6)	442 (6)
38	1470 (4)	63·4 (4)	8412 (4)	104 (4)
5 (2°)‡	16240 (4)	4480 (4)	215,650 (4)	345 (4)

* Mice were injected intraperitoneally with antigen in FCA, killed at the time indicated and their spleens assayed for anti-DNP–PFC. Data are presented as the geometric mean of the number of animals studied.

† Geometric mean of the reciprocal of the concentration of hapten required for 50 per cent inhibition of the number of PFC.

‡ Assayed 5 days after boosting with 0·5 mg of DNP_{50}–BGG intraperitoneally 38 days after priming.

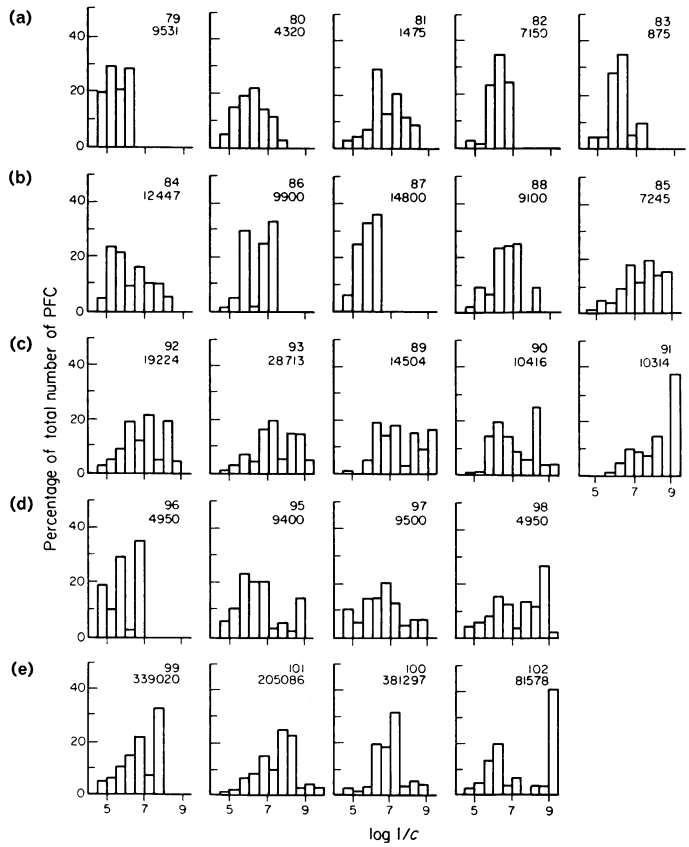

FIG. 6. Each histogram illustrates the distribution of indirect PFC with respect to avidity in the spleen of an individual mouse. The animals had been lethally irradiated and reconstituted with normal, adult, syngeneic spleen and thymus cells 1 day before immunization with 500 μg of DNP–BGG in FCA. Each row presents data on animals killed at a different time after immunization. The time of killing after immunization was: (a) 9 days; (b) 13 days; (c) 20 days; (d) 38 days. (e) Animals killed 5 days after boosting on day 38 with 500 μg of DNP–BGG. The abscissa represents the log of the inverse of the free hapten concentration used in the plaque inhibition assay. The ordinate represents the percentage of the total population of PFC present in each subpopulation. Animal identification number (top) and the total number of PFC per spleen are shown in the right upper corner of each histogram. Avidity increases to the right.

determine if there is any association between the average avidity of direct and indirect PFC from the same animal. The rank correlation coefficient was calculated according to Spearman as described in Snedecor and Cochran (1971) to be 0·437. According to Fisher (1970) this indicates a highly significant, positive correlation ($P<0·01$).

DISCUSSION

Changes in average avidity and heterogeneity of avidity during the immune response were described, at the PFC level, in the mouse. With time after immunization there is a progressive increase in average avidity associated with an increase in heterogeneity of PFC with respect to avidity. Both an absolute and a relative increase in the number of high avidity PFC occurs. Low avidity PFC continue to be present throughout the course of the immune response. Thus, the data in general confirm the reports of previous workers studying mice (Paul *et al.*, 1970; Andersson, 1970, 1972; Miller and Segre, 1972; Kim and Siskind, 1974; Huchet and Feldmann, 1973; Möller *et al.*, 1973; Claflin *et al.*, 1973) and other species (Eisen and Siskind, 1964; Siskind *et al.*, 1968; Goidl *et al.*, 1968; Lamelin and Paul, 1971; Davie and Paul, 1972; Werblin *et al.*, 1973).

Several observations described in the present paper should be noted. Late after immunization (approximately 1 month) with 500 μg of DNP_{50}–BGG there is a relative decrease in the number of high avidity anti-DNP–PFC. This late decrease in average avidity is mainly seen when a large dose of antigen is used for immunization. A decrease in avidity late after immunization has previously been reported in mice by Doria *et al.* (1972). We have observed a similar decrease in average affinity 6–12 months after immunization of rabbits (Werblin *et al.*, 1973). Both in the studies reported here and in our previous studies on rabbits (Kim *et al.*, 1974) it was found that high avidity memory cells persist since boosting promptly elicited high avidity PFC. The mechanism of the late fall in antibody affinity is not known. However, these observations do suggest the existence of some process controlling the pattern of differentiation into antibody-producing versus memory cells.

An increase in affinity of IgG antibody with time after immunization has been demonstrated in many studies both with respect to serum antibody and indirect PFC (reviewed by Siskind and Benacerraf, 1969; Werblin and Siskind, 1972). However, the situation with respect to IgM antibody production is less clear. While some workers (Doria *et al.*, 1972; Claflin and Merchant, 1972; Claflin *et al.*, 1973; Wu and Cinader, 1972, 1973) have reported an increase in avidity of direct PFC with time after immunization other investigators (Voss and Eisen, 1968; Huchet and Feldmann, 1973; Möller *et al.*, 1973; Roszman, 1974; Smith *et al.*, 1974) have failed to observe any change in avidity of IgM-producing cells in the primary response. Huchet and Feldmann (1973) observed a small increase in avidity of PFC following boosting. The data presented here support the contention that there is an increase in avidity of direct PFC similar to that seen with indirect PFC. In fact, a highly significant correlation was noted between the avidity of direct and indirect PFC in the present study.

Finally, the changes in avidity and heterogeneity of avidity were studied in a cell transfer system. Since such cell transfer systems are frequently employed in immunological investigation it was regarded as important to establish that a similar process of cell selection occurs in a lethally irradiated, reconstituted mouse as takes place in a normal animal. Compared with intact animals, the reconstituted mice have a PFC response of

somewhat reduced magnitude. In addition, the peak response may be slightly delayed and significant numbers of direct PFC persist for a long time after immunization. The reconstituted mice, at both antigen doses, appeared to produce somewhat higher avidity direct and indirect PFC in the primary immune response when compared with intact animals. The increase in affinity is in most cases too small to permit one to be confident of the validity of the observation. Upon secondary antigenic stimulation, reconstituted animals mount an immune response of clearly higher magnitude than that produced by similarly boosted intact animals. Although we have not formally tested the possibility, the magnitude of the secondary response would appear to be inversely related to the magnitude of the primary response.

Despite the slight differences, the changes in heterogeneity of avidity and average avidity in the reconstituted mice are basically similar to what is seen in intact animals. Thus, one can infer that a similar process of cell selection occurs in the intact as in the irradiated reconstituted animal. It thus would appear possible to employ irradiated reconstituted mice in studies of affinity, heterogeneity and cell selection.

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