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Studies on the Control of Antibody Synthesis

XI. Effect of Antigen on the Localization of Cells Secreting Different Avidity Antibodies[1] (39774)

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Introduction. In considering the process by which B cells are selected by antigen to proliferate, differentiate, and secrete antibody, a distinction should be made between antigen passively diffusing to interact with specific lymphocytes and migrating lymphocytes being trapped by localized antigen. Relatively little is known about the dynamics of the selection process. It has been shown, for example, that the migration of lymphocytes to the lymphoid tissues draining the site of antigen injection is important in initiating an immune response (1, 2). What fraction of these cells is specific for the particular antigen is not known. While some investigators have clearly demonstrated antigen-specific-lymphocyte trapping (3–10) others have suggested that the trapping is, at least in part, nonspecific (11–15). Specific trapping might result in the depletion of specific lymphocytes from the remaining lymphoid tissues (16–18). Nossal *et al.* (19) have presented evidence that antigen localizes on dendritic macrophages in regional lymph nodes. In such circumstances the lymph node could act as an immunoadsorbent to trap antigen-specific T and B lymphocytes which are migrating through it. The purpose of the present study was twofold: (a) to determine whether specific B-cell trapping leads to a depletion of potential antibody-forming cells from other sites; (b) to determine the extent to which the "avidity of the B cells" influences their propensity to localize in lymph nodes draining the site of antigen injection.

Materials and methods. Antigens and haptens. Dinitrophenylated bovine γ-globulin (DNP-BGG) was prepared (20) by reacting BGG (Miles Laboratories, Kankakee, Ill.) with 1-fluoro-2,4-dinitrobenzene (DNFB; Eastman Organic Chemicals Co., Rochester, N. Y.). The protein concentration was determined from the "dry weight," and the degree of substitution was estimated spectrophotometrically (ε for DNP-lysine = 17,400 at 360 nm). The preparation of DNP-BGG used throughout these studies had approximately 44 DNP groups per molecule. DNP-ε-amino-*n*-caproic acid (DNP-EACA) was prepared, as described previously (21), by the reaction of DNFB with EACA (Sigma Chemical Co., St. Louis, Mo.) under alkaline conditions.

Animals and immunization. Three-to-four killogram, female New Zealand white rabbits were used. They were immunized with DNP-BGG emulsified in complete Freund's adjuvant (CFA) containing 2 mg/ml of *Mycobacterium butyricum*. Emulsion (0.5 ml) was injected into one or both hind footpads. The animals were sacrificed 1 to 7 weeks following immunization; their popliteal lymph nodes were removed and were assayed for anti-DNP plaque-forming cells (PFC).

Assay of anti-DNP PFC and PFC avidity distribution. Anti-DNP direct PFC were assayed by the Dresser and Greaves modification (22) of the Jerne *et al.* plaque assay (23) as described previously (24). The avidity distribution of anti-DNP PFC was estimated by the hapten inhibition of plaque formation according to the technique of Andersson (25). The assay is based upon the observation that plaque formation around high affinity antibody-secreting cells is inhibited by a low concentration of hapten,

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while a high concentration of hapten is required to inhibit plaque formation around low affinity antibody-secreting cells. Concentrations of DNP-EACA ranging from 1 $\times 10^{-9}$ to 1 $\times 10^{-5}$ *M* in half-log increments were used. The details of the procedure have been described and discussed previously (24).

Results. Number and avidity of PFC in lymph nodes draining, and distant to, the site of antigen injection. DNP-BGG (100 μg) in CFA was injected into the rabbits left rear footpad and CFA without antigen was injected into the other footpad. One to seven weeks later the rabbits were sacrificed and their popliteal lymph nodes, ipsilateral and contralateral to the site of DNP-BGG injection, were assayed for the number and avidity distribution of anti-DNP PFC. The results are summarized in Table I and Fig. 1.

There are clearly more PFC in the ipsilateral node than in the node contralateral to the antigen injection. In addition, the popliteal node from the side of the antigen injection contained considerably higher avidity PFC than were detected in the contralateral node. This difference between the popliteal nodes ipsilateral and contralateral to the antigen injection was maintained from 1 to 7 weeks after immunization, and was present in all but one of the 18 rabbits studied. The difference in avidity distribution is made particularly apparent if the percentages of the total population inhibited by 3 $\times$ 10^{-7} M DNP-EACA (percentages of high avidity components) are compared (Table I). Surgical excision of the ipsilateral node 1 week after antigen injection did not alter the number or avidity of the anti-DNP PFC in the contralateral node when assayed 2 to 7 weeks after immunization (data not shown). It would thus appear that high avidity antibody-secreting cells, or their precursors, are preferentially localized to the lymph node draining the site of antigen injection. Distant nodes contain mainly low avidity antibody-secreting cells.

Effect of injecting different doses of antigen into the two rear foot pads on the number and avidity of the PFC. DNP-BGG (1000 μg) in CFA was injected into one hind footpad and 100 μg of the same antigen was injected into the opposite footpad. The rabbits were sacrificed and the anti-DNP PFC responses in their popliteal nodes were assayed 3 weeks later. The results are illustrated in Table II and Fig. 2. The popliteal node from the side which received the larger dose of antigen had a greater PFC response and higher avidity PFC in every case.

The results suggest that high avidity PFC, or precursors of high avidity PFC, are captured and retained in regional lymph nodes as a function of antigen availability. In addition, high avidity cells appear to be preferentially retained in the regional lymph nodes and do not become distributed to distant nodes as readily as do low avidity cells. This hypothesis was further examined by studying the response when one dose of antigen was injected into one footpad and a different dose was injected into the other rear footpad 1 week later. These data are also summarized in Table II and Fig. 2. If a high dose of antigen is given 1 week before a low dose, the popliteal node on the side

TABLE I. ANTI-DNP PFC RESPONSE IN POPLITEAL LYMPH NODES IPSILATERAL AND CONTRALATERAL TO AN INJECTION OF DNP-BGG IN CFA INTO ONE FOOTPAD.[a]

Time after immunization (weeks)	Number of rabbits	Direct PFC/node		Percent inhibition by 3 $\times$ 10^{-7} *M* DNP-EACA	
		Ipsilateral $\times 10^3$	Contralateral $\times 10^3$	Ipsilateral (%)	Contralateral (%)
1	3	52.6	12.5	53	0
2-4	9	25.2 ± 25.2	5.32 ± 8.81	58 ± 13	5 ± 9
5-7	6	7.76 ± 6.42	2.73 ± 1.75	34 ± 23	1 ± 3

[a] Rabbits were injected with 100 μg of DNP-BGG in CFA into one hind footpad and with CFA without antigen into the other hind footpad. They were sacrificed at various times thereafter and the anti-DNP PFC were assayed in the popliteal nodes ipsilateral and contralateral to the antigen injection. The data are presented as arithmetic means ± standard deviations (except in the 1-week group, where only three animals were studied so standard deviations are not indicated). Lymph nodes from unimmunized animals have fewer than 100 anti-DNP plaques/node.

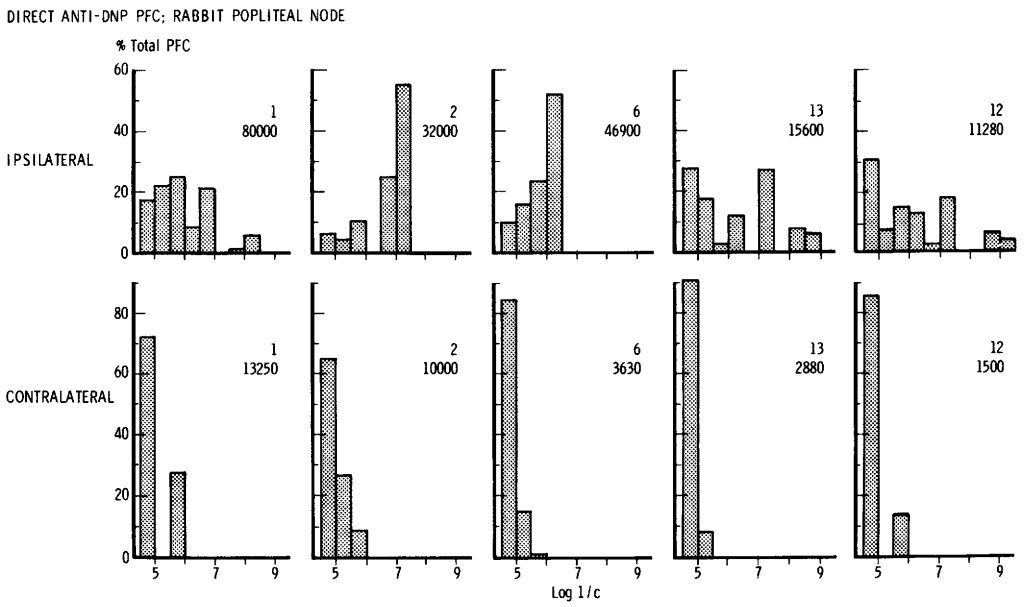

FIG. 1. Each histogram illustrates the distribution of anti-DNP direct PFC with respect to avidity in a rabbit popliteal lymph node 3 weeks after immunization. In the top row are data from the node on the same side as the footpad injected with 100 μg of DNP-BGG in CFA. In the bottom row are data from the contralateral popliteal node of the same rabbit. The abscissa represents the log of the inverse of free hapten concentration used in the plaque inhibition assay. The ordinate represents the percentage of the total population of PFC present in each subpopulation. Rabbit identification number (top) and the total direct PFC per node are indicated in the upper right corner of each histogram. Avidity increases to the right.

TABLE II. EFFECT OF INJECTING DIFFERENT DOSES OF DNP-BGG INTO THE TWO FOOTPADS ON THE NUMBER AND AVIDITY OF THE ANTI-DNP PFC IN THE POPLITEAL NODES.[a]

Order of injection	Number of rabbits	Direct PFC/node		Percent inhibition by 3 × 10^{-7} *M* DNP-EACA	
		1000-μg side × 10^3	100 μg side × 10^3	1000-μg side (%)	100-μg side (%)
100 and 1000 μg simultaneously	4	20.8	5.28	64	12
1000 before 100 μg	3	11.0	5.13	66	39
100 before 1000 μg	5	7.46	14.5	45	49

[a] Rabbits were injected with either 1000 or 100 μg of DNP-BGG into one rear footpad and 1 week later were given the other dose of antigen into the other rear footpad. The order of injection is indicated in the first column; another group received the two doses of antigen simultaneously into different footpads. The popliteal lymph nodes were assayed for PFC 3 weeks after the second injection. The data are presented as arithmetic means.

receiving the high dose has more and higher avidity PFC than does the popliteal node on the low dose side. On the other hand, when the low dose is given first, then the low dose side has the greater number of PFC and the avidity of the PFC are roughly equal on the two sides. It would thus appear that high avidity B cells, or their precursors, tend to be trapped in the node draining the initial site of antigen injection.

Discussion. In this study it was demonstrated that high avidity B cells tend to be located in lymph nodes draining the site of antigen injection. Lymph nodes distant to the site of antigen injection become populated mainly with low avidity PFC. It would appear that high avidity PFC (or their precursors) are preferentially retained in the node draining the site of antigen injection while low avidity B cells escape from that

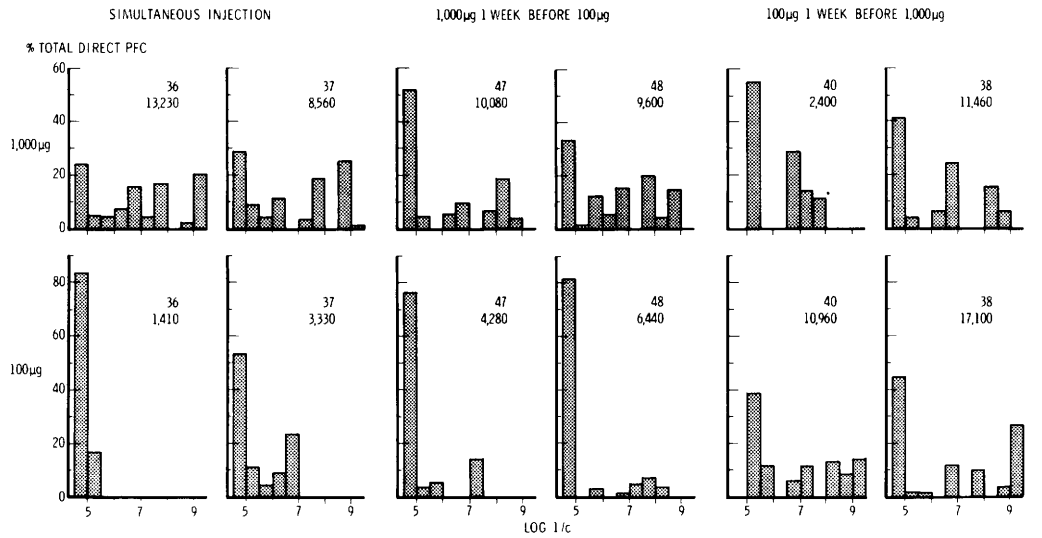

FIG. 2. Each histogram illustrates the distribution of anti-DNP direct PFC with respect to avidity in a rabbit popliteal lymph node. Animals were injected with 100 μg of DNP-BGG in CFA into one rear footpad and 1000 μg of DNP-BGG into the other rear footpad. The injections were given simultaneously or 1 week apart. Three weeks after the simultaneous or second injection of antigen the anti-DNP PFC response in each of the two popliteal nodes was assayed and the data are presented in a pair of histograms, one above the other. In each pair, the top histogram represents the lymph node on the same side as the footpad injected with 1000 μg of antigen and the bottom histogram illustrates data from the lymph node on the same side as the footpad injected with 100 μg of antigen. The two pairs of histograms on the left were obtained with rabbits given the two doses of antigen simultaneously; the two pairs in the center were obtained with rabbits given 1000 μg 1 week before 100 μg of antigen; the two pairs on the right were obtained with rabbits given 100 μg 1 week before 1000 μg of antigen. The abscissa represents the log of the inverse of free hapten concentration used in the plaque inhibition assay. The ordinate represents the percentage of the total population of PFC present in each subpopulation. Rabbit identification number (top) and the total anti-DNP direct PFC per node are indicated in the upper right corner of each histogram. Avidity increases to the right.

node and are thus detected at distant sites. Numerous studies have indicated that a lower dose of antigen tends to favor the rapid selection of cells producing high affinity antibody (26–28). If the difference between the two nodes were related to such a cell selection process, one would expect the distant node, which is undoubtedly exposed to lower concentrations of antigen, to have higher avidity PFC. The fact that the distant node consistently had only low avidity PFC suggests that a simple process of trapping of specific cells is operating to produce the distribution of PFC observed here. Other workers (3–10) have demonstrated an antigen-dependent arrest of recirculating cells in lymph nodes. Introduction of antigen has been shown to alter the flow of lymphocytes through individual lymph nodes (6, 29).

When different doses of antigen were injected into different footpads, it was found that the high avidity B cells were present in the node exposed to the higher concentration of antigen. It is of interest that when different doses of antigen were given 1 week apart into different footpads, the node draining the initial site of antigen injection tended to have greater numbers of and higher avidity PFC regardless of which dose of antigen was administered first. This would suggest that the preferential trapping of high avidity antibody-producing cell precursors leads to a temporary depletion of the available pool of the specific precursor cells. Thus, a subsequent injection of antigen into the opposite footpad tends to stimulate relatively fewer high avidity PFC in the node draining the site of the second antigen injection.

It would thus appear that antigen availability is a critical factor in trapping B cells in regional lymph nodes. Both high and low

avidity cells appear to be localized wherever antigen is most abundant. Low avidity B cells seem more likely to migrate to distant sites. It should be noted that in these studies only direct PFC (presumably IgM-producing cells) were examined. Thus, it is not certain how these results relate to the distribution of IgG antibody-secreting cells.

It might be mentioned that no significant increase in the avidity of direct PFC was detected in this study. Maturation of IgM avidity has been a variable finding, some workers observing an increase in avidity (30) and others reporting no change in avidity during the primary response (31–35). The precise explanation for these differences is not clear but seems to be related to the kinetics of the switch from IgM to IgG production. It appears that if the switch is delayed IgM maturation can be demonstrated.

Summary. It was shown that rabbit popliteal lymph nodes draining the site of antigen injection tend to contain high avidity PFC while the PFC present in sites distant to the antigen injection tend to be of low avidity. When different doses of antigen are injected into the two rear footpads the popliteal node draining the side receiving the higher dose of antigen tends to contain more and higher avidity PFC. If antigen injections are given serially, 1 week apart, into different footpads, then the popliteal node draining the site of the first antigen injection tends to contain more and higher avidity PFC regardless of the dose of antigen used. Thus, antigen availability appears to be a critical factor in determining the localization of precursors of PFC. Following an injection of antigen, high avidity PFC (or their precursors) appear to be preferentially retained in the node draining the site of antigen injection; only low avidity PFC are found at distant sites. In addition, there is a depletion of the available pool of high avidity B-cell precursors of PFC so that a second injection of antigen, at a distant site, stimulates mainly low avidity PFC in the lymph nodes draining the site of the second antigen injection.

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