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Manipulation of the Immune Response*

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The immune response is generally characterized by the synthesis of a highly heterogeneous population of specific antibodies belonging to a variety of different immunoglobulin classes and subclasses. In addition, within a single immunoglobulin class a striking degree of heterogeneity (3, 16, 18) can be demonstrated with respect to the affinity of the antibody for the antigenic determinant (table 1). Finally, the magnitude of the immune response varies under different conditions of immunization. According to current theory and experimental evidence, each antibody-forming cell secretes a homogeneous antibody product with respect to affinity (7, 10, 19). (It is likely that an individual cell or clone can switch with respect to the immunoglobulin class of the antibody it synthesizes.) Thus, the production of a heterogeneous population of molecules with respect to affinity implies that a heterogeneous population of cells (B-lymphocytes) is involved in the immune response. In this paper, some of the procedures will be discussed by which one can manipulate the population of B-lymphocytes participating in the immune response, that is, experimental maneuvers by which the magnitude of the antibody response or the subpopulation of B-lymphocytes selected to secrete antibody or both can be altered in a predictable manner.

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In table 2 is indicated the effect of antigen dose and time after immunization on the amount and affinity of the antihapten antibody synthesized by rabbits in response to dinitrophenylated bovine γ-globulin (DNP-BGG) in complete Freund's adjuvant (CFA). It is clear that there is a progressive increase in the average affinity of the anti-DNP antibody with increasing time after immunization and that the rate of increase in affinity is inversely related to the dose of antigen (3, 4, 11, 16). Early after immunization larger doses of antigen elicit the production of a higher concentration of antibody. However, later after immunization the largest concentration of antibody is the result of immunization with a relatively low dose of antigen. Immunization with a single injection of 0.5 mg of DNP-BGG in CFA resulted in an optimal immune response with respect to both the magnitude and affinity of the antibody synthesized at 6 weeks after antigen injection.

Thus far we have been discussing changes in average affinity. Recently, we have developed computer techniques for approximating the actual distribution of affinities in an antibody sample with data obtained by equilibrium dialysis (18). Such an approximation to the distribution of affinities is illustrated in figure 1. Initially, there is a relatively normal distribution of low affinity antibodies. With time after immunization the distribution becomes skewed towards the high affinity end of the distribution. A subpopulation of high affinity antibody, of relatively restricted heterogeneity,

TABLE 1

*Fractionation of anti-DNP antibody according to affinity**

Fraction	K_0
	l/mole × 10^6
1	>1000
2	330
3	89
4	19
5	8.1
6	1.0
7	0.53
8	0.23
9	0.17
10	0.11

* Serum from a single bleeding of a rabbit immunized with 5 mg of DNP-BGG was separated by fractional precipitation with limiting amounts of DNP-BGG. The anti-DNP antibody was recovered from each precipitate by DNP-OH elution and its affinity for E-DNP-L-lysine measured by fluorescence quenching at 30°C. (Data are taken from H. N. Eisen and G. W. Siskind: Variations in affinities of antibodies during the immune response. Biochemistry 3: 996–1008, 1964.)

TABLE 2

*Effect of antigen dose and time after immunization on antibody concentration and affinity**

Antigen Dose (mg)	Antibody Concentration (mg/ml)			Affinity $-\Delta F^0$ (kcal/mole)		
	13 days	20 days	41 days	13 days	20 days	41 days
0.05	0.02	0.08	0.54		9.88	11.1
0.5	0.26	0.61	4.23	8.72	10.3	12.7
5.0	1.06	1.16	1.98	8.96	9.70	11.0
50.0	1.78	1.14	1.36	8.46	8.06	9.54

* Groups of 5 to 20 rabbits were immunized with the indicated dose of DNP-BGG in CFA and bled at various times after immunization. The antibody concentration was measured by quantitative precipitin reaction with DNP-bovine fibrinogen as antigen. Affinities were measured by fluorescence quenching at 20°C with DNP-lysine as ligand. (Data taken from G. W. Siskind, P. Dunn, and J. G. Walker: Studies on the control of antibody synthesis. II. Effect of antigen dose and of suppression by passive antibody on the affinity of antibody synthesized. J. Exp. Med. 127: 55–66, 1968.)

frequently comes to be a major portion of the total antibody present. Very late after immunization (1 year) a highly heterogeneous population of antibodies is observed ranging in affinity from the lowest to the highest affinity detectable at any time throughout the course of the response. Consequently, very late after immunization there is actually a decrease in average affinity (16). If animals are boosted at this point, there is a rapid synthesis of a high affinity subpopulation of antibody molecules (8, 12).[1]

This pattern of response can be understood in terms of a simple selectional theory of antibody synthesis (2, 10, 13, 19) according to which B-lymphocytes bear on their surface antibody molecules identical in binding properties to that of the antibody which that cell or its progeny or both will secrete after stimulation by antigen. High affinity antibody-producing cells preferentially capture antigen and are thus preferentially stimulated to proliferate or secrete antibody or both. With time, as antigen concentration decreases, such "high affinity" cells tend to become predominant in the population of antibody forming cells and the average affinity increases. A type of microevolution, on the cellular level, thus takes place during the immune response.

Injection of a large dose of soluble antigen into a neonatal animal causes a specific depression in the magnitude of the immune response. This phenomenon, referred to as immunological tolerance, mainly effects high affinity antibody forming cells (14) which would be expected to capture antigen preferentially during tolerance induction. The antibody formed by a partially tolerant animal is thus of extremely low averge affinity (table 3).

Injection of soluble antigen intravenously 2 days to 2 weeks prior to immunization with the antigen in CFA causes a specific depression of the magnitude of the immune response (6, 17). This type of tolerance induction also results in a depression in the affinity of the residual antibody formed (table 4). However, the extent of the depres-

[1] Y. T. Kim and G. W. Siskind: Unpublished observations.

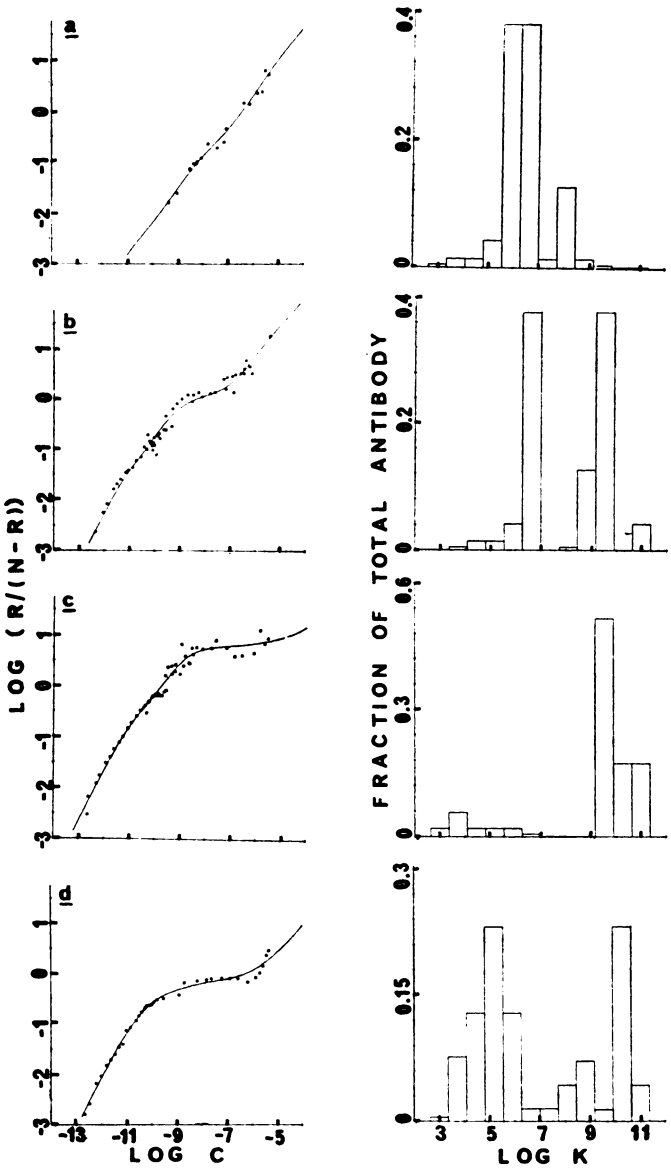

FIG. 1. Binding data and distributions of antibody affinities in serum of a rabbit immunized with 5 mg of DNP-BGG in complete Freund's adjuvant and bled at: a) 7 days, b) 42 days, c) 90 days, and d) 360 days. The left hand column of the figure shows the actual data points (dots) plotted along with the generated binding curves (solid line) which were calculated by an approximation procedure. The logarithm of the ratio of the concentration of bound to free antibody sites (ordinate) is plotted against the logarithm of the free hapten concentration in millimoles/ml (abscissa). The right hand column of the figure shows the computed distributions of affinities calculated from the actual binding data by an approximation procedure. The percent of the total amount of antibody present (ordinate) in each of the subpopulations is plotted against the logarithm of the affinity (abscissa) of each antibody subpopulation. (From T. P. Werblin and G. W. Siskind: Distribution of antibody affinities: technique of measurement. Immunochemistry 9: 987-1011, 1972.)

TABLE 3

*Effect of neonatal tolerance induction on antibody affinity**

	Antibody Concentration	Affinity $-\Delta F^0$
	mg/ml	*kcal/mole*
Normal (6)	1.02	8.67
Tolerant (10)	0.54	6.61

* Neonatal rabbits received 45 mg of DNP-HrSA intraperitoneally over the first 12 days of life. Tolerant animals together with littermate controls were immunized with 5 mg of DNP-HrSA in CFA at 5 to 6 weeks of age and bled 21 days later. Affinities were determined by fluorescence quenching with E-DNP-L-lysine as ligand. (Data are taken from G. A. Theis and G. W. Siskind: Selection of cell population in induction of tolerance: affinity of antibody formed in partially tolerant rabbits. J. Immunol. 100: 138–141, 1968.)

sion in affinity appears to be less marked in this type of tolerance induction as compared with neonatally induced tolerance despite essentially equivalent or greater depression in the amount of antibody formed.

Passive antibody is well known to cause a specific depression in the amount of antibody formed in response to a simultaneous injection of antigen (9, 15). Passive antibody appears to act mainly by binding antigen and thus preventing its interaction with specific lymphoid cells. One would predict that passive antibody would depress mainly low affinity antibody synthesis. Data obtained with several different systems have been found to be consistent with this prediction (6, 11).

In addition to specific modifications of the immune response resulting from variations in antigen dose, tolerance induction, or passive antibody, a variety of experimental manipulations can non-specifically modify the magnitude of the immune response. Three such methods have been studied by us: 1) use of cytotoxic drugs (5)[2]; 2) treatment with heterologous antilymphocyte serum[3]; and 3) antigenic competition (1, 5).

[2] J. Mond and G. W. Siskind: Unpublished observations.

[3] M. E. Weksler and G. W. Siskind: Unpublished observations.

TABLE 4

Effect of adult tolerance induction on antibody affinity

Normal		Tolerant	
Antibody concentration	Antibody affinity	Antibody concentration	Antibody affinity
μg/ml	$-\Delta F^0_{I_{10\%}}$ *kcal/mole*	*μg/ml*	$-\Delta F^0_{I_{10\%}}$ *kcal/mole*
405	11.58	50	
704	11.83	139	11.48
763	11.29	158	10.97
799	11.11	249	10.84
903	11.38	254	10.58
1094	11.55	367	11.03
1148	10.83	388	11.27
1318	11.62	420	11.31
1431	13.10	531	11.50
1460	11.87	535	11.16
1465	11.53	601	11.50
1480	12.16	619	11.36
1587	12.57	689	10.73
2122	11.66	754	11.04
2350	11.52	764	11.47
2406	11.41	820	11.54
2420	11.30	822	10.87
2860	12.04	1129	10.86
2958	11.54	——	——
4968	11.37	516 ± 68	11.15 ± 0.07
——	——		
1732 ± 237	11.66 ± 0.11		

* New Zealand white rabbits were immunized with 0.5 mg of DNP-BGG in CFA and bled 4 weeks later. Tolerant rabbits had been given 40 mg of DNP-BGG intravenously 3 days prior to immunization. Anti-DNP antibody concentration and affinity were measured by the Farr technique at 20°C with DNP-EACA as ligand. Mean ± standard error of the mean is given for each group. The difference in average affinities of the tolerant and normal animals are statistically, significantly different ($P < .001$). (Data are summarized in M. E. Weksler, L. L. Merritts, T. P. Werblin and G. W. Siskind: Studies on the control of antibody synthesis. IV. Effect of tolerance induction in adult rabbits on antibody binding affinity. J. Immunol. 110: 897–904, 1973.)

In general, non-specific depression of the immune response does not effect the average affinity of the residual antibody formed (table 5). Thus, as a rule, it appears that non-specific depression of the immune response has relatively little effect on antibody affinity. Apparently no special sub-

TABLE 5

*Effect of antigenic competition on antibody affinity**

	Antibody Concentration	Affinity $-\Delta F^0$
	mg/ml	*kcal/mole*
Normal (17)	0.97	8.55
Competed (17)	0.66	8.68

* Normal rabbits received 5 mg of DNP-rabbit γ-globulin (DNP-RGG) in CFA. Competed animals received 5 mg of DNP-RGG mixed with 5 mg of arsanilate-azo-RGG in CFA. Animals were bled 20 days later. Concentration of antibody was determined by quantitative precipitin reaction with DNP-bovine fibrinogen and affinity was determined by fluorescence quenching at 20°C with DNP-lysine as ligand. (Data are taken from N. I. Brody and G. W. Siskind: Studies on antigenic competition. J. Exp. Med. 130: 821–832, 1969.)

TABLE 6

*Effect of marked antigenic competition on antibody affinity**

	Antibody Concentration	Affinity $-\Delta F^0_{I_{10\%}}$
	mg/ml	*kcal/mole*
Normal (8)	1.09	10.96
Competed (9)	<0.12	8.46

* Normal guinea pigs were immunized with 0.05 mg of DNP-ovalbumin (DNP-EA) in CFA. Competed animals received 0.05 mg of DNP-EA mixed with 5.0 mg arsanilate-azo-BGG in CFA. Animals were bled 14 days after immunization and the concentration and average affinity of their anti-DNP antibody determined by the Farr technique with DNP-ε-amino-caproic acid as ligand. The lowest concentration of antibody measurable by the procedure is 0.02 mg/ml. Four of the competed animals had less than this concentration of antibody. For the purposes of calculating averages, these animals were assumed to have 0.02 mg/ml of antibody. Affinities were only determined for the five competed animals having greater than 0.02 mg/ml of antibody. All of the normal animals had measurable amounts of antibody. (Data are from Y. T. Kim, N. Merrifield, T. Zarchy, N. I. Brody and G. W. Siskind: Unpublished observations.)

population of B-cells is selectively depressed by these procedures. However, when the degree of antigenic competition becomes very marked we[4] and other workers (5) have noted a significant depression in average affinity (table 6).

Thus, in conclusion, specific modifications in the immune response (tolerance, antibody mediated immune suppression, or variations in antigen dose) result in changes in the average affinity of the antibody produced during the course of the immune response. These changes in average affinity are predictable on the basis of a simple selectional theory in which antigen selects B-lymphocytes to proliferate or secrete antibody or both on the basis of its interaction with "cell-associated" antibody. Nonspecific modifications of the immune response generally have little effect on affinity. However, if a very profound degree of non-specific depression of the immune response is produced, there is a significant depression in affinity. An efficient selection of "high affinity" cells requires not only the selective pressure of decreasing antigen concentration but also a marked degree of cell proliferation. If the magnitude of the response is markedly depressed, cell selection is inefficient, and a rapid progressive increase in antibody affinity with time is not seen.

[4] Y. T. Kim, N. Merrifield, T. Zarchy, N. I. Brody and G. W. Siskind: Unpublished observations.

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