# RECEPTORS ON IMMUNOCOMPETENT CELLS

## II. Specificity and Nature of Receptors on Dinitrophenylated Guinea Pig Albumin-$^{125}$I-Binding Lymphocytes of Normal Guinea Pigs

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The existence of cell-associated antigen-binding receptors is central to selective theories of the immune response. Clonal selection theories further postulate that individual immunocompetent cells are committed, before their initial antigen contact, to respond to a limited range of antigens and that the commitment is expressed by the existence of surface antigen-binding receptors of a single specificity (1–3).

Direct evidence that such precommitted lymphocytes exist and bear detectable amounts of antigen-binding receptors has been obtained recently by several independent methods. Passage of lymphoid cells from nonimmunized mice over columns of glass beads to which a given antigen has been adsorbed diminishes the ability of the recovered cells to adoptively transfer primary immune responsiveness to that antigen to irradiated syngeneic mice (4). Similarly, incubation of lymphoid cells from nonimmune mice with radioiodinated antigens of high specific activity causes a specific diminution in the capacity of these cell populations to transfer responsiveness to that antigen to irradiated recipients (5). In the former instance, specific cells are believed to bind to glass bead-associated antigen by virtue of their receptors; in the latter case, the binding of highly radioactive antigen to the cell surface is thought to result in the death of the cell. Both methods appear to remove a class of antigen-specific cells by virtue of the fact that such cells possess a given antigen-binding receptor.

It has also been demonstrated that nonimmune animals possess small numbers of lymphoid cells which bind a given radioactive antigen to their surface and which can be visualized and enumerated by radioautography. Among the antigens for which such cells have already been demonstrated are bovine serum albumin (6), hemocyanin (7), flagellin (7), and a synthetic branched polypeptide consisting of iodotyrosine, glutamic acid, DL alanine, and lysine (8). Studies on flagellin antigen-binding cells of mice strongly suggest that the surface binding is dependent on cell-bound immunoglobulin, largely of the IgM type (9).

We have examined antigen-binding lymphocytes of guinea pigs with a view to defining the specificity and nature of the membrane-bound antigen receptors existing on various classes of immunocompetent lymphocytes. The first paper (10) of this series demonstrated that, in guinea pigs immunized to dinitrophenylated guinea pig albumin (DNP-GPA),[1] cells which mediate a cellular immune response (i.e. in vitro antigen-

[1] *Abbreviations used in this paper:* ABC, antigen-binding cell; BSA, bovine serum albumin;

stimulated DNA synthesis) possess receptors which have specificity encompassing elements of hapten and carrier. The characteristics of these receptors appear to account largely for the "carrier specificity" associated with cellular immune responses to hapten-carrier conjugates. Anti-hapten antibody-secreting cells obtained from the same animals possessed receptors which appeared to be highly hapten specific and, thus, to be similar to serum-type anti-hapten antibody.

In the present paper, we describe studies on lymphocytes, from nonimmunized guinea pigs, which bind sufficient DNP-GPA–$^{125}$I to their surfaces to be detected by radioautography. The receptors of these cells appear to be largely hapten specific and do not exhibit the high degree of carrier specificity which the receptors of cells mediating a cellular immune response displayed. Further, these relatively rare cells (~40/100,000 lymphocytes) are found in the lymph nodes, spleen, peripheral blood, and bone marrow, but not in thymus. In addition, most possess surface immunoglobulin (Ig) molecules, largely of the $\gamma_2$ class. The latter molecules appear to be the cellular receptors since anti-$\gamma_2$ serum inhibits antigen binding. Finally, the adoptive transfer of anti-DNP responsiveness by cells from nonimmunized donors into irradiated syngeneic recipients can be prevented either by adsorbing the cells onto DNP-protein agarose beads, in which the carrier is different from that used in subsequent immunization, or by killing the cells with anti-Ig antiserum and complement. Thus, at least one cell type involved in immune responsiveness has receptor characteristics similar to those of the DNP-GPA–$^{125}$I–binding cells.

## *Materials and Methods*

*Animals and Preparation of Cells.*—Adult Hartley, strain 2 and strain 13 guinea pigs were obtained from the Division of Research Services of the National Institutes of Health. Animals which were used either had not been immunized or had received, 2–4 wk previously, injections into the footpads of 0.4 ml of complete Freund's adjuvant-saline emulsions.

Cell suspensions were prepared from lymph nodes, spleen, bone marrow, and thymus by teasing the tissues in minimal essential medium (MEM) (Grand Island Biological Co., Rockville, Md.) supplemented with 10% fetal bovine serum (Industrial Biological Laboratories, Rockville, Md.). Tissue fragments were allowed to settle; the cells which remained in suspension were washed twice and resuspended in medium containing sodium azide (1 mg/ml). Peripheral blood leukocytes were prepared by layering heparinized blood over a solution of 1.25% methyl cellulose in 12.5% sodium diatrizoate (Hypaque-M, 90%, Winthrop Laboratories, New York) (11). Erythrocytes aggregated and sedimented in this solution. The leukocyte-rich upper phase was removed and centrifuged at 1000 rpm for 10 min; the sedimented cells were washed twice in MEM with serum and resuspended in the same medium containing azide.

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C, rabbit complement; CFA, complete Freund's adjuvant; DNP-GPA, dinitrophenylated guinea pig albumin; Fl, fluorescein; HGG, human gamma globulin; KLH, keyhole limpet hemocyanin; MEM, minimal essential medium; OVA, ovalbumin; PBS, phosphate-buffered saline; RAGIG, rabbit polyvalent anti-guinea pig Ig.

*Antigens, Agarose Conjugates, and Iodination.*—The preparation of dinitrophenylated derivatives of guinea pig albumin ($DNP_{16}$-GPA or $DNP_{23}$-GPA), keyhole limpet hemocyanin ($DNP_9$-KLH), bovine serum albumin ($DNP_{19}$-BSA), and the coupling of conjugates to agarose beads (Sepharose 2B, Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) have been described previously (10). Subscripts refer to the degree of derivatization (moles DNP per mole protein or, in the case of KLH, moles DNP per 100,000 molecular weight units).

DNP-GPA–$^{125}$I and human gamma globulin-$^{125}$I (HGG-$^{125}$I) were prepared by the chloramine-T method (12). Ovalbumin-$^{125}$I (OVA-$^{125}$I) was prepared utilizing $KI_3$ as previously described (13). Several preparations of each antigen were used during the course of this study. Specific activities of DNP-GPA–$^{125}$I and HGG-$^{125}$I varied from approximately 5 to 56 $\mu$Ci/$\mu$g and 13 to 30 $\mu$Ci/$\mu$g respectively.

*Antibodies.*—Antisera were produced in New Zealand white rabbits, obtained from the Division of Research Services of the National Institutes of Health, by multiple subcutaneous injections of purified guinea pig immunoglobulins emulsified in complete Freund's adjuvant. Rabbit polyvalent anti-guinea pig Ig (RAGIG) was produced by immunization of a rabbit with a total of 5 mg of guinea pig gamma globulin (Fraction II, Pentex Biochemical, Kankakee, Ill.) which had been passed over a diethylaminoethyl (DEAE)-cellulose column equilibrated with 0.05 M phosphate buffer, pH 7.6. The antiserum contained 4.90 mg of antibody/ml as determined by quantitative precipitin analysis. Immunoelectrophoresis and double diffusion in agar gel demonstrated that antibodies in the serum were directed primarily to light (L) chains and to the $\gamma_2$ class of heavy (H) chains.

H chains were prepared by reduction of the DEAE-purified immunoglobulin with 0.2 M mercaptoethanol for 1 hr at room temperature and alkylation with a 50% molar excess of recrystallized iodoacetamide (prepared by Dr. J. Inman). The chains were separated by filtration through G-100 Sephadex (Pharmacia) in 1 M acetic acid. Yields of heavy and light chains were the same as previously noted (14). A rabbit was immunized with a total of 3 mg of purified heavy chain. The resulting antiserum contained 1.24 mg of antibody/ml. As determined by immunoelectrophoresis and double diffusion in agar gel, this antiserum precipitated $\gamma_2$ H chains but failed to precipitate either $\gamma_1$ H chains or L chains. This antiserum has therefore been regarded as specific for $\gamma_2$ H chains. An anti-$\gamma_1$ antiserum was the gift of Dr. V. Nussenzweig. It was prepared by immunizing rabbits with purified anti-DNP antibodies and was rendered specific for $\gamma_1$ H chains by absorption. This antiserum contained approximately 1 mg of anti-$\gamma_1$ antibody/ml. Anti-$\mu$antiserum was raised in a goat by immunization with partially purified guinea pig macroglobulin and was made specific for $\mu$-determinants by absorption. This antiserum, a gift from Dr. R. Asofsky, contained 2.7 mg of antibody/ml. RAGIG and anti-$\gamma_2$ antibodies were specifically purified by adsorption onto immunoadsorbent columns of agarose to which purified guinea pig immunoglobulin had been covalently linked by the cyanogen bromide activation method (15). Purified antibodies were eluted from the columns with 1 M acetic acid and were dialyzed against phosphate-buffered saline (PBS). These antibodies were then fluoresceinated by the dialysis method of Clark and Shepard (16) using fluorescein isothiocyanate (Nutritional Biochemicals Corp., Cleveland, Ohio). The conjugates were not absorbed before use. Each fluorescein conjugate was kept at a concentration of 2 mg of antibody/ml in PBS containing sodium azide (1 mg/ml).

*Radioautography and Immunofluorescent Radioautography.*—Lymphoid cells (20–40 $\times$ $10^6$) were suspended in 0.2 ml of MEM which contained 10% fetal bovine serum and 1 mg of sodium azide/ml. $^{125}$I-labeled antigen (20–100 ng in 5–10 $\mu$l) was added. After 30 min incubation in an ice bath, the cell suspension was diluted to 1 ml with fresh medium and layered over 3 ml of fetal bovine serum in a 15 ml plastic culture tube (Falcon Plastics, Los Angeles, Calif.). The cells were sedimented by centrifugation at 1000 rpm for 10 min. The washing cycle through serum was repeated three more times. The amount of antigen adsorbed to the cells was determined by measuring the radioactivity of the final pellet in a gamma ray

spectrometer. The cells were smeared on gelatin-coated microscope slides, air dried, fixed for 30 min in 1% glutaraldehyde in PBS, and washed for 30 min in water. Slides were dipped in NTB-2 Nuclear Track Emulsion (Eastman Kodak, Rochester, New York) which had been diluted with an equal volume of water and heated to 48°C. The slides were stored in a light-free, stainless steel container *in vacuo* for appropriate exposure times (1–12 days) and developed in D-19 developer (Kodak). The cells were then stained with methyl green-pyronin and examined for silver grains.

Since the frequency of lymphocytes which bind antigen specifically is very low in nonimmune animals, it was impractical to count individually all cells which had been examined for the presence of grains. Rather, the total number of cells examined was determined by first counting 10 or more representative fields (in a 10 × 10 mm grid at × 400–500) and then counting the number of fields which were inspected for antigen-binding cells. An antigen-binding cell (ABC) was defined as a morphologically intact cell with three or more grains directly over it or on its margins. Cells in clumps were not considered due to the difficulty in assigning a given grain to a given cell.

Certain experiments (discussed in the Results section) utilized immunofluorescence in addition to radioautography. The details of this double evaluation method have been published previously (17). Briefly, $^{125}$I-labeled antigen was allowed to adsorb to cells as described above. After unadsorbed antigen was removed by sedimentation through serum, the cells were resuspended in 0.2 ml of MEM with 10% fetal bovine serum and 1 mg/ml of azide which contained purified, fluorescein-labeled antibody. The cell suspension was incubated at room temperature for 30 min and washed twice through serum. The cells were then processed as usual for radioautography but were not stained. Silver grains were identified as white dots by dark-field microscopy with a tungsten light source. After an antigen-binding cell was found, the tungsten light source was replaced with an ultraviolet source and the cell evaluated for fluorescence. Cell morphology was subsequently determined by staining with methyl green-pyronin, relocating the field, and examining the cell.

*Adsorption to Antigen-Agarose Conjugates.*—Cell suspensions were adsorbed to antigen-linked agarose beads in a batch procedure as described previously (10). In brief, 200–800 × $10^6$ lymphoid cells were mixed in a 15 x 60 plastic petri dish (Falcon) with about 2 ml of packed volume of antigen-coated agarose beads in a total volume of 5 ml of MEM with 10% fetal bovine serum. The suspension was rotated horizontally at about 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 5 ml syringe barrel. When large numbers of cells were required, as in the adoptive primary immunization experiments, the beads were removed by filtration through wire mesh (325 mesh size).

*Adoptive Primary Response.*—Lymphoid cell suspensions were prepared from spleen and lymph nodes of either strain 2 or strain 13 guinea pigs which had been injected 2–4 wk earlier with an emulsion of saline and complete Freund's adjuvant. The cells were either adsorbed with antigen-agarose conjugates (see previous section) or treated with anti-immunoglobulin. In the latter experiments, about 1.5 × $10^9$ cells were suspended in 2.5 ml of MEM containing 20% anti-$\gamma_2$, anti-$\mu$, RAGIG, or normal rabbit serum and incubated for 30 min at 37°C. Rabbit complement (C) (Grand Island Biological Co.) was then added to give a final concentration of 20% and the cells incubated a further 30 min. The cells were then washed three times in MEM and, as in experiments involving adsorption on antigen agarose beads, injected intravenously into syngeneic guinea pigs which had received 500 rads of irradiation from a Westinghouse 250 kvp machine which delivered 139 R/min with a penetrance of 0.9 mm of Cu hvl. The recipients were immunized the next day with either 50 $\mu$g of DNP-BSA, 50 $\mu$g of DNP-KLH, or 50 $\mu$g of DNP-KLH and 100 $\mu$g of OVA. The antigens were administered, as complete Freund's adjuvant emulsions, in the four footpads. On the 4th day after receiving the cells, the recipients were given either 50 × $10^6$ or 200 × $10^6$ syngeneic bone

marrow cells. The animals were bled at various times after immunization and serum binding capacity for ε-DNP-L-lysine or OVA determined by the Farr technique (18, 19) using ε-DNP-L-lysine-$^{3}$H ($1 \times 10^{-8}$ M) or OVA-$^{125}$I (4 μg/ml) as ligands. Antigen-binding capacities were determined by plotting the per cent binding of ligand against the log of serum dilution. The product of the serum dilution factor required for 33% binding and the bound antigen concentration at this dilution is antigen-binding capacity. Geometric means and standard errors were calculated and groups were compared by Student's $t$ test.

## RESULTS

The lymphoid organs of guinea pigs were found to contain rare cells which bound DNP-GPA–$^{125}$I to their surface as determined by the presence of silver

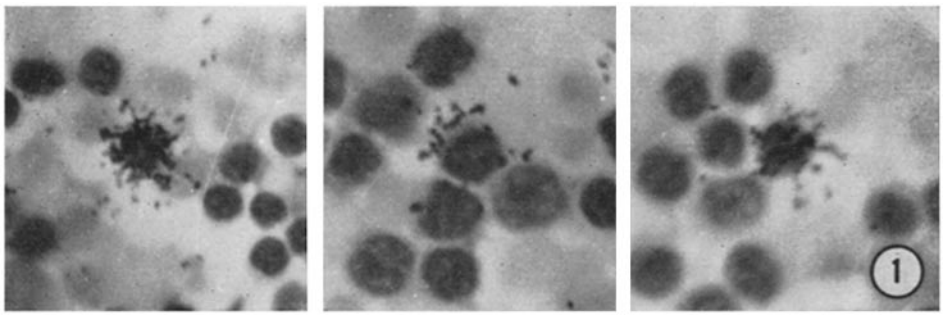


FIG. 1. DNP-GPA–$^{125}$I ABC among lymph node lymphocytes from nonimmune guinea pigs. (× 2700–3500). The ABC in the center panel has grains over its surface but, because of the focal plane of the photograph, only grains on the edge of the cell are visible.

grains over these cells. In animals which had not been immunized with DNP-GPA, these cells almost always had the appearance of small lymphocytes. Three typical DNP-GPA–$^{125}$I ABC are illustrated in Fig. 1. Plasma cells or large pyroninophilic cells binding DNP-GPA–$^{125}$I were rarely encountered in cell suspensions from these animals. The frequency of DNP-GPA ABC among lymph node lymphocytes of strain 2 guinea pigs injected with saline-adjuvant emulsions 2–4 wk previously was 39 ± 6 (mean ± SE) per 100,000 lymphocytes as determined from nine consecutive experiments. Although cells with as few as three silver grains were scored as positive, the bulk of the ABC generally had six or more grains over their surface and these grains were usually distributed diffusely.

DNP-GPA–$^{125}$I ABC were found in the lymph nodes, spleen, peripheral blood, and bone marrow of adult guinea pigs but were conspicuously absent from the thymus. Frequencies of DNP-GPA–$^{125}$I ABC (per 100,000 lymphocytes or thymocytes) in the various lymphoid organs of two adult strain 13 guinea pigs, which had not been immunized with any DNP compound, are presented in Table I. The thymus possessed fewer than one DNP-GPA ABC per 100,000

thymocytes; the lymph nodes, spleen, bone marrow, and peripheral blood possessed from 23 to 48 DNP-GPA ABC per 100,000 lymphocytes.

DNP-GPA-$^{125}$I ABC are much more frequent, in nonimmunized animals, than are antibody-secreting cells. The latter, as judged from the incidence of trinitrophenyl-specific plaque-forming cells in the spleen,[2] occur with frequency of less than $0.5/10^5$ cells.

*Specificity of Binding of DNP-GPA-$^{125}$I by ABC.*—The binding of DNP-GPA-$^{125}$I to specific ABC of nonimmunized animals can be shown to involve, to a considerable degree, an interaction of receptors with ε-DNP-L-lysyl groups. This can be demonstrated both by inhibition of antigen binding by

TABLE I

*Distribution of DNP-GPA-$^{125}$I-Binding Lymphocytes in Adult Guinea Pigs*

| Organ* | Total cells counted | Grains/ABC | | | Total ABC |
|---|---|---|---|---|---|
| | | 3–5 | 6–9 | ≥10 | |
| | | *Cells/$10^5$*‡ | | | |
| Thymus | >100,000 | 0 | 0 | 0 | < 1 |
| Lymph node | 59,040 | 5.1 | 11.9 | 16.9 | 33.9 |
| | 46,812 | 6.4 | 17.1 | 21.4 | 44.9 |
| | | | | | (38.6)§ |
| Spleen | 51,039 | 2.0 | 9.8 | 17.6 | 29.4 |
| | 53,300 | 9.4 | 16.9 | 11.3 | 37.6 |
| Bone marrow | 39,000 | 2.6 | 5.1 | 17.9 | 25.6 |
| | 48,010 | 8.3 | 8.3 | 6.2 | 22.8 |
| Peripheral blood | 54,500 | 12.8 | 12.8 | 22.0 | 47.6 |
| | 51,100 | 5.9 | 11.7 | 11.7 | 29.3 |

* From two adult strain 13 guinea pigs immunized with CFA 4 wk before sacrifice.

‡ Number of cells possessing the indicated number of grains per $10^5$ lymphocytes.

§ Mean number of ABC from nine strain 2 guinea pigs.

ε-DNP-L-lysine or by DNP-BSA and by specific removal of DNP-GPA-$^{125}$I ABC by agarose beads to which either DNP-GPA, DNP-BSA, or DNP-KLH has been covalently linked.

Table II demonstrates that the addition of $5 \times 10^{-3}$ M ε-DNP-L-lysine to lymph node cells before and during incubation with DNP-GPA-$^{125}$I diminished the number of lymphocytes which bound the radioiodinated antigen by 72–82% and caused a diminution in grain counts over cells which were scored as positive. No inhibition of binding of HGG-$^{125}$I to HGG ABC was caused by this hapten concentration.

The lack of significant carrier specificity of DNP-GPA-$^{125}$I receptors was shown when DNP-GPA and DNP-BSA proved equally capable of inhibiting

[2] Katz, D. H. Personal communication.

DNP-GPA–$^{125}$I binding to specific lymphocytes (Fig. 2). When 0.5 μg/ml of either $DNP_{23}$-GPA or $DNP_{19}$-BSA was mixed with 0.2 μg/ml of $DNP_{23}$-GPA–$^{125}$I during exposure to lymphocytes from nonimmune guinea pigs, approximately half the ABC were inhibited from binding sufficient DNP-GPA–$^{125}$I to be detected as ABC by radioautography. A 25-fold excess of nonradioactive DNP-BSA or DNP-GPA caused 85 and 90% inhibition of ABC, respectively. In addition to demonstrating the hapten-specific nature of the DNP-GPA–$^{125}$I-binding receptors, these results suggest that a concentration of

TABLE II

*Inhibition by DNP Lysine of DNP-GPA–$^{125}$I-Binding by Lymphocytes from Nonimmune Guinea Pigs*

| Expt* | Cells | Total cells counted | Grains/ABC | | | Total ABC | % Inhibition‡ |
|---|---|---|---|---|---|---|---|
| | | | 3–5 | 6–9 | ≥10 | | |
| | | | *Cells/$10^5$* | | | | |
| A | Control | 175,076 | 7.3 | 5.8 | 4.5 | 17.6 | — |
| | Hapten treated§ | 204,678 | 2.9 | 1.0 | 1.0 | 4.9 | 72 |
| B | Control | 42,765 | 18.7 | 25.7 | 16.3 | 60.7 | — |
| | Hapten treated | 49,691 | 8.1 | 8.1 | 0 | 16.2 | 82 |
| C | Control | 56,090 | 37.4 | 8.9 | 16.0 | 62.3 | — |
| | Hapten treated | 73,210 | 14.1 | 1.3 | 0 | 15.4 | 75 |
| D | HGG-$^{125}$I-binding cells | | | | | | |
| | Control | 52,800 | — | 5.7 | 1.9 | 7.6 | — |
| | Hapten treated | 54,000 | — | 5.6 | 1.9 | 7.5 | 1 |

* Exposure times for the experiments are A, 3 days; B, 8 days; C and D, 12 days.

‡ $100\left(1 - \frac{\text{Hapten treated}}{\text{Control}}\right)$.

§ $5 \times 10^{-3}$ M DNP lysine added to cells before the addition of the radiolabeled antigen.

0.2 μg/ml of DNP-GPA–$^{125}$I effectively saturates the receptors of at least the ABC seen by radioautography.

Agarose beads coated with DNP-GPA or with DNP on other carriers remove, essentially to the same degree, DNP-GPA–$^{125}$I ABC from lymph node cell populations. Thus, in three experiments (Table III), DNP-GPA–$^{125}$I ABC in cell populations incubated with DNP-GPA agarose were 94, 95, and 58% lower than in cell populations incubated with HGG agarose or with KLH agarose. Cell populations incubated with DNP-BSA agarose were diminished 79, 96, and 75% in their DNP-GPA ABC; cells incubated with DNP-KLH agarose were depleted by 95% in their DNP-GPA ABC. In one of these experiments, the incidence of HGG-$^{125}$I ABC was also determined; HGG agarose caused a 68% diminution of these cells as compared to DNP-GPA agarose, thus demonstrating the specificity of cell depletion and, further, showing

that DNP-GPA–$^{125}$I ABC and HGG-$^{125}$I ABC are largely independent cell populations.

The "hapten-specific" nature of the interaction of DNP-GPA with ABC stands in striking contrast to the specific antigen agarose depletion, in lymph node cell populations from immunized animals, of cells mediating an in vitro DNA synthetic response to DNP-GPA. In those experiments (10), incubation

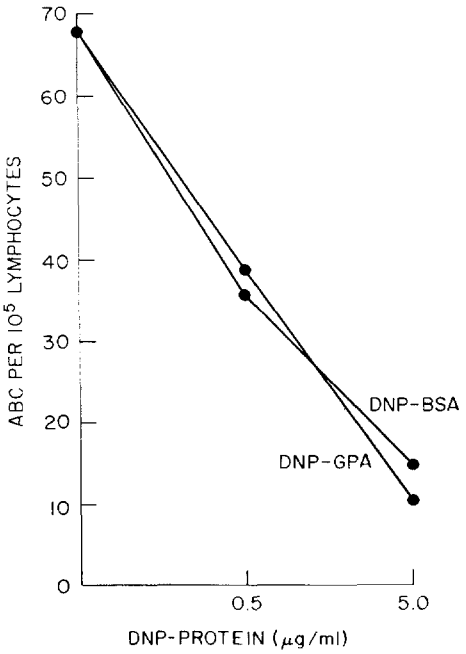


FIG. 2. Inhibition by DNP-GPA and DNP-BSA of DNP-GPA–$^{125}$I binding to lymphocytes from nonimmune guinea pigs. Shown is the frequency of DNP-GPA–$^{125}$I ABC when cells are incubated with 0.2 μg/ml of DNP-GPA–$^{125}$I alone or in association with various concentrations of DNP-GPA or DNP-BSA.

with DNP-GPA agarose caused striking diminution, in recovered cells, of the response to DNP-GPA whereas DNP-BSA agarose had no detectable effect.

In order to demonstrate that the hapten-specific depletion of DNP-GPA–$^{125}$I ABC in the current experiments had immunologic relevance, the capacity of lymph node and spleen cell suspensions which had been treated with various antigen-agarose bead conjugates to transfer primary immunologic responsiveness to irradiated syngeneic recipients was determined. Lymph node and spleen cell suspensions were prepared from inbred guinea pigs that 2 wk previously had been injected with a saline-adjuvant emulsion. Portions of the pooled cells were adsorbed with agarose beads coated with DNP-GPA, DNP-

KLH, KLH, or BSA as described above. The nonadsorbed cells were then injected intravenously into lethally irradiated (500 R) syngeneic recipients. The recipients were protected from radiation death by transfusion of syngeneic bone marrow cells 4 days after irradiation. The animals were immunized with 50 μg of DNP-KLH in complete Freund's adjuvant (CFA) (Expt A) or 50

TABLE III

*Agarose Depletion of Antigen-Binding Cells from Nonimmune Guinea Pigs*

| Expt | Agarose | Total cells counted | Grains/ABC | | | Total ABC | % Depletion |
|---|---|---|---|---|---|---|---|
| | | | 3–5 | 6–9 | ≥10 | | |
| | | | *Cells/10⁵* | | | | |
| A | DNP-GPA–$^{125}$I-binding cells | | | | | | |
| | HGG | 120,637 | 17.9 | 8.6 | 9.1 | 35.6 | 0 |
| | DNP-GPA | 142,360 | 2.1 | 0 | 0 | 2.1 | 94* |
| | DNP-BSA | 72,621 | 5.2 | 2.1 | 0 | 7.3 | 79 |
| | HGG-$^{125}$I-binding cells | | | | | | |
| | DNP-GPA | 178,457 | 7.5 | 3.5 | 5.4 | 16.4 | 0 |
| | HGG | 186,165 | 1.2 | 1.2 | 2.9 | 5.3 | 68‡ |
| B | DNP-GPA–$^{125}$I-binding cells | | | | | | |
| | HGG | 26,100 | 11.5 | 23.0 | 46.0 | 80.5 | 0 |
| | DNP-GPA | 73,200 | 0 | 4.1 | 0 | 4.1 | 95* |
| | DNP-BSA | 66,750 | 1.5 | 1.5 | 0 | 3.0 | 96 |
| | DNP-KLH | 80,100 | 3.7 | 1.2 | 0 | 4.9 | 94 |
| C | DNP-GPA–$^{125}$I-binding cells | | | | | | |
| | KLH | 105,000 | 14.0 | 5.0 | 5.0 | 24.0 | 0 |
| | DNP-GPA | 100,000 | 5.0 | 5.0 | 0 | 10.0 | 58* |
| | DNP-BSA | 105,000 | 5.0 | 1.0 | 0 | 6.0 | 75 |

* % depletion for DNP-GPA ABC is:

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

‡ % depletion for HGG ABC is:

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

μg of DNP-KLH plus 100 μg of OVA in CFA (Expt B) the day after they had received lymphoid cells; the ε-DNP-L-lysine–binding capacity of serum obtained 12–25 days later was measured. Table IV summarizes two such studies: experiment A employed strain 2 guinea pigs; experiment B, strain 13 guinea pigs. In experiment A, $400 \times 10^6$ agarose-depleted lymph node and spleen cells and $200 \times 10^6$ bone marrow cells were injected into recipients. 12 and 16 days after injection of lymph node and spleen cells, animals that had received KLH agarose-depleted cells had serum DNP lysine-binding

capacities considerably greater than animals which had only received bone marrow. Guinea pigs which had received DNP-GPA agarose-depleted cells demonstrated lower DNP lysine serum-binding capacities than the animals which had received KLH agarose-adsorbed cells. Indeed, at 16 days, the

TABLE IV

*Specific Depletion of Adoptive Primary Response by Antigen Agarose*

EXPERIMENT A

| Group | Treatment of transferred cells | No. of recipients | Serum DNP lys-binding capacity ($10^{-8}$ M) | |
|---|---|---|---|---|
| | | | Day 12 | Day 16 |
| A | KLH Agarose | 4 | 8.3 (6.4–10.7)* | 35.3 (23.2–53.6) |
| B | DNP-GPA Agarose | 4 | 2.9 (2.3–3.6) | 10.2 (8.3–12.5) |
| C | No cells | 4 | <0.33 | 7.0 (4.4–10.9) |

Strain 2 guinea pigs were irradiated and received $400 \times 10^6$ lymph node and spleen cells, treated as described, intravenously. 1 day later (day 1) they were immunized with 50 $\mu$g of DNP-KLH emulsified in CFA. $200 \times 10^6$ bone marrow cells were infused on day 4 and the recipients were bled on days 12 and 16.

* Geometric mean and range encompassed by $\pm$ 1 standard error. $P$ value for comparison of groups A and B at 12 days was <0.02 and at 16 days was <0.05.

TABLE IV

*Specific Depletion of Adoptive Primary Response by Antigen Agarose*

EXPERIMENT B

| Group | Treatment of transferred cells | No. of recipients | Serum antigen-binding capacity (day 25) | |
|---|---|---|---|---|
| | | | Anti-DNP ($10^{-8}$ M) | Anti-OVA ($\mu$g/ml) |
| A | BSA Agarose | 3 | 1847 (1600–2132)* | 27 (23–32) |
| B | DNP-GPA Agarose | 3 | 344 (297–399) | 37 (24–55) |
| C | DNP-KLH Agarose | 3 | 535 (375–762) | 36 (24–54) |
| D | No Cells | 3 | 384 (204–725) | 16 (13–18) |

Strain 13 guinea pigs were irradiated and received $400 \times 10^6$ lymph node and spleen cells, treated as described above, intravenously. 1 day later (day 1) they were immunized with 50 $\mu$g of DNP-KLH and 100 $\mu$g of OVA emulsified in CFA. $50 \times 10^6$ bone marrow cells were infused on day 4 and the recipients were bled on day 25.

* Geometric mean and range encompassed by $\pm$ 1 standard error. $P$ values for comparison of anti-DNP binding were A vs. B, <0.005; A vs. C, <0.05.

group which received DNP-GPA agarose-depleted cells was not statistically different from the group of animals that had received only bone marrow cells. Experiment B compares DNP-GPA agarose to DNP-KLH agarose in diminishing the capacity of cell populations to transfer a primary response to DNP-KLH. At day 25, animals which had received cells adsorbed with either DNP agarose had serum DNP-binding capacities not significantly different from those of animals which had received only bone marrow cells. At the same

time, no significant diminution of the primary response to OVA was found in DNP-depleted populations. A striking increase in the serum DNP lysine-binding capacity of all groups occurs in the course of the immune response, as may clearly be seen by comparing the results shown in Tables IV A and IV B. Nonetheless, the relative differences we have described between control and

TABLE V

*Inhibition by Anti-Immunoglobulin of DNP-GPA-$^{125}$I-Binding by Lymphocytes from Non-immune Guinea pigs*

| Expt | Antiserum* | Total cells counted | Grains/ABC | | | Total ABC | % Inhibition‡ |
|---|---|---|---|---|---|---|---|
| | | | 3–5 | 6–9 | ≥10 | | |
| | | | *Cells/10$^5$* | | | | |
| A | NRS§ | 74,368 | 12.1 | 9.4 | 17.5 | 39.0 | — |
| | RAGIG (2.5 mg/ml) | 66,400 | 15.1 | 1.5 | 0 | 16.6 | 57 |
| B | NRS | 56,238 | 10.7 | 7.1 | 24.9 | 42.7 | — |
| | Anti-$\gamma_2$ (500 μg/ml) | 56,017 | 1.8 | 1.8 | 1.8 | 5.4 | 87 |
| | Anti-$\gamma_1$ (500 μg/ml) | 48,600 | 6.2 | 8.2 | 24.7 | 39.1 | 8 |
| | Anti-$\mu$ (1.35 mg/ml) | 48,070 | 10.4 | 6.2 | 16.6 | 33.2 | 22 |
| C | NRS | 56,600 | 12.4 | 7.1 | 24.7 | 44.2 | — |
| | Anti-$\gamma_1$ (500 μg/ml) | 44,622 | 11.2 | 6.7 | 11.2 | 29.1 | 34 |
| | Anti-$\gamma_2$ (500 μg/ml) | 46,800 | 6.4 | 0 | 2.1 | 8.5 | 81 |
| | (50 μg/ml) | 49,500 | 2.0 | 2.0 | 4.0 | 8.0 | 82 |
| | (5 μg/ml) | 37,100 | 8.1 | 2.7 | 29.6 | 40.4 | 9 |

* Antiserum added before and during exposure to DNP-GPA-$^{125}$I.

‡ $100\left(1 - \frac{\text{antibody treated}}{\text{NRS}}\right)$.

§ NRS, normal rabbit serum.

specifically depleted groups are noted at each time shown here. These results substantiate the data obtained from morphological studies alone and show that the depletion of antigen-binding cells of nonimmune animals is accompanied by a specific diminution in the capacity of the cell population to transfer a primary immune response. The two sets of data, taken together, show that the receptors of these cells have a considerable degree of hapten specificity.

*Immunoglobulin Nature and Class of Receptors of DNP-GPA-$^{125}$I-Binding Lymphocytes.*—The demonstration that the receptors of most DNP-GPA-

$^{125}$I–binding cells have specificity for hapten indicates a similarity to the binding characteristics of serum anti-DNP antibody molecules; this similarity,

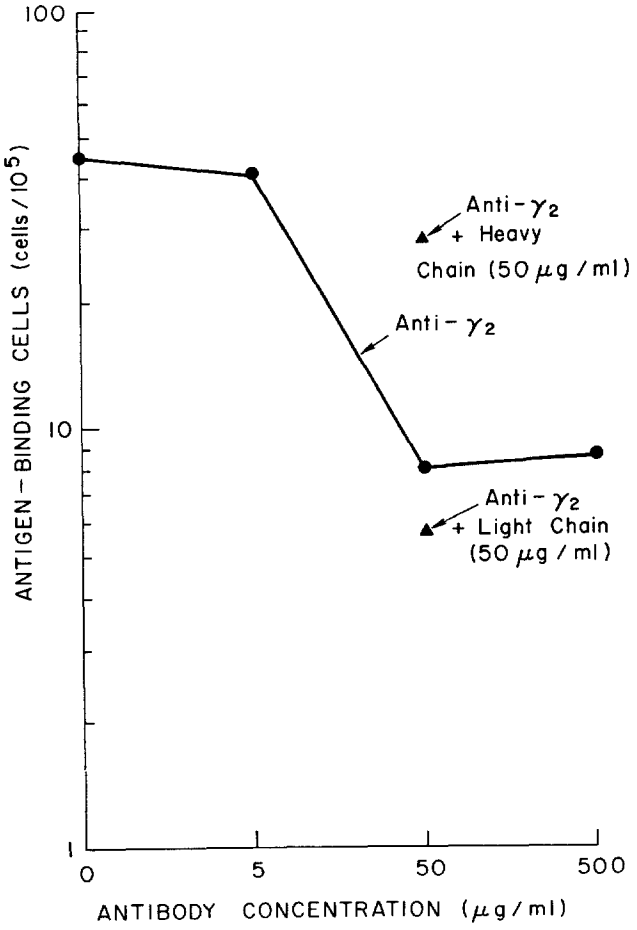


FIG. 3. Inhibition by anti-$\gamma_2$ of DNP-GPA–$^{125}$I binding to lymphocytes from nonimmune guinea pigs. Plotted is the frequency of DNP-GPA–$^{125}$I ABC when cells are exposed to various concentrations of anti-$\gamma_2$ antibody before and during incubation with DNP-GPA–$^{125}$I. The two filled triangles (▲) represent the number of ABC found when either 50 $\mu$g/ml of heavy chain or 50 $\mu$g/ml of light chain are added to 50 $\mu$g/ml of anti-$\gamma_2$ antibody before and during exposure to radiolabeled antigen and cells.

and a variety of other considerations (20–23), suggests that the receptors may be immunoglobulin. Experimental support for this concept derives from the capacity of anti-Ig antibody to inhibit the binding of DNP-GPA–$^{125}$I to ABC. Polyvalent rabbit anti-guinea pig Ig (2.5 mg/ml) was added to lymph node

cell suspensions from nonimmune guinea pigs before and during incubation with DNP-GPA–$^{125}$I. A considerable inhibition in the number of antigen-binding lymphocytes was seen and the number of grains per positive cell was diminished (Table V, experiment A). The polyvalent antiserum contained,

TABLE VI

*Effect of Anti-Ig and C on Adoptive Primary Response*

EXPERIMENT A

| Group | Treatment of transferred cells | No. of recipients | Serum DNP Lys-binding capacity ($10^{-8}$ M) | |
|---|---|---|---|---|
| | | | Day 16 | Day 25 |
| A | NRS + C | 4 | 392 (341–451)* | 936 (872–1005) |
| B | Anti-$\gamma_2$ + C | 5 | 98 (55–174) | 305 (235–397) |
| C | Anti-$\mu$ + C | 3 | 282 (180–443) | 1497 (1166–1923) |
| D | No cells | 4 | 32 (21–50) | 146 (97–219) |

Strain 13 guinea pigs were irradiated and received 400 × $10^6$ lymph node and spleen cells, treated as described above, intravenously. 1 day later (day 1) they were immunized with 50 $\mu$g of DNP-KLH emulsified in CFA. 50 × $10^6$ bone marrow cells were infused on day 4 and the recipients were bled on days 16 and 25.

* Geometric mean and range encompassed by ±1 standard error. $P$ value for comparison of Groups A and B were: >0.05; <0.1 on day 16 and <0.01 on day 25.

TABLE VI

*Effect of Anti-Ig and C on Adoptive Primary Response*

EXPERIMENT B

| Group | Treatment of transferred cells | No. of recipients | Serum DNP Lys-binding capacity ($10^{-8}$ M) Day 22 (individual values) |
|---|---|---|---|
| A | NRS + C | 2 | 160, 190 |
| B | RAGIG + C | 3 | 30, 20, 36 |
| C | No cells | 2 | 8, 25 |

Strain 2 guinea pigs were irradiated and received 500 × $10^6$ lymph node and spleen cells, treated as described above, intravenously. 1 day later (day 1) they were immunized with 50 $\mu$g of DNP-BSA emulsified in CFA. 50 × $10^6$ bone marrow cells were infused on day 4 and the recipients were bled on day 22.

primarily, antibodies directed to light chains and to the $\gamma_2$ class of heavy chains. To determine which, if any, of the heavy chain classes normally found in guinea pig serum immunoglobulin might be associated with the DNP-GPA–$^{125}$I–binding receptors of cells, inhibition experiments with specific anti-heavy chain sera were performed (Table V, experiment B). Anti-$\gamma_2$ antiserum markedly reduced the number of cells binding DNP-GPA–$^{125}$I, whereas anti-$\gamma_1$ caused little, if any, inhibition; anti-$\mu$, at higher concentration, inhibited far fewer cells from binding DNP-GPA than did anti-$\gamma_2$ serum. In a third experiment,

anti-$\gamma_2$ at 50 $\mu$g/ml inhibited 82% of the DNP-GPA-$^{125}$I-binding cells while anti-$\gamma_1$, in this cell population, inhibited about $\frac{1}{3}$ of the ABC. Purified light chains added to anti-$\gamma_2$ antiserum before the addition of DNP-GPA-$^{125}$I had no effect on the capacity of the serum to block antigen binding whereas pure heavy chains, at 50 $\mu$g/ml, inhibited by approximately 80% the blocking effect of 50 $\mu$g/ml anti-$\gamma_2$ (Fig. 3). This substantiates the fact that the anti-$\gamma_2$ antiserum acted by combining with heavy chain determinants on the antigen-binding cell, and rules out the possibility that undetected anti-light chain antibodies in the anti-$\gamma_2$ serum were responsible for the inhibition.

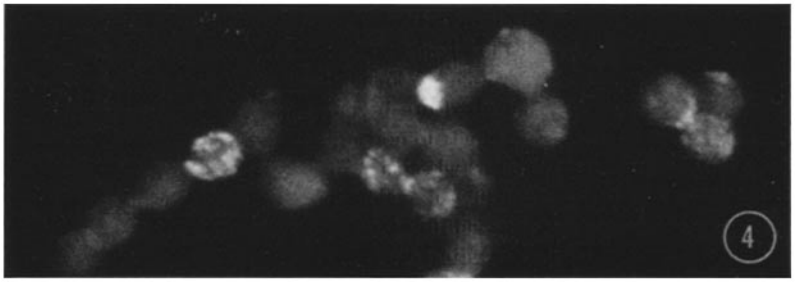


FIG. 4. Membrane-associated immunoglobulin on lymph node lymphocytes from guinea pigs. Viable lymph node cells are stained with Fl-RAGIG as described in Materials and Methods to demonstrate membrane-associated immunoglobulin. Cells are shown which have immunoglobulin in speckled patches over their surface as well as cells with immunoglobulin limited to a single pole. × 2700–3500.

If many of the immunologically relevant lymphoid cells possess receptors with $\gamma_2$ heavy chains, then treatment of such cells with anti-$\gamma_2$ antiserum and complement might result in their lysis and, thus, in a diminished adoptive primary response to DNP proteins. Lymph node and spleen cell suspensions from guinea pigs which had previously received injections of saline-adjuvant emulsions were suspended in either 20% normal rabbit serum, rabbit anti-$\gamma_2$ antiserum, goat anti-$\mu$ antiserum (Table VI, experiment A), or rabbit polyvalent anti-Ig antiserum (Table VI, experiment B) and incubated 30 min at 37°C. The cells were incubated another hour in the presence of rabbit complement and washed twice. 400 × $10^6$ cells were injected intravenously into two to five lethally irradiated recipients. The recipients were immunized the next day with 50 $\mu$g of DNP-KLH (experiment A) or 50 $\mu$g of DNP-BSA (experiment B) in CFA, and the serum DNP-binding capacities measured on days 16–25. The animals received syngeneic bone marrow cells on day 4. Treatment of cells with anti-$\gamma_2$ or RAGIG significantly reduced the subsequent anti-DNP response, whereas anti-$\mu$ caused no significant effect.

Thus, the ability to mount a primary immune response to dinitrophenylated proteins depends, in part, on cells which possess $\gamma_2$ surface determinants, just

as a majority of DNP-GPA–$^{125}$I ABC, evaluated by radioautography, appear to possess these molecules.

*Simultaneous Cellular Localization of DNP-GPA–$^{125}$I and Immunoglobulin by a Double Label.*– To demonstrate further that cells, in nonimmune animals, which bind DNP-GPA–$^{125}$I also possess surface Ig, double-label experiments were performed utilizing fluorescein (Fl)-labeled purified anti-Ig and DNP-GPA–$^{125}$I. Before double-label experiments, the number of immunoglobulin-bearing lymphocytes in various lymphoid organs was determined. Fluoresceinated RAGIG was added to suspensions of thymocytes, peripheral blood leukocytes, lymph node cells, and spleen cells. After incubation for 30 min at

TABLE VII

*Membrane Ig Localization and Class*

| Antibody | Organ | Staining pattern | | Total positive cells |
|---|---|---|---|---|
| | | Speckled | Capped | |
| | | %* | %* | %* |
| Fl-RAGIG | Thymus | 0.1 | 0 | 0.1 |
| | Blood | 7.6 | 0.5 | 8.1 |
| | Node | 22.9 | 3.6 | 26.5 |
| | Spleen | 28.2 | 4.3 | 32.5 |
| Fl-anti-$\gamma_2$ | Thymus | 0 | 0 | 0 |
| | Blood | 3.8 | 0 | 3.8 |
| | Node | 9.7 | 0.7 | 10.4 |
| | Spleen | 15.3 | 0.3 | 15.6 |

* (Immunoglobulin positive lymphocytes/total lymphocytes) × 100.

room temperature in the presence of sodium azide, unbound antibody was removed by washing the cells twice and cell smears were prepared. It should be emphasized that the cells were viable during incubation with the fluorescent reagent and that the presence of sodium azide should inhibit pinocytosis. Therefore, we assume that only membrane-bound immunoglobulin is detected. Fig. 4 depicts a typical dark-field photograph showing guinea pig lymph node cells after incubation with Fl-RAGIG. It can be seen that many of the small lymphocytes have patches of immunoglobulin over their surface (speckled pattern) while a few have immunoglobulin limited to one pole of the cell (capped pattern). This distribution of immunoglobulin has been demonstrated previously for normal mouse and rabbit lymphocytes (17, 24–26) and malignant human lymphocytes (27).

The per cent of lymphocytes bearing immunoglobulin and the staining pattern of the positive cells is presented in Table VII. Thymus has few, if any, lymphocytes which bear detectable immunoglobulin, while 8% of peripheral

blood, 27% of lymph nodes, and 33% of splenic lymphocytes have membrane-bound Ig. The relative proportions of speckled and capped distribution of Ig is similar in these organs. Lymphocytes with $\gamma_2$ heavy chain determinants were found in the same organs, but the frequency of the cells was about half of that demonstrated with the polyvalent anti-Ig. In this regard, it should be noted that $\gamma_2$ is the major class of immunoglobulin in the serum of nonimmunized

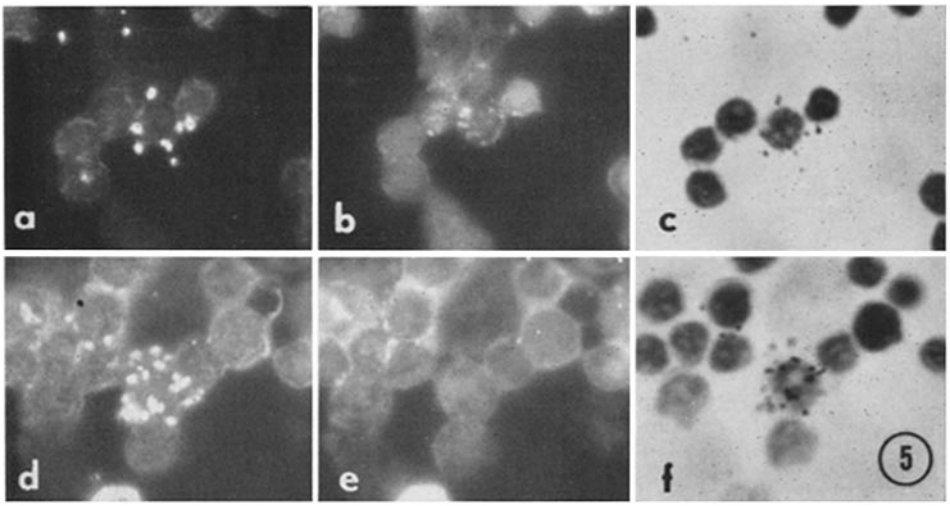


FIG. 5. Simultaneous localization of DNP-GPA–$^{125}$I and anti-immunoglobulin by double label. Lymph node lymphocytes from nonimmune guinea pigs were treated with DNP-GPA–$^{125}$I and Fl–anti-$\gamma_2$ as described in Materials and Methods. DNP-GPA–$^{125}$I ABC were located in a dark field by the presence of silver grains which appear white under tungsten light. By changing the light source from tungsten to ultraviolet, ABC can be evaluated for fluorescence. Two DNP-GPA–$^{125}$I ABC are depicted here. One (*a, b, c*) has $\gamma_2$ determinants (*b*) on its surface. The other (*d, e, f*) has DNP-GPA–$^{125}$I receptors (*d*), but lacks $\gamma_2$ determinants (*e*). Both (*c* and *f*), after staining with methyl green-pyronin, appear to be small lymphocytes. × 2700–3500.

guinea pigs (28). Most $\gamma_2$-bearing lymphocytes had a speckled fluorescent pattern.

By first labeling lymph node cell suspensions with DNP-GPA–$^{125}$I and then with Fl–anti-Ig, it was possible to determine precisely whether DNP-GPA–$^{125}$I–binding lymphocytes had membrane-bound immunoglobulin and what fraction of these cells had surface Ig of the $\gamma_2$ class. These observations were done on smears not stained with methyl green-pyronin in order to preserve fluorescence. Antigen-binding cells could be identified easily by dark-field illumination with a tungsten light source since silver grains appeared as white dots (Fig. 5 *a, d*). After locating an antigen-binding cell, the light source was changed

from a tungsten lamp to an ultraviolet emitter and barrier filters were inserted. Silver grains then became invisible and the presence of the fluorescent label could be evaluated. Finally, cover slips were removed, the cells stained with methyl green-pyronin, the field relocated by stage coordinates, and the morphology of the ABC determined. The upper panel of Fig. 5 demonstrates an DNP-GPA–$^{125}$I-binding cell (Fig. 5 *a*) which also binds fluoresceinated anti-$\gamma_2$ antibody (Fig. 5 *b*) and appears to be a small lymphocyte (Fig. 5 *c*). The lower panel of Fig. 5 demonstrates an DNP-GPA–$^{125}$I ABC (Fig. 5 *d*) which does not bind fluoresceinated anti-$\gamma_2$ antibody (Fig. 5 *e*). Table VIII summarizes our experience with the double-label experiments. All ($^{12}/_{12}$) DNP-GPA–$^{125}$I-binding cells were stained by fluoresceinated RAGIG. One cell had a capped

TABLE VIII

*Coincident Staining by DNP-GPA–$^{125}$I and Fl-Anti-Ig*

| Antibody | DNP-GPA–$^{125}$I-binding cells* | | |
|---|---|---|---|
| | Fluorescent | | Not fluorescent |
| | Speckled | Capped | |
| Fl-RAGIG | 11‡ | 1 | 0 |
| Fl-anti-$\gamma_2$ | 19 | 0 | 3 |
| | HGG-$^{125}$I-binding cells | | |
| Fl-anti-$\gamma_2$ | 8 | 0 | 0 |

* $\geq$ 5 grains/ABC.
‡ Number of cells.

distribution of immunoglobulin and also had silver grains limited to the fluorescent end of the cell. All the other cells had a speckled distribution of immunoglobulin and had no apparent regionalization of silver grains. As expected from the previous data, most ($^{19}/_{22}$) DNP-GPA–$^{125}$I-binding lymphocytes had $\gamma_2$ determinants on their surface. These cells had speckled fluorescence and silver grains scattered over their surface. The small number of DNP-GPA ABC which did not have $\gamma_2$ determinants presumably possess heavy chains of other classes, but this has not been studied in these experiments. To demonstrate that the association of $\gamma_2$ determinants with antigen-binding cells of nonimmune animals was not limited to hapten-specific cells, a second antigen was utilized in the double-label technique. As shown in Table VIII, cells ($^{8}/_{8}$) which bind HGG–$^{125}$I also bear $\gamma_2$ heavy chains.

## DISCUSSION

The observation, from a number of laboratories, that normal, unimmunized animals possess rare lymphoid cells which bind large amounts of individual radiolabeled antigens (6-8) has suggested that these cells represent immunolog-

ically competent, precursor lymphocytes which, upon antigenic stimulation, proliferate and differentiate into cells involved either in antibody synthesis, cellular immunity, or both. It should be emphasized that such a role for these cells is based on inference. However, the present studies, and prior work (5), provide experimental support for the immunologic relevance of these antigen-binding cells in nonimmune animals.

Before considering the results of our study of the receptors of antigen-binding cells, an examination of our methods is appropriate. Antigen binding was done by methods which should evaluate binding to plasma membranes alone. Further, as demonstrated in the subsequent paper (29), and as shown in our previous work (17), such antigen binding is not the result of passive coating of the cell by circulating immunoglobulin. Our approach differs somewhat from that of previous workers in the evaluation of ABC. By utilizing short exposure periods for radioautography, obliteration of cell morphology by silver grains was avoided. Therefore, it was possible for us to be confident both of the intactness of the cell and of its morphology. However, with the short exposure times employed, some of the ABC had few grains over their surface. Therefore, we have designated, arbitrarily, an ABC as any lymphoid cell having three grains or more over its surface. The majority of ABC which we encountered had considerably more than this minimum number of grains. It should be emphasized that the extensive washing procedure and the short exposure periods for radioautography may not allow us to detect cells having relatively few receptors or receptors of low affinity.

Our studies describe the existence of lymphocytes in nonimmunized guinea pigs which bind sufficient DNP-GPA–$^{125}$I to their surface to be detected by radioautography. These cells occur with a frequency of ~40/100,000 lymphocytes in lymph nodes and with a similar frequency in peripheral blood, bone marrow, and spleen. They are strikingly absent in the thymus. This is consistent with observations of others concerning ABC in mice (7, 8). Further, anti-Ig (polyvalent) and anti-$\gamma_2$ antibodies cause a marked reduction in the number of DNP-GPA–$^{125}$I ABC which bind sufficient antigen to be detected, indicating that the surface antigen-binding receptor is an immunoglobulin, generally of the $\gamma_2$ class. This result is supported by the capacity of anti-$\gamma_2$ antiserum and C to markedly diminish the capacity of lymph node and spleen cells to transfer an adoptive primary response to DNP-KLH or DNP-BSA to irradiated syngeneic guinea pigs.

These DNP-GPA–$^{125}$I ABC appear to possess receptors with a considerable degree of specificity for $\epsilon$-DNP-L-lysine as demonstrated by the ability of $\epsilon$-DNP-L-lysine to inhibit the binding of DNP-GPA–$^{125}$I to the ABC and the equivalent capacity of DNP-GPA and DNP-BSA to inhibit such binding. Further, DNP-GPA agarose, DNP-BSA agarose, and DNP-KLH agarose are similar in their ability to specifically adsorb these cells, and treatment of lymph node and spleen cells with DNP-GPA agarose or DNP-KLH agarose

causes significant diminution in the capacity of these cells to transfer primary responsiveness to DNP-KLH to irradiated syngeneic recipients. Hapten-specific depletion of adoptive primary responses has also been reported by Wigzell and Mäkelä (4).

The highly hapten-specific receptors possessed by DNP-GPA–$^{125}$I ABC in nonimmunized animals resembles in specificity the "receptors" possessed by antibody-secreting cells (10). The characteristics displayed here could well be explained if a molecule identical to or resembling humoral antibody were integrated into the cell membrane.

On the other hand, our previous studies (10) have indicated that cells mediating a cellular immune response (the antigen-stimulated synthesis of DNA in vitro), which were obtained from guinea pigs immunized with DNP-GPA, possess receptors with very different specificity characteristics. These cells can be specifically adsorbed onto DNP-GPA agarose, but not onto DNP-BSA agarose. However, they distinguish DNP-GPA agarose, trinitrophenyl GPA agarose, and *o*-nitrophenyl GPA agarose. Their receptors appear, therefore, to have a complex specificity pattern recognizing elements of both hapten and carrier.

In view of the evidence that cellular immune responses are generally associated with thymus function (30–32) and thymus-derived cells (33) and that in the mouse, cells bearing easily detectable amounts of immunoglobulin on their surface appear to be thymus independent (34), the differential specificity of the receptors described in this and the previous paper (10) may be related to the different cell types on which they appear to exist. The receptors possessed by precursors of antibody-secreting cells ("thymus independent lymphocytes") would, in this schema, be immunoglobulin molecules essentially equivalent to the antibodies to be secreted by the progeny of that cell. The bulk of DNP-GPA–$^{125}$I ABC are, most likely, representatives of this cell type. The nature of the receptors present on the other general cell type is more difficult to deal with. Preliminary evidence in the mouse suggests that the receptors of cells mediating cellular immune responses may be immunoglobulin of an unusual heavy chain class which is present in very small amounts on the cell surface (21, 35, 36). Our data, in that the antigen-binding cells which we detect have specificity characteristics resembling precursors of antibody-forming cells (37) and very different from the cells involved in cellular responses, suggests either that the latter cells have too few receptors to be detected by the radioautography method we employ or that, relative to the frequency of the precursors of antibody-secreting cells, they are scarce. Unpublished data demonstrating that anti-Ig-binding cells and DNP-GPA–$^{125}$I ABC can largely be removed from a population of DNP-GPA immune cells without diminishing the antigen-mediated DNA synthetic response[3] is consistent with either of these possibilities.

[3] Rosenthal, A. S., J. M. Davie, D. L. Rosenstreich, and J. T. Blake. Manuscript in preparation.

Data obtained in the mouse (34) showing that the $\theta$-isoantigen, an apparent marker of thymus and thymus-derived cells, tends to be present on different cells than those bearing large amounts of Ig suggests that the cells involved in cellular responses have fewer receptors than the precursors of antibody-secreting cells. The factors underlying the major difference in specificity, in the DNP-GPA system at least, between the receptors possessed by these cell types remain a major challenge.


## SUMMARY

Nonimmunized guinea pigs possess rare lymphocytes which bind sufficient 2,4-dinitrophenyl–guinea pig albumin–$^{125}$I (DNP-GPA) to their surface to be detected by short-term radioautography. The cells occur in the lymph nodes, spleen, peripheral blood, and bone marrow with a frequency of ~40/100,000 lymphocytes, but are absent from the thymus. The receptors of these cells are largely specific for the haptenic group ($\epsilon$-DNP-L-lysine) as shown by inhibition of DNP-GPA–$^{125}$I binding with $\epsilon$-DNP-L-lysine and with DNP bovine serum albumin (DNP-BSA). Furthermore, these cells specifically adsorb to agarose beads to which either DNP-GPA, DNP-BSA, or DNP-keyhole limpet hemocyanin (KLH) has been covalently linked. This hapten specific depletion of DNP-GPA–$^{125}$I antigen-binding cells (ABC) correlates with a similar diminution in the capacity of adsorbed populations to transfer primary responsiveness to DNP-KLH to irradiated syngeneic recipients.

Fluoresceinated anti-immunoglobulin binds to the surface of some guinea pig lymphocytes, and all DNP-GPA–$^{125}$I ABC, as shown by a double-label technique. The great majority of DNP-GPA ABC and human $\gamma$-globulin ABC possess surface Ig molecules of the $\gamma_2$ heavy chain class. Preincubation of cell suspensions with anti-$\gamma_2$ antibody markedly diminishes the number of DNP-GPA–$^{125}$I ABC which are detected, strongly suggesting that the receptors of these cells are immunoglobulin molecules, most of which possess $\gamma_2$ heavy chains.

The specificity characteristics of DNP-GPA–$^{125}$I ABC are strikingly different from those of cells mediating a cellular immune response to DNP-GPA, indicating major differences in the specificity and nature of the receptors of these cell types.


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