RECEPTORS ON IMMUNOCOMPETENT CELLS

III. Specificity and Nature of Receptors on Dinitrophenylated Guinea Pig Albumin-^{125}I-Binding Cells of Immunized Guinea Pigs

By JOSEPH M. DAVIE, M.D., ALAN S. ROSENTHAL, M.D., and WILLIAM E. PAUL, M.D.

(*From the Laboratory of Immunology and Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014*)

(Received for publication 7 April 1971)

The interaction of antigen with receptors on lymphoid cells is thought to be the key event in the specific activation of immunocompetent cells (1–3). Direct evidence for the existence of specific cell surface receptors has been obtained through the specific depletion of lymphoid cell populations with antigen linked to solid particles (4–7) and through the binding of radioiodinated antigen to the surface of individual lymphoid cells (8–11).

We have utilized these techniques to characterize the receptors possessed by different types of lymphoid cells in the guinea pig. In the preceding paper (7), we demonstrated that rare individual lymph node lymphocytes (~40/100,000) bind sufficient 2,4-dinitrophenyl–guinea pig albumin–^{125}I (DNP-GPA)[1] to their surface so that they may be enumerated by short-term radioautography. These cells have been referred to as antigen-binding cells (ABC) although no inference has been intended that other lymphoid cells do not exist which bind antigen in smaller quantities. The receptors of DNP-GPA–^{125}I ABC appeared to be immunoglobulin in nature and generally of the γ_2 heavy (H) chain class. Furthermore, these receptors were in large part specific for the ϵ-DNP-L-lysyl group as demonstrated by the capacity of the free hapten to inhibit binding of DNP-GPA–^{125}I to these cells and by the equivalent ability of DNP-GPA agarose beads, DNP bovine serum albumin (BSA) agarose beads, and DNP keyhole limpet hemocyanin (KLH) agarose beads to remove these cells from lymphoid populations.

Animals which have been immunized with DNP-GPA, emulsified in complete Freund's adjuvant, display cellular immune responses to DNP-GPA and have high serum concentrations of anti-DNP antibody. The lymphocytes which mediate an in vitro correlate of cellular immunity, the antigen-mediated stimulation of DNA synthesis, possess receptors with a more complex specificity than is true of the highly hapten-specific receptors of the DNP-GPA–^{125}I ABC of immunized animals. As

[1] *Abbreviations used in this paper:* ABC, antigen-binding cells; BSA, bovine serum albumin; CFA, complete Freund's adjuvant; DNP-GPA, dinitrophenyl-guinea pig albumin; HGG, human gamma globulin; KLH, keyhole limpet hemocyanin; MEM, minimal essential medium; RAGIG, rabbit polyvalent anti-guinea pig Ig.

evaluated by specific adsorption onto antigen-agarose bead conjugates, the receptors of these "cellular immunity" cells recognized elements of both hapten and carrier and required the presence of both for an effective interaction. Thus, DNP-GPA agarose removed these cells from a lymphoid cell population whereas both DNP-BSA agarose and *o*-nitrophenyl GPA agarose did not bind these cells (6).

In the current paper, we consider the nature of the receptors possessed by DNP-GPA–^{125}I ABC in DNP-GPA–immunized guinea pigs in order to determine whether the receptors of these cells resemble those of ABC in nonimmunized animals or those possessed by cells of immunized animals which mediate the in vitro DNA synthetic response. Our data reveal that the DNP-GPA–^{125}I ABC of immunized animals are similar to ABC in nonimmunized animals in that their receptors are highly hapten-specific and that anti-γ_2 immunoglobulin antibodies inhibit antigen binding to most cells. They differ from the ABC of nonimmune animals in that they are much more numerous and in that their receptors have a greater affinity for ϵ-DNP-L-lysine. These studies, coupled with our previous work (6), show that DNP-GPA–immunized guinea pigs possess two classes of specific lymphocytes with receptors of quite different fine specificity. It is likely that the two classes of cells have very different biologic functions.

Materials and Methods

Animals and Immunization.—Adult Hartley, strain 2 and strain 13 guinea pigs were obtained from the Division of Research Services of the National Institutes of Health. Animals were immunized by injection, into the four footpads, of a total of 50 μg of DNP-GPA (containing an average of 16 DNP groups/molecule) or of 100 μg of human gamma globulin (HGG) emulsified in 0.4 ml complete Freund's adjuvant (CFA) (Difco Laboratories Inc., Detroit, Mich.). The preparation of DNP-GPA has been described (6).

Preparation of Cells.—Suspensions of lymph node cells and of peripheral blood lymphocytes were prepared as described in the preceding paper (7).

Detection of Antigen-Binding Cells.—Antigen-binding cells were detected by radioautography of cells which had been incubated with ^{125}I-labeled DNP-GPA or HGG at 4°C in the presence of sodium azide (1 mg/ml), as described in detail in the preceding paper (7). Radioautographs were exposed for 1–4 days so that specific antigen-binding cells demonstrated 2–20 grains directly over their surface. By limiting the exposure time, the morphology of the ABC was not obscured by silver grains. The total amount of antigen bound to a cell suspension was determined by the amount of radioactivity which remained bound after an extensive washing procedure consisting of four cycles of centrifugation after layering the cells on fetal calf serum (7, 9).

Adsorption of Antigen-Binding Cells to Antigen-Coated Agarose Beads.—The preparation of antigen-coated agarose beads and the method of adsorption of cell suspensions to these beads have been described in detail (6). Briefly, agarose beads (Sepharose 2B, Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) were activated with cyanogen bromide and covalently linked to either DNP-GPA, DNP-BSA, or HGG. The beads were extensively washed and 2 ml of packed volume of beads were mixed with 200–800 $\times$ 10^6 lymphoid cells in a total volume of 5 ml of minimal essential medium (MEM) containing 10% fetal calf serum. The suspension was rotated horizontally at 1 revolution per second on a rotary shaker. Unadsorbed

cells were separated from beads by filtration through a thin pad of glass wool in a syringe barrel.

Measurement of Antigen-Stimulated DNA Synthesis.—Lymph node cells were cultured for 2 days in the presence of various concentrations of DNP-GPA or DNP-BSA as described in detail elsewhere (6). For the final 12 hr of culture, 1 μCi of thymidine-^{3}H (specific activity 2 Ci/mM, New England Nuclear Corp., Boston, Mass.) was added to each culture. The amount of thymidine-^{3}H incorporated into DNA was then determined. Stimulation of DNA synthesis by antigen was expressed as the ratio of the amount of thymidine-^{3}H incorporated into DNA in the presence of antigen to that amount incorporated in the absence of antigen. This is denoted as E/C.

Anti-Immunoglobulins.—The preparation of rabbit polyvalent anti-guinea pig immunoglobulin (RAGIG), rabbit anti-γ_2, rabbit anti-γ_1, and goat anti-μ as well as the isolation of guinea pig light and heavy chains was described in the preceding paper (7).

Antibody Determination.—Serum ϵ-DNP-L-lysine-binding capacity was determined using a Farr procedure (12, 13) with ϵ-DNP-L-lysine-^{3}H (10^{-8} M) as ligand. Data was calculated as previously described (7).

RESULTS

Effect of Immunization on the Frequency of Antigen-Binding Lymphocytes.—The preceding paper described the characteristics of the receptors of DNP-GPA–^{125}I–binding lymphocytes of nonimmune guinea pigs. These cells occur with a frequency of $\sim 0.39/10^3$ lymphocytes and are chiefly small lymphocytes (Table I). Guinea pigs sacrificed 2–4 wk after immunization with 50 μg of DNP-GPA in complete Freund's adjuvant have more than 100 times the frequency of antigen-binding cells in the draining lymph nodes than do nonimmune animals, and these cells morphologically are more diverse. Small lymphocytes are the most common cell type observed but both larger cells demonstrating intense cytoplasmic pyroninophilia and typical plasma cells which bind antigen are also encountered. The detailed morphology of these cells will be reported separately.[2] Since the characteristics of the antigen-binding receptors for these morphologically diverse cells have proven to be identical, our results will be presented as total ABC without regard to morphology.

The increased number of DNP-GPA–^{125}I ABC in immunized animals does not appear to be due to passive coating of cells by cytophilic antibody. This may be demonstrated by the failure of anti-DNP antibodies, either in vivo or in vitro, to bind to lymphocytes sufficiently tightly so that these cells may subsequently bind detectable amounts of DNP-GPA–^{125}I. Thus, incubation of lymph node cells from a nonimmunized guinea pig with serum from a DNP-GPA–immune animal for 2 hr at 4°C, followed by two washings resulted in no increase in the number of DNP-GPA–^{125}I ABC detected as compared to the same cells not incubated in antiserum (Table II). Infusion of 5 ml of serum from guinea pigs immunized 2–3 wk earlier with DNP-GPA in CFA into nonimmunized guinea pigs 18 hr before sacrifice of the latter animals led to no increase in DNP-GPA–^{125}I ABC in the lymph node lymphocytes of the re-

[2] Rosenthal, A. S., J. M. Davie, and W. E. Paul. Unpublished observations.

cipients as compared to control nonimmunized animals. It should be emphasized that the washing procedure utilized in these studies is extensive and any cytophilic antibody which may have been adsorbed is apparently washed away before the evaluation of antigen binding. Therefore, the antigen-binding ability

TABLE I

Effect of Immunization on Frequency of Antigen-Binding Cells

Lymphocytes	No. of animals	Small lymphocytes which bind DNP-GPA-^{125}I	Pyroninophilic* cells which bind DNP-GPA-^{125}I
		ABC/10^3 lymphocytes‡	ABC/10^3 lymphocytes
Nonimmune§	9	0.39 ± 0.06	0
Immune‖	10	46.5 ± 4.9	15.8 ± 2.6

* Pyroninophilic cells are defined as any lymphoid cell having 1/4 or more of its visible surface stained with pyronin.

‡ Mean and standard error.

§ Guinea pigs immunized 2–4 wk before with CFA or 50 μg HGG in CFA.

‖ Guinea pigs immunized 2–4 wk before with 50 μg DNP-GPA in CFA.

TABLE II

Effect of Passive Antibody on Antigen Binding

Cells	Radioactivity bound	Grains/cell (5 day exposure)		
		3–5	6–9	≥10
	cpm/10^6 cells	ABC/10^5 cells		
Control*	7.2	40	12	10
Control + Anti-DNP‡	7.7	18	4	8
Infused Anti-DNP§	5.6	10	2	2
	46.0	13	6	5

* Nonprimed, untreated lymph node cells.

‡ Control cells incubated 2 hr at 4°C with high titer anti-DNP antiserum; washed twice before addition of DNP-GPA-^{125}I.

§ Lymph node cells from two animals that received 5 ml of high titer anti-DNP antiserum 18 hr before sacrifice. A 1/10 dilution of recipients serum, at the time of sacrifice, bound 60% of 10^{-8} M DNP-lys-^{3}H.

of cells described in this study appears to be a characteristic of the cells themselves and is independent of humoral antibody.

Furthermore, antigen-binding cells in peripheral blood appear before circulating anti-DNP antibody can be detected. Thus, at day 5 after immunization the frequency of DNP-GPA-^{125}I ABC in peripheral blood is 0.6/1,000 lymphocytes, considerably greater than the frequency in nonimmunized animals (7), and 60-fold greater than their frequency 2 days after immunization (Fig. 1). Subsequently, the rate of increase in frequency of DNP-GPA-^{125}I ABC diminishes. On the other hand, serum anti-DNP antibody cannot be

detected on day 5 by a sensitive technique, the Farr method, and the greatest rate of increase of antibody activity appears to occur between days 8 and 12.

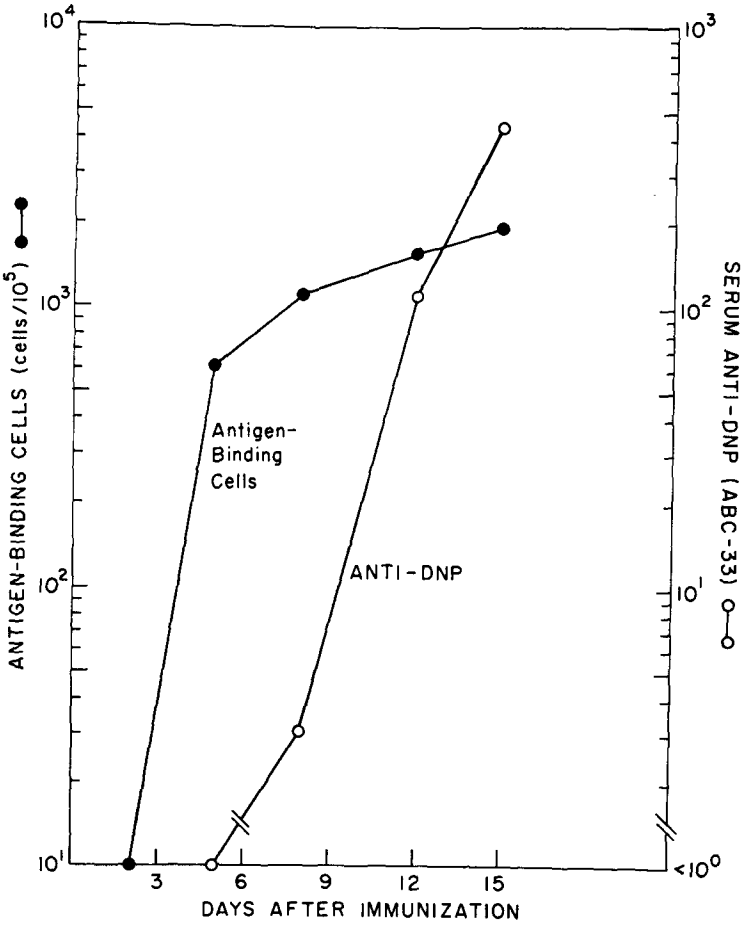

FIG. 1. Frequency of ABC in peripheral blood after immunization. Three Hartley guinea pigs were immunized by injection of 50 μg of DNP-GPA emulsified in CFA into the four footpads. At various times after immunization, the animals were bled from the retro-orbital sinus into heparinized tubes. Leukocytes were isolated and the frequency of ABC was determined as described in Materials and Methods. Serum anti-DNP antibody levels were also measured.

This data strengthens the contention that ABC are not cells with passively adsorbed antibody. Further, the temporal sequence in the appearance of ABC and serum antibody may reflect a transition of some, or all, ABC into antibody-secreting cells.

Receptor Specificity of ABC from Immune Guinea Pigs.—The first two papers of this series have outlined a dichotomy in the antigen-binding receptors on cells participating in an immune response to DNP-GPA. Thus, the binding of DNP-GPA–^{125}I to ABC of nonimmunized guinea pigs involves an interaction between an immunoglobulin receptor, generally of the γ_2 H chain class, with antigen, largely by virtue of ϵ-DNP-L-lysyl specificity. Similarly, anti-DNP

TABLE III

Agarose Depletion of DNP-GPA–^{125}I ABC from Immune Guinea Pigs

Expt	Agarose	DNP–GPA–^{125}I bound		ABC				
		cpm/10^6 cells	% Depletion	Total cells counted	grains/ABC 2–5	grains/ABC ≥ 6	grains/ABC Total	% Depletion*
					ABC/10^3			
A	BSA	114	—	967	26	2	28	—*
	DNP-GPA	19	83	998	2	0	2	93
	DNP-BSA	22	81	1131	5	0	5	82
B	KLH	2573	—	892	113	20	133	—*
	DNP-GPA	407	84	1014	20	2	22	83
	DNP-BSA	208	92	1236	13	1	14	89
C	HGG	1178	—	1566	56	10	66	—*
	DNP-GPA	621	47	2082	12	2	14	79
	DNP-BSA	166	86	1662	2	0	2	97
D	HGG-^{125}I binding to HGG ABC							
	DNP-GPA			2802	14	2	16	—‡
	DNP-BSA			1512	7	3	10	38
	HGG			1930	3	0	3	81

* % Depletion for DNP-GPA ABC is:

$$100\left(1 - \frac{\text{ABC present after DNP protein agarose treatment}}{\text{ABC present after BSA, KLH, or HGG agarose treatment}}\right).$$

‡ % Depletion for HGG ABC is:

$$100\left(1 - \frac{\text{ABC present after HGG agarose or DNP-BSA agarose treatment}}{\text{ABC present after DNP-GPA agarose treatment}}\right).$$

antibody-secreting cells of immunized guinea pigs bind to antigen agarose beads with a high degree of DNP specificity. On the other hand, the lymphoid cells of DNP-GPA–immunized guinea pigs which mediate in vitro DNA synthetic responses to DNP-GPA appear to have receptors which require elements of hapten and carrier for antigen-receptor interaction.

A study was therefore made of the specificity of receptors of DNP-GPA–^{125}I ABC of immunized guinea pigs. First, the relative capacity of DNP-GPA agarose beads and DNP-BSA agarose beads to specifically remove DNP-GPA–^{125}I ABC from immune cell populations was determined. In each in-

stance, this was compared to the number of such cells present after adsorption with an agarose containing a non-dinitrophenylated protein. DNP-GPA agarose caused, in three experiments (Table III), a 93, 83, and 79% depletion of DNP-GPA–^{125}I ABC whereas DNP-BSA agarose caused 82, 89, and 97% depletion. A comparison of the amount of DNP-GPA–^{125}I bound to these various cell populations yielded similar results. Thus, no detectable difference between the two DNP agarose conjugates could be demonstrated in the deple-

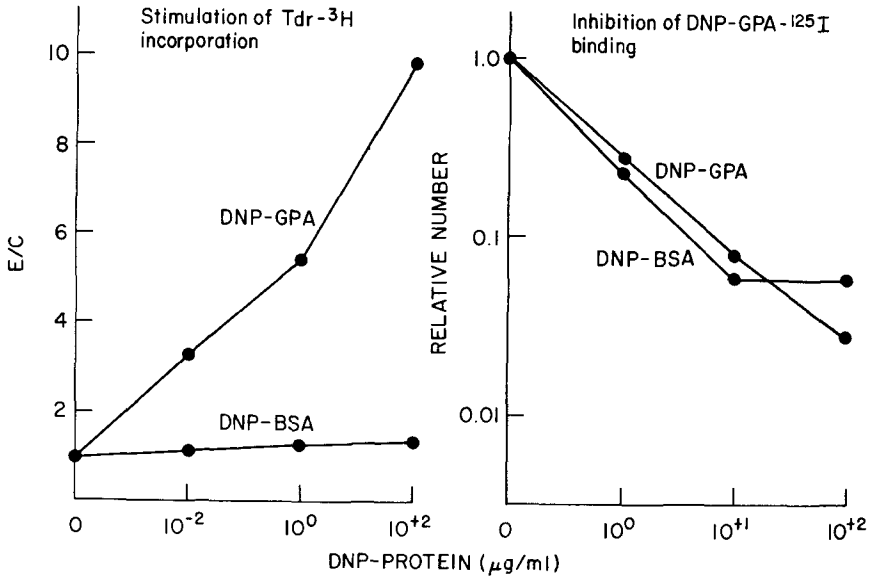

FIG. 2. Comparison of DNP-GPA and DNP-BSA in their ability to stimulate in vitro DNA synthesis by lymph node cells from a DNP-GPA–immune guinea pig and to inhibit ABC from this animal from binding DNP-GPA–^{125}I. Lymph node cells from a guinea pig immunized 3 wk previously with 50 μg of DNP-GPA in CFA were tested for stimulation of thymidine-^{3}H incorporation into DNA by DNP-GPA or DNP-BSA (shown on the left). Relative stimulation of antigen stimulated cultures compared to control cultures in the absence of antigen is denoted as E/C. On the right is the comparison of DNP-GPA and DNP-BSA in the ability to inhibit DNP-GPA ABC from binding DNP-GPA–^{125}I (0.2 μg/ml). Plotted is the relative number of ABC found at various concentrations of DNP-GPA or DNP-BSA.

tion of DNP-GPA–^{125}I ABC from immune cell populations suggesting that interaction with DNP groups was most important in the depletion. As a control, HGG-^{125}I ABC were markedly (81%) depleted by HGG agarose as compared to DNP-GPA agarose; a modest, nonspecific depletion was caused by DNP-BSA agarose.

Furthermore, DNP-BSA and DNP-GPA are equivalent in their capacity to inhibit the binding of DNP-GPA–^{125}I (0.2 μg/ml) to lymph node ABC of an

immunized animal (Fig. 2). This is in striking contrast to the failure of DNP-BSA to stimulate a DNA synthetic response by lymph node cells from the same animal in the face of a 10-fold stimulation by DNP-GPA. This comparison, plus the depletion studies of in vitro DNA synthetic responsiveness presented previously (6), illustrates the marked difference in receptor specificity of DNP-GPA–^{125}I ABC and of cells mediating a cellular immune response to DNP-GPA.

An additional test of the hapten specificity of the interaction of DNP-GPA–^{125}I with antigen-binding receptors of the lymphocytes under study is the capacity of ε-DNP-L-lysine to compete with DNP-GPA–^{125}I for binding to these receptors. Lymph node cells from guinea pigs immunized either 21 or 29 days earlier with DNP-GPA were incubated with a constant concentration of DNP-GPA–^{125}I (0.2 μg/ml) and varying concentrations ε-DNP-L-lysine (Table IV). Cells obtained from an animal immunized 21 days earlier were virtually completely inhibited in their binding of DNP-GPA–^{125}I by 5×10^{-3} M ε-DNP-L-lysine and were approximately 50% inhibited by 5×10^{-7} M. Cells obtained from guinea pig immunized 29 days earlier were almost completely inhibited in their binding of DNP-GPA–^{125}I by as little as 5×10^{-7} M and were 81% inhibited in their binding by 5×10^{-9} M. Most interestingly, 50% inhibition of DNP-GPA–^{125}I ABC from nonimmune animals requires more than 5×10^{-5} M

TABLE IV

Inhibition by DNP-Lysine of DNP-GPA–^{125}I Binding by Lymphoid Cells from Immune Guinea Pigs

Expt	Days after immunization	DNP lysine	Antigen-binding cells Grains/ABC				% Inhibition*
			3–5	6–9	≥ 10	Total	
		(M)	ABC/10^3				
A	Nonimmune	0	0.09	0.06	0.06	0.21	—
		5×10^{-5}	0.08	0	0.08	0.16	24
		5×10^{-3}	0	0.03	0	0.03	86
B	21	0	14	7	16	37	—
		5×10^{-9}	8	6	13	27	27
		5×10^{-7}	5	1	11	17	54
		5×10^{-5}	7	2	0	9	76
		5×10^{-3}	1	0	1	2	95
C	29	0	30	5	7	42	—
		5×10^{-9}	4	4	0	8	81
		5×10^{-7}	2	2	0	2	95
		5×10^{-5}	0	0	0	0	100

* % Inhibition is $100\left(1 - \frac{\text{Hapten treated}}{\text{Control}}\right)$.

ε-DNP-L-lysine. A clear trend of increasing capacity of ε-DNP-L-lysine to inhibit binding of DNP-GPA-^{125}I to ABC is noted as a result of immunization.

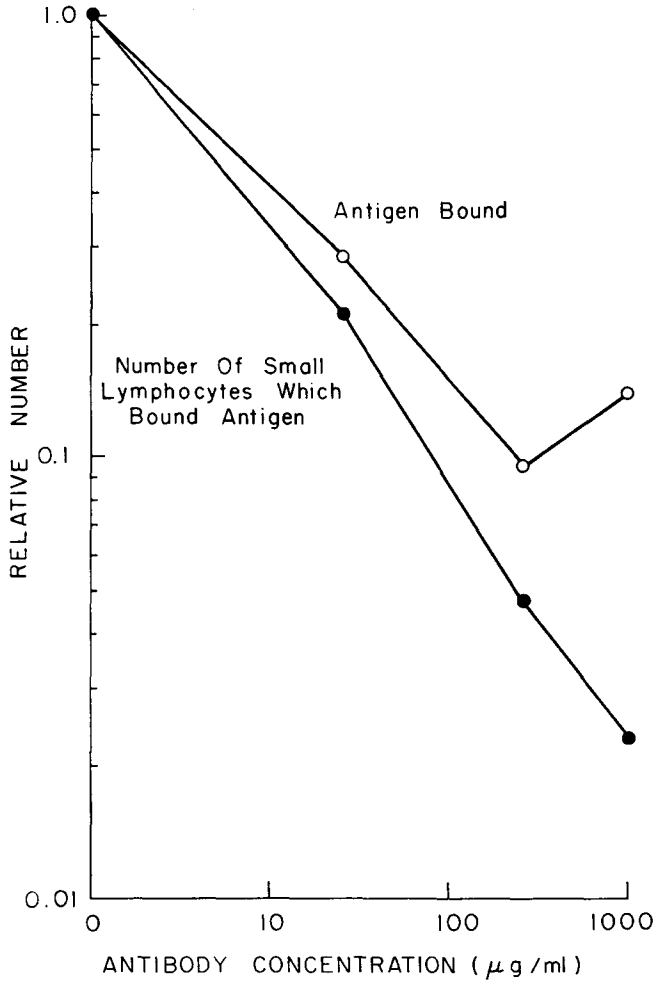

FIG. 3. Inhibition of DNP-GPA-^{125}I binding to ABC by RAGIG. The ability of RAGIG to inhibit DNP-GPA—^{125}I binding to lymphocytes from immune guinea pigs either when measured by the total antigen bound per 10^6 cells or as specific DNP-GPA-^{125}I ABC is demonstrated.

This most likely reflects an increase in affinity for ε-DNP-L-lysine of receptors possessed by DNP-GPA ABC during the course of immunization. An extensive study of this phenomenon and of its potential significance will be presented in a subsequent publication.[3]

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

Immunoglobulin Nature and Class of the DNP-GPA–^{125}I Antigen-Binding Receptors of Lymphocytes from DNP-GPA–Immune Guinea Pigs.—The hapten specificity of the receptors of DNP-GPA–^{125}I–binding cells is suggestive in itself that the receptor is similar to circulating antibody. In addition, the demonstration that the binding of DNP-GPA–^{125}I to receptors of lymphocytes from nonimmune guinea pigs may be inhibited by anti-immunoglobulin, and, particularly, anti-γ_2 serum, suggests that receptors are immunoglobulins,

TABLE V

Inhibition by Anti-Immunoglobulin of DNP-GPA–^{125}I Binding by Lymphoid Cells from Immune Guinea Pigs

Expt	Antiserum	Concentration of antibody	Total cells counted	ABC grains/ABC				% Inhibition*
				3–5	6–9	≥10	Total	
		μg/ml		ABC/10^3				
A	NRS‡	—	736	29	7	7	43	—
	Polyvalent	1000	852	1	0	0	1	98
	Polyvalent	250	810	3	0	0	3	93
	Polyvalent	25	802	9	0	0	9	79
B	NRS	—	475	30	6	13	49	—
	Anti-γ_2	500	441	9	0	0	9	82
	Anti-γ_1	500	470	30	2	6	38	22
	Anti-μ	1350	440	52	2	2	56	−14
C	NRS	—	511	30	20	8	58	—
	Anti-γ_2	500	512	4	0	0	4	93

* % Inhibition is $100\left(1 - \frac{\text{Antiserum treated)}}{\text{NRS treated}}\right)$.

‡ NRS, normal rabbit serum.

primarily of the γ_2 class. When varying concentrations of rabbit polyvalent anti-guinea pig immunoglobulin are added to lymph node suspensions from immunized guinea pigs before and during exposure of the cells to DNP-GPA–^{125}I, a striking inhibition both of the number of ABC which bind sufficient DNP-GPA to be detected and of the absolute amount of antigen bound is seen (Fig. 3). 25 μg/ml of RAGIG inhibits by 72% the amount of DNP-GPA–^{125}I bound and diminishes the number of ABC detected by 79%.

The relative distribution of immunoglobulin classes among the DNP-GPA–^{125}I receptors can be estimated by competitive binding between DNP-GPA–^{125}I and antibodies specific for the various heavy chain classes. Table V summarizes several experiments. Experiment A presents the data for RAGIG illustrated in Fig. 3. Anti-γ_1 and anti-μ (Experiment B) appear to block only a small percentage, if any, of receptors from binding antigen. However, anti-γ_2

(Experiments B and C) appears to be particularly effective in inhibition of antigen binding. 50 μg/ml of anti-γ_2 antibody blocks half the ABC from binding DNP-GPA-^{125}I (Fig. 4). Higher concentrations are more effective. The blocking effect of the anti-γ_2 antiserum can be partially inhibited by 50 μg/ml of pure heavy chains but is unaffected by a comparable concentration of light

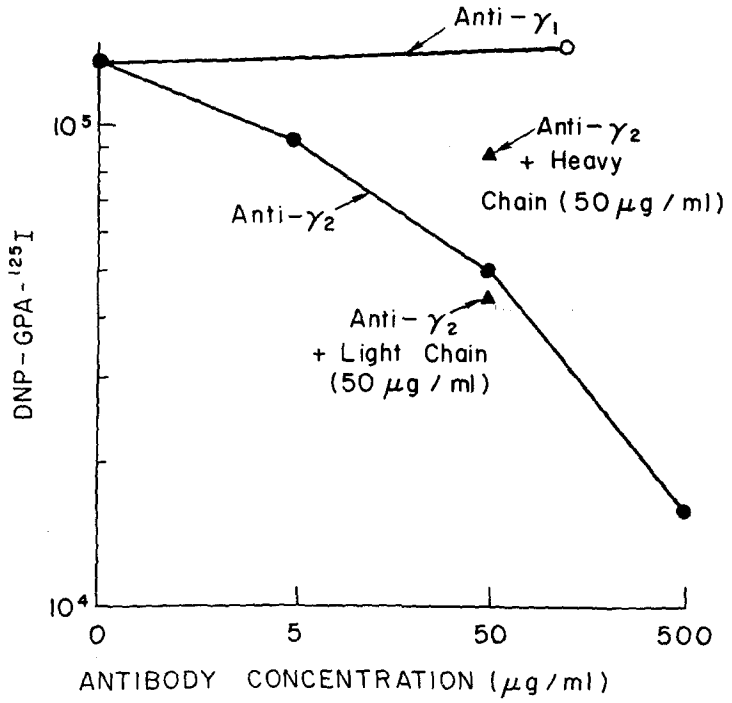

FIG. 4. Inhibition of DNP-GPA-^{125}I binding by anti-γ_1 and anti-γ_2. Lymph node cells from a guinea pig immunized 3 wk previously with DNP-GPA were exposed to DNP-GPA-^{125}I in the presence or absence of anti-γ_1 or anti-γ_2. The amount of antigen bound per 10^6 cells was determined. The two filled triangles (▲) represent the amount of antigen bound when 50 μg/ml of either heavy chain or light chain was included in suspensions containing 50 μg/ml of anti-γ_2 antibody.

chains. Therefore, it is clear that the majority DNP-GPA-^{125}I ABC from guinea pigs immunized with DNP-GPA in complete Freund's adjuvant have γ_2 immunoglobulin on their surface. It is most likely that this immunoglobulin is the surface receptor for this antigenic determinant. It has previously been reported (14) that immunization with antigens in CFA favor production of γ_2 antibodies. One would expect that immunization protocols which favor the synthesis of γ_1 anti-DNP antibodies would lead to the presence of a significant number of DNP-GPA ABC with γ_1 determinants on their surface. This prediction remains to be tested.

DISCUSSION

In this and the two preceding papers of this series (6, 7) we have outlined the specificity characteristics of antigen-binding receptors on several cell types. Cells which bind sufficient DNP-GPA–^{125}I to their surface to be detected by radioautography have been called DNP-GPA–^{125}I antigen-binding cells. These cells, present in low numbers in nonimmune animals and in much greater frequency in immunized animals, possess surface immunoglobulin molecules and bind DNP-GPA through receptors with a high degree of hapten specificity. Further, anti-Ig and anti-γ_2 sera prevent most ABC from binding DNP-GPA–^{125}I suggesting that the receptors are immunoglobulin and that most contain γ_2 type heavy chains. The binding of DNP-GPA–^{125}I to receptors of ABC of immunized animals is exquisitely sensitive to inhibition by ϵ-DNP-L-lysine. The receptors of DNP-GPA–^{125}I ABC of nonimmune animals, although inhibitable by ϵ-DNP-L-lysine, require much greater concentrations for inhibition. This difference is consistent with the increase in affinity which occurs in the population of serum anti-DNP antibody molecules during the course of immunization (3, 15) and likely reflects increase in the affinity for ϵ-DNP-L-lysine of the receptors of ABC due to selective stimulation of precursors bearing high affinity receptors.

Anti-DNP antibody-secreting cells are bound by DNP-agarose conjugates equally well whether GPA or BSA is the protein carrier (6) and it has been shown that anti-Ig inhibits binding of antibody-secreting cells to antigen-glass bead columns (16). Thus, ABC and antibody-secreting cells appear to have similar surface receptors and these receptors resemble circulating antibody molecules in that they are immunoglobulins, they are hapten specific, and an avidity increase occurs in the course of the immune response. ABC therefore have receptors consistent with those postulated for precursors of antibody-forming cells (1–3); that is, the receptors of such cells should be essentially equivalent to the antibody to be secreted by the descendants of the precursor cells. Finally, specific removal of DNP-GPA–^{125}I from a population of nonimmune lymphoid cells markedly diminishes the capacity of the population to transfer primary immune responses to DNP-KLH to an irradiated syngeneic recipient (7). This provides biologic evidence for the likelihood that most ABC are precursors of antibody-forming cells.

As shown in an earlier paper, cells from DNP-GPA–immunized guinea pigs, which mediate in vitro antigen-stimulated DNA synthetic responses, have very different specificity (6). Lymphoid cell populations from animals sensitive to DNP-GPA are depleted of responsive cells by DNP-GPA agarose and not by DNP-BSA agarose. This is in striking contrast to the character of the ABC presented in this paper. DNP-GPA–^{125}I ABC, obtained from the same lymphoid organ of animals immunized in the same way, are adsorbed equally

well by DNP-GPA agarose, DNP-BSA agarose, and DNP-KLH agarose. Furthermore, although ε-DNP-L-lysine has little capacity to inhibit the binding to DNP-GPA agarose of cells mediating a cellular response to DNP-GPA, very low concentrations of ε-DNP-L-lysine inhibit binding of DNP-GPA–^{125}I to ABC. Finally, DNP-BSA and DNP-GPA inhibit binding of DNP-GPA–^{125}I to ABC equally well, whereas DNP-GPA stimulates DNA synthesis on the part of the cellular immune cells but DNP-BSA has little or no effect.

As pointed out in the discussion of the previous paper (7), the fact that DNP-GPA–^{125}I ABC and cells involved in a cellular immune response to DNP-GPA have different specificity characteristics implies either that the cellular immune cells bind too little antigen to be detected by the radioautographic technique or that the cellular immune cells are a minor subpopulation within the DNP-GPA–^{125}I ABC. It seems more likely that the former explanation is correct in view of the demonstrated segregation of peripheral lymphocytes into a population bearing easily demonstrable amounts of surface Ig and one with either no Ig or too little to be detected by standard immunofluorescent and radioautographic methods (7, 17). Further, the absence of sufficient Ig on thymocytes to be detected by these methods (7, 17, 18) also suggests the paucity of this molecule on cellular immune cell surfaces, as such cells are apparently derived from the thymus (19, 20).

This clear delineation of a distinction in specificity of receptors possessed by two cell types, in the same animal, involved in an immune response to DNP-GPA gives a cellular basis to the well-known distinction in the specificity of serum anti-hapten antibody and of hypersensitivity reactions mediated by antibody, on the one hand, and of cellular immune responses, such as delayed hypersensitivity, on the other (21). Further, the helper cells which are either required for or markedly enhance the stimulation of precursors of antibody-forming cells by antigen, very likely have surface receptors resembling cellular immune cells (22), although a direct demonstration of this has not yet been obtained.

We have in the past suggested (6, 20) that the difference in specificity of receptors of these cell types in immunized animals might be a selection phenomenon and that the two cell types might have generally similar receptors in nonimmunized animals. In view of the requirement for carrier function in primary responses (23, 24), it was postulated that if a common prototype receptor existed it would be similar to that of the cellular immune cells in immune animals. The demonstration in the previous paper that the DNP-GPA–^{125}I ABC of nonimmune guinea pigs have hapten-specific receptors renders this thesis very unlikely. We are now attempting to directly assess the receptors of precursors of cellular immune cells in nonimmunized animals to complete the symmetry of this experiment but it would seem highly unlikely that these cells would not be carrier specific.

SUMMARY

Guinea pigs immunized with 2,4-dinitrophenyl–guinea pig albumin (DNP-GPA) possess lymphocytes which specifically bind sufficient DNP-GPA–^{125}I to their surface to be detected by radioautography. These lymphocytes are present in the draining lymph nodes in a frequency of ~50/1000 lymphocytes in animals immunized 2–4 wk earlier with DNP-GPA in complete Freund's adjuvant. Nonimmunized animals have ~0.4 DNP-GPA antigen-binding cells (ABC) per 1000 lymphocytes. An increase in the frequency of DNP-GPA ABC in peripheral blood is detectable by 5 days after immunization, which is before the time that serum anti-DNP antibody is measurable.

The receptors of these ABC are hapten specific in that free ϵ-DNP-L-lysine, at low concentration, inhibits the binding of DNP-GPA–^{125}I; DNP bovine serum alumbin (DNP-BSA) is equivalent to DNP-GPA in the inhibition of binding of DNP-GPA–^{125}I to ABC; and both DNP-GPA agarose beads and DNP-BSA agarose beads specifically adsorb DNP-GPA–^{125}I ABC. Anti-immunoglobulin antisera, particularly anti-γ_2 sera, inhibit the binding of DNP-GPA–^{125}I to these cells implying that the receptors are immunoglobulin, primarily of the γ_2 heavy chain class.

DNP-GPA–^{125}I ABC appear to represent precursors of antibody-secreting cells and have specificity characteristics which are very different from cells, of similarly immunized guinea pigs, which mediate a cellular immune response to DNP-GPA.

We thank Barbara Johnson and Robert McCleskey for assistance with these experiments.

REFERENCES

1. Burnet, F. M. 1959. The Clonal Selection Theory of Acquired Immunity. Vanderbilt University Press, Nashville, Tenn. 209.
2. Mitchison, N. A. 1967. Antigen recognition responsible for the induction *in vitro* of the secondary response. *Cold Spring Harbor Symp. Quant. Biol.* **32**:431.
3. Siskind, G. W., and B. Benacerraf. 1969. Cell selection by antigen in the immune response. *Advan. Immunol.* **10**:1.
4. Wigzell, H., and B. Andersson. 1969. Cell separation on antigen-coated columns. Elimination of high rate antibody-forming cells and immunological memory cells. *J. Exp. Med.* **129**:23.
5. Truffa-Bachi, P., and L. Wofsy. 1970. Specific separation of cells on affinity columns. *Proc. Nat. Acad. Sci. U.S.A.* **66**:685.
6. Davie, J. M., and W. E. Paul. 1970. Receptors on immunocompetent cells. I. Receptor specificity of cells participating in a cellular immune response. *Cell. Immunol.* **1**:404.
7. Davie, J. M., and W. E. Paul. 1971. Receptors on immunocompetent cells. II. Specificity and nature of receptors on dinitrophenylated guinea pig albumin-^{125}I–binding lymphocytes of normal guinea pigs. *J. Exp. Med.* **134**:495.
8. Naor, D., and D. Sulitzeanu. 1967. Binding of radioiodinated bovine serum albumin to mouse spleen cells. *Nature* (*London*). **214**:687.
9. Byrt, P., and G. L. Ada. 1969. An *in vitro* reaction between labelled flagellin or

haemocyanin and lymphocyte-like cells from normal animals. *Immunology*. **17:** 503.

10. Humphrey, J. H., and H. U. Keller. 1970. Some evidence for specific interaction between immunologically competent cells and antigens. *In* Developmental Aspects of Antibody Formation and Structure. J. Šterzl and I. Riha, editors. Academia, Publishing House of the Czechoslovak Academy of Sciences, Prague. **2:**485.
11. Dwyer, J. M., and I. R. Mackay. 1970. Antigen-binding lymphocytes in human blood. *Lancet*. **1:**164.
12. Farr, R. S. 1958. A quantitative immunochemical measure of the primary interaction between I*BSA and antibody. *J. Infec. Dis.* **103:**239.
13. Green, I., B. Benacerraf and S. H. Stone. 1969. The effect of the amount of mycobacterial adjuvants on the immune response of strain 2, strain 13 and Hartley strain guinea pigs to DNP-PLL and DNP-GL. *J. Immunol.* **103:**403.
14. Benacerraf, B., Z. Ovary, K. J. Bloch, and E. C. Franklin 1963. Properties of guinea pig 7S antibodies. I. Electrophoretic separation of two types of guinea pig 7S antibodies. *J. Exp. Med.* **117:**937.
15. Eisen, H. N., and G. W. Siskind. 1964. Variations in affinities of antibodies during the immune response. *Biochemistry*. **3:**996.
16. Walters, C. S., and H. Wigzell. 1970. Demonstration of heavy and light chain antigenic determinants on the cell-bound receptor for antigen. Similarities between membrane-attached and humoral antibodies produced by the same cell. *J. Exp. Med.* **132:**1233.
17. Raff, M. C., M. Sternberg, and R. B. Taylor. 1970. Immunoglobulin determinants on the surface of mouse lymphoid cells. *Nature (London)*. **225:**553.
18. Coombs, R. R. A., B. W. Gurner, C. A. Janeway, Jr., A. B. Wilson, P. G. H. Gell, and A. S. Kelus. 1970. Immunoglobulin determinants on the lymphocytes of normal rabbits I. Demonstration by the mixed antiglobulin reaction of determinants recognized by anti-γ anti-μ, anti-Fab and anti-allotype sera, anti-As4 and anti-As6. *Immunology*. **18:**417.
19. Cerottini, J. C., A. A. Nordin, and K. T. Brunner. 1970. Specific *in vitro* cytotoxicity of thymus-derived lymphocytes sensitized to alloantigens. *Nature (London)*. **228:**1308.
20. Stutman, O., E. J. Yunis, and R. A. Good. 1970. Studies on thymus function. I. Cooperative effect of thymic function and lymphohemopoietic cells in restoration of neonatally thymectomized mice. *J. Exp. Med.* **132:**583.
21. Paul, W. E. 1970. Functional specificity of antigen-binding receptors of lymphocytes. *Transplant. Rev.* **5:**130.
22. Paul, W. E., D. H. Katz, E. A. Goidl, and B. Benacerraf. 1970. Carrier function in anti-hapten immune responses. II. Specific properties of carrier cells capable of enhancing anti-hapten antibody responses. *J. Exp. Med.* **132:**283.
23. Green, I., W. E. Paul, and B. Benacerraf. 1966. The behavior of hapten–poly-L-lysine conjugates as complete antigens in genetic responder and as haptens in nonresponder guinea pigs. *J. Exp. Med.* **123:**859.
24. Schlossman, S. F., J. Herman, and A. Yaron. 1969. Antigen recognition: *in vitro* studies on the specificity of the cellular immune response. *J. Exp. Med.* **130:** 1031.