HAPTEN-SPECIFIC TOLERANCE

Preferential Depression of the High Affinity Antibody Response

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The treatment of guinea pigs with 2,4-dinitrophenyl (DNP)[1] derivatives of the copolymer of D-glutamic acid and D-lysine (D-GL) induces a state of specific immunologic tolerance in the recipient (1). The type of tolerance induced is unusual in that it is DNP specific. That is, challenge of such guinea pigs with a DNP conjugate of ovalbumin (DNP-OVA) elicits anti-DNP responses markedly smaller than those of animals which have not been pretreated with DNP-D-GL. The degree of depression in responsiveness is related to the dose of tolerogen, to the mode of immunization with DNP-OVA, and to the interval between the two events. A similar type of hapten-specific tolerance, in mice, has recently been described by both Hraba et al. (2) and by Golan and Borel (3).

The tolerant state, in this model, appears to be largely expressed in the precursors of antibody-secreting cells. The evidence for this view is the following:

(*a*) The number of DNP-specific antigen-binding lymphocytes in the lymph nodes of tolerized animals is markedly reduced (1). These antigen-binding lymphocytes are members of the bone marrow-derived (B) lymphocyte pool and include the precursors of antibody-secreting cells (4).

(*b*) The tolerance is hapten specific (1). Thymus-derived (T) lymphocytes acting as "helpers" in the activation of precursor cells are almost exclusively carrier or conjugate specific (5, 6); on the other hand, hapten-specific precursors of antibody-secreting cells are relatively common (4, 7). Hapten-specific tolerance thus implies precursor cell tolerance.

(*c*) Even when an excess of helper cells are provided through an immunization with carrier after tolerance induction with DNP-D-GL but before challenge with DNP-carrier conjugate, the subsequent anti-DNP response is markedly diminished (1). Thus, in a situation in which helper T cells are not limiting, the hapten-specific tolerance is easily demonstrable, again implying a defect in the population of precursors of antibody-secreting cells.

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[1] *Abbreviations used in this paper:* B lymphocytes, bone marrow-derived lymphocytes; DNP, 2,4-dinitrophenyl; DNP-GPA, dinitrophenylated guinea pig albumin; DNP-OVA, dinitrophenyl conjugate of ovalbumin; D-GL, copolymer of D-glutamic acid and D-lysine; PFC, plaque-forming cells; T lymphocytes, thymus-derived lymphocytes; TNP, trinitrophenyl.

(*d*) DNP-D-GL is either nonimmunogenic or only marginally immunogenic in guinea pigs, presumably because a pool of functional T cells specific for DNP-D-GL or for D-GL is lacking[2] (1). If functional T cells specific for the tolerogen are lacking, a T cell locus for tolerance would be excluded.

(*e*) Tolerant animals immunized to DNP-guinea pig albumin (DNP-GPA) have no deficit in delayed hypersensitivity and their lymphocytes transform normally in response to DNP-GPA in culture.[3]

This system of hapten-specific tolerance offers a model to test the hypothesis that the induction of tolerance, just as the activation of cells, depends upon the interaction of antigen and antibody-like surface receptors. In such a situation, a regimen leading to partial tolerance in the pool of precursors of antibody-forming cells should preferentially eliminate the precursor cells bearing high affinity receptors. Thus, a subsequent immune response should be characterized by the production of diminished amounts of antibody and the antibody which is produced should be of low affinity.

A previous study of the affinity of antibody produced by partially tolerant animals has revealed that such antibody is, indeed, of lower affinity than that produced by control immunized animals (8). In this study, however, tolerance was induced by the hapten-protein conjugate subsequently used for immunization and thus tolerance may have been induced in both the helper and precursor cell pools. Indeed, in view of the demonstration, in mice, that thymus cells are rendered tolerant by lower concentrations of antigen and appear to remain tolerant for longer periods of time than bone marrow cells (9), it seems likely that helper cell (T cell) tolerance existed in the previously investigated model. As helper cells play an important role in the activation of specific B cells (5, 6, 10–12a) and, moreover, may indirectly affect the affinity of the antibody produced (13), these previous studies cannot be interpreted unequivocally.

In the present studies, strain 13 guinea pigs were injected with 0.3 or 3.0 mg of DNP-D-GL, in divided doses, and immunized with DNP-GPA 2 wk or 2 months later. The number of plaque-forming cells (PFC) in the draining lymph nodes and the serum antibody levels were measured 10–11, 18, and 30–33 days after DNP-GPA immunization. In addition, the avidity of antibody secreted by PFC was measured by a hapten-inhibition technique which allows the enumeration of PFC producing antibody of any given avidity (14). The affinity of the serum antibody produced was determined by a Farr assay. Although the diminution in total antibody response was only modest, especially 2 months after tolerance induction, a very marked depression both in PFC secreting high affinity anti-DNP antibody and in high affinity serum anti-DNP antibody was noted. This result indicates a preferential tolerization of precursors bearing high affinity receptors.

Materials and Methods

Hapten-Carrier Conjugates.—A copolymer of D-glutamic acid and D-lysine (D-GL) was obtained from New England Nuclear Corp., Boston, Mass. Guinea pig albumin (GPA) was

[2] Davie, J. M., A. M. Kask, B. Cohen, W. E. Paul, and I. Green. Unpublished observations.

[3] Cohen, B., J. M. Davie, and W. E. Paul. Manuscript in preparation.

prepared from strain 13 guinea pig serum by the method of Schwert (15). Dinitrophenyl conjugates of these materials, DNP_{53}-D-GL and DNP_{23}-GPA, were prepared as previously described (1, 16). Subscripts refer to the number of moles of hapten per mole of carrier.

Animals, Tolerance Induction, and Immunization.—Adult strain 13 guinea pigs were obtained from the Division of Research Services of the National Institutes of Health. Hapten-specific tolerance was induced by the intraperitoneal injection of either 1.0 mg or 0.1 mg of DNP_{53}-D-GL in saline on 3 successive days (1). 2 wk or 2 months after tolerance induction, tolerant animals and untreated animals were immunized by injection, into the footpads, of 50 μg of DNP_{23}-GPA emulsified in 0.4 ml of complete Freund's adjuvant (Difco Laboratories, Inc., Detroit, Mich.). On days 10–11, 18, and 30–33 after immunization, animals were exsanguinated and draining lymph nodes were removed. Single cell suspensions were prepared by teasing the lymph nodes in minimal essential medium (Grand Island Biological Co., Rockville, Md.) supplemented with 10% fetal bovine serum (Industrial Biological Laboratories, Rockville, Md.). Tissue fragments were allowed to settle; cells which remained in suspension were washed three times in fresh medium.

Detection of Anti-DNP Antibody-Secreting Cells and Determination of the Avidity for Hapten of the Secreted Antibody.—Anti-DNP antibody-secreting cells were detected by a modification of the localized hemolysis-in-gel technique, using trinitrophenyl (TNP)-coated sheep erythrocytes (17); rabbit polyvalent anti-guinea pig immunoglobulin antiserum was used for facilitation of hemolysis. Only facilitated plaques were measured.

The avidity for hapten of the antibody secreted by the plaque-forming cells (PFC) was determined by a hapten-inhibition method (14). ϵ-DNP-L-lysine incorporated into the agar can compete with TNP-erythrocytes for anti-DNP antibody and thereby inhibit the formation of plaques. Plaques inhibitable by low concentrations of free hapten represent high avidity antibody formers; plaques requiring high concentrations of hapten for inhibition represent low avidity antibody-forming cells. This is based upon the fact that high affinity antibodies may be saturated by low concentrations of hapten and thus prevented from binding to and lysing the TNP-erythrocytes. Low affinity antibodies, on the other hand, require high ligand concentrations for saturation and thus PFC secreting low affinity antibody are inhibited by high, but not low, concentrations of ϵ-DNP-L-lysine incorporated in the agar. By utilizing a series of ligand concentrations, increasing at tenfold steps, the number of PFC secreting antibody of any given avidity can be enumerated. Thus, the PFC secreting the highest avidity antibody are those inhibited by 10^{-7} M ϵ-DNP-L-lysine. The number of these cells is determined by subtracting the number of PFC in the presence of 10^{-7} hapten from the number detected with no inhibitor present. The PFC secreting antibody of the next highest avidity range are those inhibited by 10^{-6} M ϵ-DNP-L-lysine but not by 10^{-7} M ϵ-DNP-L-lysine. In this case the number of PFC detected in the presence of 10^{-6} M hapten is subtracted from the number detected in the presence of 10^{-7} M hapten. We routinely evaluate PFC in five avidity ranges; the lowest are those which are not inhibited by 10^{-4} M ϵ-DNP-L-lysine and the highest are those inhibited by 10^{-7} M hapten. A detailed analysis of this method and its relation to the affinity of serum anti-DNP antibody has been published (14).

Binding Capacity and Affinity of Serum Anti-DNP Antibody for ϵ-DNP-L-Lysine.—The affinity of serum anti-DNP antibody for ϵ-DNP-L-lysine was evaluated by the comparison of antigen-binding capacity in a Farr assay (18) at a standard concentration of bound and free ligand with that at several other concentrations of bound and free ligand. ϵ-DNP-L-lysine-^{3}H (14–25 Ci/mM), prepared as previously described (12, 19), at a final concentration of either 0.5×10^{-6}, 0.5×10^{-7}, or 0.5×10^{-8} M, was mixed with antiserum or antiserum diluted in 10% normal guinea pig serum. After 30 min, an equal volume of saturated ammonium sulfate was added; 30 min later the suspension was centrifuged at 2500 rpm at 4°C, and a measured aliquot of supernatant added to 6 ml of Aquasol (New England Nuclear Corp.). Radioactivity was measured in a liquid scintillation spectrometer. Per cent of ligand bound was determined

and the antigen-binding capacity then calculated according to the relation:

Antigen-binding capacity = (concentration of bound ligand × 1/dilution of antiserum required to yield such binding).

Affinity was determined by the following equation:[4]

$$K = \frac{R\cdot(B/F)_x\cdot B_i - (B/F)_i\cdot B_x}{(1\text{-}R)(B_i)(B_x)}$$

where, K is the association constant, R the ratio of antigen-binding capacity at any experimental concentration of bound and free ligand to antigen-binding capacity at index condition (0.333×10^{-6} M free ligand; 0.167×10^{-6} M bound ligand); $(B/F)_i$ is the ratio of bound to free ligand at index condition (0.5); $(B/F)_x$ is the ratio of bound to free ligand at test condition; B_i is bound ligand concentration at index condition (0.167×10^{-6} M); and B_x is bound ligand concentration at test condition. Antibody populations are evaluated by plotting log R against log K. For homogeneous antibodies, log K is independent of log R but for thermodynamically heterogeneous populations log K rises as log R decreases.

RESULTS

Pretreatment of guinea pigs with DNP-D-GL diminishes the subsequent response of these animals to DNP-proteins. Guinea pigs immunized with DNP-GPA 2 wk after treatment with a total of 3.0 mg of DNP-D-GL had fewer indirect PFC in their draining lymph nodes and their serum had a lower DNP-lysine binding capacity than nonpretreated controls. In the current experiments in which 50 μg of DNP-GPA emulsified in complete Freund's adjuvant was used as immunogen, the quantitative depression of the response was only modest. Thus, 10–11 days after immunization, the animals pretreated with 3.0 mg of tolerogen had approximately 30% as many PFC as control animals and their serum DNP-lysine binding capacity, when tested at high (0.5×10^{-6} M) ligand concentration, was approximately 50% of that of control animals (Table I). Although affinity as well as concentration is important in antigen-binding capacity, the contribution of affinity is diminished at higher ligand concentrations and the values obtained with 0.5×10^{-6} M ε-DNP-L-lysine more closely reflect antibody concentrations than do values obtained with lower ligand concentrations. Guinea pigs pretreated with 0.3 mg of DNP-D-GL were intermediate between the control and the 3.0 mg group in both numbers of PFC and their serum DNP-lysine binding capacity. With increasing time after immunization, the degree of depression in the group pretreated with 3.0 mg of DNP-D-GL increased so that 30–33 days after immunization these animals had 10.8% as many PFC as the control group and their serum DNP-lysine binding capacity was 15.0% of control.

If challenge is delayed until 2 months after pretreatment with DNP-D-GL, little depression in total PFC in draining lymph nodes is seen although the

[4] Paul, W. E. Manuscript in preparation.

serum DNP-lysine binding capacity is still depressed in both groups of tolerized guinea pigs.

The avidity of antibody secreted by individual PFC was measured by the ε-DNP-L-lysine inhibition technique described in Materials and Methods. The data is reported as the number of PFC per 10^6 cells which are inhibited at a given ligand concentration but not at a tenfold lower concentration. The highest

TABLE I

Degree of Tolerance in Guinea Pigs Pretreated with DNP-D-GL

Interval between tolerance induction and immunization	Time after immunization	Pretreatment	Indirect PFC		Serum ε-DNP-L-lysine binding capacity*			
					0.5×10^{-6} M Ligand		0.5×10^{-8} M Ligand	
	days		*per 10^6 cells*	*% of Control‡*	*10^{-6} M*	*% of Control‡*	*10^{-6} M*	*% of Control‡*
2 wk	10–11	0	642 ± 133§	—	8.0§	—	0.49§	—
		0.3 mg DNP-D-GL	452 ± 94	70.3	5.2	65.0	0.19	38.8
		3.0 mg DNP-D-GL	201 ± 28	31.3	4.1	51.3	0.19	38.8
	18	0	139 ± 50	—	21.7	—	3.2	—
		0.3 mg DNP-D-GL	101 ± 4	73.0	10.8	49.8	0.68	21.3
		3.0 mg DNP-D-GL	37 ± 5	26.4	5.2	24.0	0.25	7.8
	30–31	0	253 ± 20	—	28.0	—	7.6	—
		0.3 mg DNP-D-GL	116 ± 25	45.8	20.3	72.5	3.9	51.3
		3.0 mg DNP-D-GL	27 ± 3	10.8	4.2	15.0	0.20	2.6
2 months	18	0	840‖		35.0¶	—	4.84	—
		0.3 mg DNP-D-GL	325		10.4	29.7	0.76	15.7
		3.0 mg DNP-D-GL	530		7.4	21.1	0.23	4.8
	32–33	0	61; 170		38.8	—	18.5	—
		0.3 mg DNP-D-GL	78		8.9	22.9	1.7	9.2
		3.0 mg DNP-D-GL	70; 146		8.3	21.4	0.65	3.5

* Serum DNP-lysine binding capacity determined utilizing 0.5×10^{-6} M or 0.5×10^{-8} M ligand concentrations.

‡ Value for cells or serum from pretreated animals/value for cells or serum from control animals × 100.

§ Indirect PFC are presented as arithmetic mean ± standard error; antigen-binding capacity is determined on pool of serum from the same animals.

‖ Values for individual animals.

¶ Arithmetic mean of individual values from two or three animals in each group.

avidity PFC are those inhibited by 10^{-7} M ε-DNP-L-lysine; the lowest avidity PFC are those not inhibited by 10^{-4} M ε-DNP-L-lysine.

In those guinea pigs immunized 2 wk after tolerance induction, there was a striking depression in the number of PFC secreting high avidity antibody (Fig. 1). At 10–11 days after immunization, animals tolerized with 3.0 mg of DNP-D-GL had only slightly fewer low avidity PFC than did either the 0.3 mg tolerant group or the control group. However, the 3.0 mg pretreated group had no PFC in the two highest avidity sets although the control animals had large numbers of PFC in both ranges. The animals pretreated with 0.3 mg were suppressed only in the PFC of the highest avidity set and, thus, were intermediate between the control and the 3.0 mg tolerant group. 18 days after

immunization, the tolerized groups still had no PFC in the highest avidity range. When tested 30–33 days after priming, high avidity PFC (inhibitable by 10^{-7} M ϵ-DNP-L-lysine) were present in the 3.0 mg tolerized group. However, the frequency of these cells was considerably less than that found for the 0.3 mg tolerized group; in turn, both tolerant groups had considerably fewer high affinity PFC than did the control animals. In each group, evidence of affinity

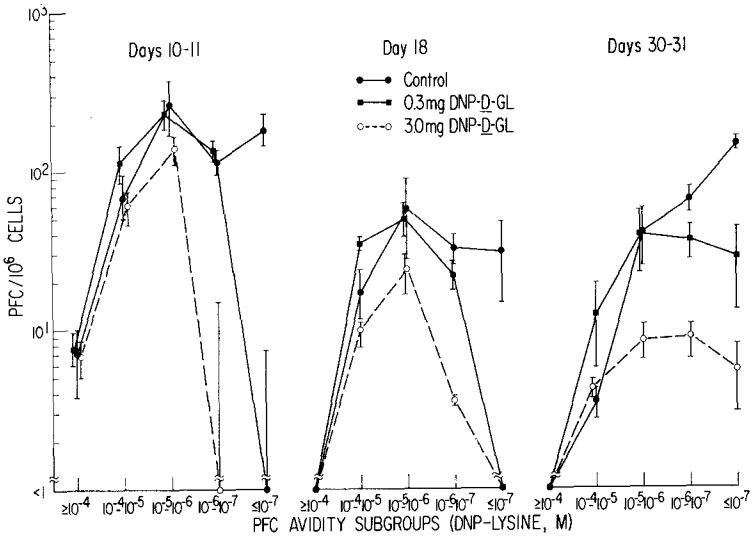

FIG. 1. Distribution of PFC among avidity groups in animals immunized 2 wk after tolerance induction. The ordinate represents the number of PFC per 10^6 cells in each avidity group. The abscissa represents the various avidity groups. Each point is the arithmetic mean obtained from the study of cells from three animals. The brackets represent the range encompassed by ± 1 standard error. The group $\leq 10^{-7}$ is the highest avidity group and represents those PFC inhibitable by 10^{-7} M ϵ-DNP-lysine. The group 10^{-6}–10^{-7} is the next highest avidity group and represents those PFC inhibitable by 10^{-6} M ϵ-DNP-L-lysine but not by 10^{-7} M ϵ-DNP-L-lysine. Each successively lower avidity group is indicated in a similar way.

"maturation" of the population of antibody-secreting cells was noted in that the frequency of low and medium avidity PFC diminished with time, while that of high avidity PFC changed relatively little in the control animals and rose in the tolerant animals.

Analysis of the binding characteristics of the serum antibody of this group of animals also demonstrated a marked diminution in the affinity of anti-DNP antibody in the tolerized groups (Fig. 2). As was the case with the PFC, the 3.0 mg tolerant group had antibody of the lowest affinity, the 0.3 mg tolerant group had antibody of intermediate affinity, and the control animals had the highest affinity antibody. In one respect, the antibody data differed from the PFC data. That is, the PFC data revealed the most striking avidity

differences between tolerized and control groups at the earliest time point (10–11 days), whereas the difference in serum antibody affinity seemed to increase with time. One possible explanation for this apparent discrepancy is that we have placed an arbitrary upper limit on the avidity of the PFC we have enumerated. It is possible that had we utilized 10^{-8} M and 10^{-9} M ε-DNP-

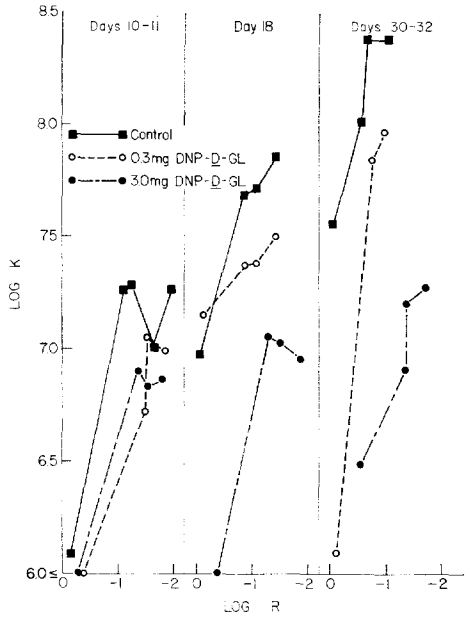

FIG. 2. Affinity of serum anti-DNP antibody from animals immunized 2 wk after tolerance induction. Each determination was performed on a pool of serum from two or three animals. For thermodynamically heterogeneous antibody preparations, log K rises as log R diminishes. A comparison of log K for any given value of log R allows a comparison of the affinity of two antibody preparations to be made.

L-lysine for inhibition, the PFC data would have more closely resembled the antibody data.

The data obtained from the study of animals 2 wk after tolerance induction suggests a marked degree of tolerance in the pool of precursor cells with high affinity receptors and little or no tolerance among precursors with low affinity receptors. In order to obtain information on the stability of this tolerant state, we immunized guinea pigs 3 months after tolerance induction. 10–11 days after immunization, animals which had been rendered tolerant with 3.0 mg of DNP-D-GL had no PFC secreting antibody of the highest affinity (inhibitable by 10^{-7} M ε-DNP-L-lysine) but they did possess essentially normal numbers of PFC inhibitable by 10^{-6} M ε-DNP-L-lysine (Fig. 3). In this respect, then, the tolerance in the 2-months group involved only the more avid of the two sets

depressed at 2 wk. Moreover, the 0.3 mg tolerant group demonstrated an essentially normal avidity distribution of PFC whereas, when immunized 2 wk after tolerance induction, this group had a selective depression in the highest avidity PFC. At 18 and at 32–33 days after immunization, the picture was essentially similar to that at 11 days. That is, the 3.0 mg tolerant group had absent or diminished numbers of high avidity PFC whereas the 0.3 mg tolerant

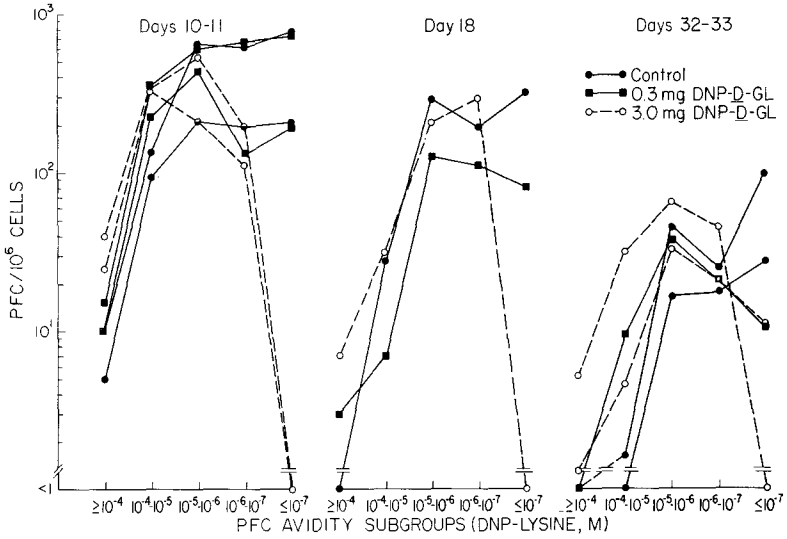

FIG. 3. Distribution of PFC among avidity groups in animals immunized 2 months after tolerance induction. The ordinate represents the number of PFC per 10^6 cells in each avidity group. The abscissa represents the various avidity groups. Each curve represents results from an individual animal. The group $\leq 10^{-7}$ is the highest avidity group and represents those PFC inhibitable by 10^{-7} M ϵ-DNP-L-lysine. The group 10^{-6}–10^{-7} is the next highest avidity group and represents those PFC inhibitable by 10^{-6} M ϵ-DNP-L-lysine but not by 10^{-7} M ϵ-DNP-L-lysine. Each successively lower avidity group is indicated in a similar way.

animals were essentially normal. As before, the low avidity PFC diminished, with time, in all groups although some were still detectable in cell populations from the tolerant animals at 18 and at 32–33 days after immunization.

The analysis of serum anti-DNP antibody in the groups immunized 2 months after tolerance induction is presented in Fig. 4. The affinity of the serum antibody in the 3.0 mg tolerant group is markedly lower than that of the control animals. Moreover, the 0.3 mg tolerant animals have serum anti-DNP antibody of intermediate affinity although that was not obvious from the analysis of avidity of PFC.

Thus, the animals immunized 2 months after tolerance induction had a depressed response even more selective than did the 2-wk animals. This suggests that tolerance in the pool of precursors of intermediate avidity was lost, per-

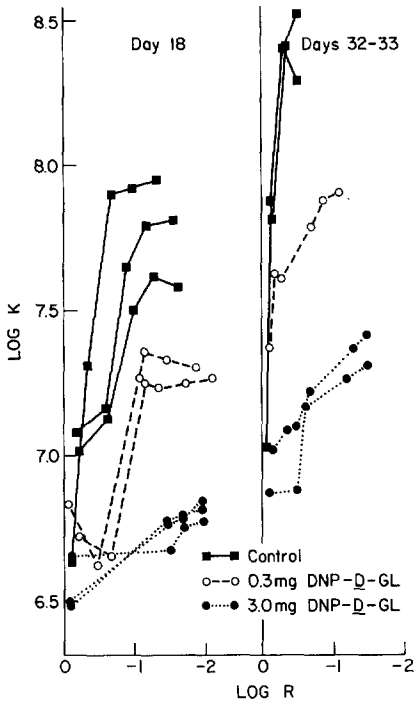

FIG. 4. Affinity of serum anti-DNP antibody from animals immunized 2 months after tolerance induction. Each curve represents results from an individual animal.

haps through replenishment from bone marrow stem cells, while the tolerant state was preserved in the pool of precursors of highest avidity.

DISCUSSION

In the experiments reported here, a DNP derivative of a copolymer of D-glutamic and D-lysine induced a state of partial tolerance demonstrable by immunizing the treated animals with a DNP derivative of guinea pig albumin. In view of the hapten-specific nature of this tolerance and our previous demonstration that animals tolerized in this manner had diminished numbers of lymphocytes capable of binding DNP-GPA-^{125}I (1), a tolerant state exists in the population of precursors of anti-DNP antibody-synthesizing cells. As the copolymer of D-glutamic acid and D-lysine is either nonimmunogenic or only weakly immunogenic in guinea pigs (1), it seems likely that thymus-derived lymphocytes specific for this compound are lacking or not functional and that tolerance induction occurs because specific B lymphocytes encounter antigen without a simultaneous influence from thymus-derived lymphocytes. In that context, the induction of tolerance should be dependent on the concentration of tolerogen and the affinity for tolerogen of the receptors of the precursor

cell. The data presented here are in keeping with such a model. Tolerance induction with DNP-D-GL preferentially depressed the high affinity response to DNP-GPA. When guinea pigs were immunized 2 wk after tolerance induction with 3.0 mg of DNP-D-GL, the PFC subsequently generated were markedly deficient in the two highest avidity categories whereas animals tolerized with 0.3 mg of DNP-D-GL showed depression only of the highest avidity PFC which we enumerated. Moreover, if 2 months elapsed between tolerance induction and immunization, the group tolerized with 3.0 mg of DNP-D-GL was depressed only in the highest avidity PFC and the 0.3 mg group was suppressed very little. The experiments of Chiller et al. (9) demonstrated that tolerance induced by deaggregated human gamma globulin persisted in the T cell population for more than 49 days, whereas bone marrow cell tolerance was lost by 49 days. Avidity measurements were not performed in that study, and the rapid loss of B cell tolerance could reflect replenishment with low avidity precursor cells or a selective recovery from a reversible state of tolerance dependent on the preferential loss of tolerogen from low affinity receptor sites. Our demonstration that specific tolerance existed for at least 2 months in the highest avidity precursor population raises the possibility that in the pool of high affinity B cells tolerance may be long lasting. On the other hand, the present model of tolerance induction utilizes a nonmetabolizable compound which may persist for 2 months or more at levels sufficient to maintain the tolerant state in the highest affinity B cell population or to tolerize newly emerging precursor cells with receptors of high affinity for DNP.

Let us now turn to certain possible objections to our model for the induction of tolerance by DNP-D-GL and of recovery from this tolerant state. Firstly, we have not formally proved that T lymphocytes specific for D-GL or for DNP-D-GL are not present in guinea pigs. Indeed, formal proof on this issue is probably not available in any current system as it requires enumeration of the specific T cell without stimulation of these cells. For T lymphocytes this is not yet routinely feasible. We have shown that DNP-D-GL does not induce a sustained anti-DNP antibody response or a state of cellular hypersensitivity nor is D-GL capable of acting as a helper or carrier molecule (1). Nonetheless, we cannot exclude the possibility that a class of suppressor T cells specific for DNP-D-GL are activated and that such cells play a role in the suppression of B cells. Definitive experiments to test this possibility are underway in mice.

The second objection which may be offered deals with the use of avidity distribution in PFC and affinity measurements in antibody molecules to evaluate the receptor characteristics of precursor cells. It seems quite clear that the binding properties of the receptor on the precursor cell and of the antibody secreted by the descendent are similar or identical (14). Furthermore, preliminary study of the binding of DNP-GPA-^{125}I by lymphoid cells in animals treated with DNP-D-GL before immunization indicates a lower affinity of

receptors when compared with the situation in control immunized guinea pigs. A similar finding has recently been observed by Möller and Mäkelä utilizing rosette formation as a measure of antigen binding.[5] However, population changes in the course of the immune response complicate extrapolation from the affinity characteristics of cells in immunized animals to those of the receptors on precursors in nonimmunized animals. In particular, it may be argued that increasing concentrations of antibody preferentially suppress low affinity precursors and that the lower avidity we observe is due completely or in part to such an antibody regulatory effect. Although ample evidence exists for the capacity of antibody to suppress low affinity precursors (20, 21), the regulatory effect of such antibody can hardly be responsible for the selective depression exhibited by tolerized animals which we have described in this report.

Thus, the prime difference exhibited by tolerized animals is the failure of high affinity PFC to appear rather than an abnormal retention of low affinity PFC. Moreover, serum anti-DNP antibody concentrations are only modestly depressed at 10–11 days after immunization. The combined effects of normal loss of low avidity PFC and absent or diminished numbers of high avidity PFC causes the degree of tolerance to increase with time after immunization. Finally, high avidity PFC are easily detectable at 6 days after immunization in normal animals, before the appearance of detectable serum anti-DNP antibody (14). Antibody regulation can hardly be important in the appearance of these cells. Thus, the lack of high avidity PFC 18 days after immunization in the tolerant groups strongly indicates a selective induction of tolerance in precursors bearing high affinity receptors.

Our data support the concept that, in the absence of thymus-derived lymphocytes, the interaction of an excess of antigen with precursors of antibody-secreting cells generally leads to a tolerant state and that the induction of tolerance depends on the interaction of some requisite number or proportion of cell receptors with antigen. Nonetheless, there are conditions, even in the absence of T cells, in which activation of precursors occurs (22, 23) but these conditions may be very precise particularly in terms of amount of antigen bound to cell receptors (24). In such a model, the T cell would be viewed as having a regulatory role and allowing a much wider range of antigen concentrations to be stimulatory for a given B cell than would be true in the absence of T cells.

SUMMARY

The induction of tolerance in guinea pigs with a 2,4-dinitrophenyl (DNP) derivative of a copolymer of copolymer of D-glutamic acid and D-lysine (D-GL) leads to a preferential depression of the capacity to produce high affinity

[5] Möller, E., and O. Mäkelä. 1972. Antigen binding cells in immune and tolerant mice. In press.

anti-DNP antibody in response to immunization with DNP-guinea pig albumin. Thus, immunization 2 wk after tolerance induction with 3 mg of DNP-D-GL results in an immune response in which individual plaque-forming cells (PFC) secreting high affinity anti-DNP antibody are absent and in which the affinity of circulating anti-DNP antibody is reduced. A similar, but less marked, suppression is seen when 0.3 mg of DNP-D-GL is used for tolerance induction. If immunization is delayed until 2 months after tolerance induction, then suppression is restricted to the highest avidity PFC group. Our data is consistent with a state of tolerance in the pool of precursors of anti-DNP antibody-secreting cells induced as a result of their interaction with DNP-D-GL in the absence of specific "helper" cells, which appear to be lacking for DNP-D-GL. In such a situation, the affinity of receptors on precursor cells for tolerogen and the concentration of tolerogen appear to be crucial determinants of whether an individual cell will become tolerant.

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