HISTOCOMPATIBILITY ANTIGENS AND GENETIC
CONTROL OF THE IMMUNE RESPONSE IN GUINEA PIGS

III. Specific Inhibition of Antigen-Induced Lymphocyte Proliferation
by Strain-Specific Anti-Idiotypic Antibodies*

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Immunization with lymphoid cells among various (2 × 13)F₂ hybrid guinea pigs resulted in the production of antisera which could specifically block antigen-induced T-cell proliferation in vitro (1). The donor lymphoid cells and the recipient animals were chosen from F₂ hybrid guinea pigs of identical (2 × 13) histocompatibility type but in which the responsiveness to low doses of aspirin anhydride (ASAN),¹ penicilloylated bovine IgG (BPO-BGG), or multichain copolymer poly-L-(Tyr, Glu)-poly-DL-Ala-poly-L-Lys [(T,G)-A--L] had segregated independently of each other. The response to low doses of these three antigens appears linked to the strain 13 major histocompatibility complex (MHC) but requires an additional gene(s) not linked to the MHC. Strain 2 guinea pigs only respond to high doses of these antigens (1). It was concluded from these studies that the antisera raised with lymphoid cells of low dose-responder (2 × 13)F₂ hybrids contain antibodies which are not directed against Ir gene products associated with the strain 13 MHC but against at least three separate recognition structures or receptors on the lymphoid cell membrane. Preliminary experiments, involving the passage of BPO-BGG antireceptor antisera (anti-RBo_B₂) through an immunoadsorbent column prepared with purified antibody (anti-BPO-BGG) suggested that the anti-R serum was not directed against immunoglobulin idiotypes. However, since this experiment was not entirely conclusive, we undertook further studies aimed at answering the question whether our anti-R sera against recognition structures are, in fact, anti-idiotypes.

Immunoadsorbent column-purified anti-BPO-BGG, raised in strain 2 and strain 13 guinea pigs, was used to immunize both strain 2 and 13 animals and the resultant antisera tested for their ability to inhibit the BPO-BGG-induced T-cell proliferation of cells from high dose-responder strain 2 and low dose-responder strain 13 guinea pigs. In this paper we examine some of the properties of these antisera and compare them with an antireceptor serum (anti-RBpo-BGG).

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¹ Abbreviations used in this paper: 2 strain 2 BPO-BGG, anti-idiotypic antibodies raised in strain 2 against strain 2 anti-BPO-BGG; 3 strain 13 BPO-BGG, anti-idiotypic antibodies raised in strain 13 against strain 13 anti-BPO-BGG; ASAN, acetylsalicylic acid anhydride; ASP-OVA, asaryl ovalbumin; BPO-BGG, penicilloylated bovine IgG; CFA, complete Freund's adjuvant; DMSO, dimethyl sulfoxide; GL, copolymer poly(L Glu, Lys); GT, copolymer poly(L Glu, Tyr); M-199, medium 199; MHC, major histocompatibility complex; PBS, 0.01 M phosphate-buffered 0.9% NaCl (pH 7.4); PEL, peritoneal exudate lymphocytes; PHA, phytohemagglutinin; R, receptors; TBS, 0.02 M Tris-buffered 0.9% NaCl (pH 8.2); (T,G)-A--L, multichain copolymer poly-L-(Tyr, Glu)-poly-DL-Ala-poly-L-Lys.
which had been raised by immunizing with lymphoid cells from an F₂ hybrid which lacked one of the genes required for the full expression of high responsiveness to BPO-BGG (1). We will provide evidence that in vitro T-cell proliferation induced by BPO-BGG may be specifically inhibited by strain-specific antibodies probably directed against cell surface-associated immunoglobulin idiootypes.

Materials and Methods

Animals. Guinea pigs of strain 2 and 13 (400-600 g) were obtained from the Institut für biologisch-medizinische Forschung AG, Füllinsdorf, Switzerland; their phenotype was checked before use by lymphocytotoxicity typing (see below).

Antigens and Immunization of Guinea Pigs. In general, strain 2 and strain 13 guinea pigs were immunized simultaneously with all antigens (high dose immunization schedule). 5 mg ASAN (Chemische Fabrik Aubing, München, Germany) were injected intradermally in 0.1 ml dimethyl sulfoxide (DMSO) on days 0, 4, and 8. BPO-BGG (100 μg) and (T,G)-A--L (5 mg; Miles-Yeda, Rehovot, Israel) dissolved in phosphate-buffered saline (PBS), 0.01 M, pH 7.4 and Tris-buffered saline (TBS), 0.02 M, pH 8.2, respectively, were emulsified with an equal volume of complete Freund's adjuvant (CFA, Difco Laboratories, Detroit, Mich.). On day 0, each animal received 0.8 ml of emulsion divided equally among the four foot pads. In addition, strain 2 animals were also immunized with a copolymer of L-glutamic acid (60%) and L-lysine (40%) (GL). Dinitrophenyl-GL (DNPrGL) was kindly supplied by Dr. I. Green, NIH, Bethesda (Md.). The subscript refers to the average number of DNP groups per molecule. Strain 13 animals were injected with a copolymer of L-glutamic acid (50%) and L-tyrosine (50%) (GT; Miles Laboratories, Inc., Miles Research Div., Kankakee, Ill.). Solutions of DNPT-GL and GT in 0.01 M PBS, pH 7.4, were emulsified with an equal volume of CFA. Animals were immunized with 100 μg of DNPT-GL or 500 μg of GT subcutaneously in multiple sites in the nuchal skin or thigh. In one series of experiments in which the influence of anti-idiotypic sera on cells from low dose- and high dose-immunized (2 × 13)/F₁ guinea pigs was compared, the following low dose immunization schedule was followed: ASAN (100 μl of a 20% solution in DMSO) was applied epicutaneously on days 0, 4, and 8. BPO-BGG (10 μg in 100 μl PBS) was injected intradermally on days 0, 4, 6, 8, and 10. (T,G)-A--L (500 μg in 100 μl TBS) was given intradermally on days 0, 4, 6, and 8, all without adjuvant.

Detection of Contact and Delayed-Type Skin Hypersensitivity. In all cases the flank was shaved with an electric clipper a few hours before testing. The increase in skin thickness at the reaction site was measured 24 h after testing with a caliper ("Schnelltaster"; Kroeplin, Schuchtern, Germany). Increase in skin thickness of more than 0.6 mm has been shown (2) to be statistically significant at the P < 0.05 level and reactions above that level were considered positive.

**ASAN.** 25 μl of a 0.4% and 4.0% (wt/vol) ASAN solution and 0.05 ml of a 10.0% (wt/vol) ASAN solution in acetone-almond oil (1:1, vol/vol) were applied with a pipette on stamped round 2 cm² surfaces of the flank. Reactions were read at 24 h and evaluated according to the following scale: 0, no reaction; 0.5, disseminated faint red papules over the test area; 1.0, confluent erythema; 2.0, marked erythema and slight edema; and 3.0, deep erythema, edema, and necrosis.

**BPO-BGG, (T,G)-A--L, DNPT-GL, AND GT.** Delayed skin reactivity was assessed by the intradermal injection into the shaved flank of 1 and 10 μg of BPO-BGG in 100 μl of PBS (0.01 M, pH 7.4); 1 and 25 μg of (T,G)-A--L in 100 μl of TBS (0.02 M, pH 8.2); and 10 and 50 μg of DNPT-GL and GT each in 100 μl of PBS.

Production of Antisera. Strain 13 antistrain 2 serum and strain 2 antistrain 13 serum were prepared by cross-immunizing animals of these strains with a homogenate of lymph node and spleen cells as previously described (1). Preparation of an antisera against specific recognition structures or "receptors" for BPO-BGG (anti-R<sub>BPO-BGG</sub>) has already been described (1). Briefly, (2 × 13) siblings from F₁ hybrid guinea pigs were cross-immunized with lymphoid cells. Thus, an anti-R<sub>BPO-BGG</sub> serum could be raised by the immunization of a (2 × 13) ASAN⁺ BPO-BGG⁺ (T,G)-A--L⁺ guinea pig with (2 × 13) ASAN⁻ BPO-BGG⁻ (T,G)-A--L⁻ lymphoid cells.

Antisera against strain 2 and strain 13 anti-BPO-BGG were prepared as follows: Firstly, anti-BPO-BGG antibodies were raised by immunizing strain 2 and strain 13 guinea pigs with 1 mg BPO-BGG in CFA, equally distributed in the four foot pads, and 2 wk later boosted with 1 mg BPO-BGG in 0.5% NaCl, administered intracutaneously in multiple sites in the nuchal skin. 2 wk
later, the sera from such animals were passed over a BPO-BGG-bearing polyacrylamide immuno-
adsorbent column prepared as previously described (3). The anti-BPO-BGG antibodies bound to
the column were eluted with glycine-HCl buffer, 0.1 M, pH 2.5, containing 0.9% NaCl and the
eluate neutralized with 1 M Tris. Antibody concentration was determined by the absorbance at 278
nm in neutral solution using an extinction coefficient (E~:m) of 14.0 as described by Eisen et al. (4)
for anti-DNP antibodies.

The immune anti-BPO-BGG antibodies were then used as immunogens for the sensitization of
four strain 2 and four strain 13 guinea pigs. Two strain 2 guinea pigs received 2 mg of anti-BPO-
BGG (raised in strain 2) and the other two strain 2 animals were given the same amount of anti-
BPO-BGG derived from strain 13 animals. Similarly, two strain 13 guinea pigs received strain 2
anti-BPO-BGG while two other strain 13 animals were immunized with anti-BPO-BGG raised in
strain 13 guinea pigs. The anti-BPO-BGG was injected in 0.8 ml CFA, equally distributed among
the four foot pads. 2 wk later the animals received a boost of 1 mg of anti-BPO-BGG in CFA
(subcutaneously in multiple sites in the nuchal skin) and a further injection (200 μg anti-BPO-
BGG intradermally) 10 days after the first boost. All animals were exsanguinated 2 wk after the
last injection, the sera sterilized by Millipore filtration, and stored at -20°C.

Cytotoxicity Testing. The details of this test have previously been reported (1). The percentage
of maximum $^{51}$Cr released was calculated as follows:

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\text{Radioactivity released by antiserum dilution} - \text{radioactivity released in absence of antiserum}
\]

\[
\text{Radioactivity released by frozen-thawed cells} - \text{radioactivity released in absence of antiserum.
}

Absorption of Anti-Idiotypic Antisera. Antisera were mixed with an equal volume of packed
lymph node and spleen cells (approximately 10$^9$ cells/ml of serum) for 4 h at 4°C. Each antiserum
was absorbed at least three times. The absorbed antisera were centrifuged at 10,000 rpm for 30 min
and sterilized by Millipore filtration.

Cell Preparation

LYMPH NODE CELLS. The regional lymph nodes were removed, freed as far as possible from fat,
and cell suspensions prepared by teasing with scissors and forceps and then by pressing the lymph
node fragments through no. 60 wire screens (Haska, Bern, Switzerland). The cell suspensions
were then washed twice with medium 199 (M-199). The cell population was composed of over 95%
lymphocytes and about 5% macrophages. For the assay of antigen-induced lymphocyte prolifera-
tion in the presence of alloantisera, lymphocyte suspensions (20 × 10$^6$/ml) were incubated with
either 100 μg of DNP-GL and GT or 400 μg of aspiryl-ovalbumin (ASP-OVA), BPO-BGG, and
(T,G)-A--L/ml of M-199 containing 10% heat-inactivated fetal calf serum. A portion of the cells was
also incubated with 10 μg phytohemagglutinin (PHA)/ml of M-199. The cells were pulsed with
antigen for 30 min at 37°C. Since ASAN is insoluble in water, ASP-OVA prepared as previously
described by de Weck (5) was used in the in vitro studies. The cells were washed three times with
18 h before the termination of

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\text{Radioactivity released by antiserum dilution} - \text{radioactivity released in absence of antiserum}
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\text{Radioactivity released by frozen-thawed cells} - \text{radioactivity released in absence of antiserum.
}

PERITONEAL EXUDATE LYMPHOCYTES (PEL). Guinea pigs were injected intraperitoneally with 30
ml of sterile paraffin oil to induce a peritoneal exudate. 4 days later, the animals were killed by
cardiac puncture and the peritoneal cavity washed with 200 ml of Hanks' balanced salt solution
containing 5 U/ml of preservative-free heparin (Hoffmann-La Roche, Basel, Switzerland). Perito-
neal exudate cells were washed three times in Hanks' solution to remove oil and finally suspended
in RPMI 1640 (Microbiological Associates, Bethesda, Md.) containing 100 U penicillin and 100 μg
of streptomycin/ml and 2 mM glutamine. The lymphocytes were separated from the whole exudate
cell population by passage over a column of cotton wool (Schaffhauser Watte, Schaffhausen,
Switzerland) packed loosely in a 50 ml plastic syringe. The population of cells after column purification consisted of 80-90% lymphocytes with a viability of over 95% as measured by trypan blue exclusion. About 90% of the PEL formed rosettes with rabbit red blood cells as previously described (1, 3). The assay of antigen-induced proliferation in the presence of antisera was the same as described for lymph node cells.

**Preparation of Sepharose Immunoadsorbents.** Sepharose 4B (Pharmacia Fine Chemicals, Ltd., Uppsala, Sweden) was activated with cyanogen bromide and coupled with protein [40% (NH₄)₂SO₄ fractions of guinea pig sera; Ig fractions] as described in the Pharmacia Fine Chemicals Instruction Manual (Affinity Chromatography, Principles and Methods). Each gram of Sepharose bound about 5 mg of protein.

**Radioimmunoassay.** Untreated Sepharose 4B and activated Sepharose bearing a normal guinea pig Ig fraction or Ig fractions prepared from strain 2 antistrain 2 anti-BPO-BGG (a strain 2 BPO-BGG) and strain 13 antistrain 13 anti-BPO-BGG (a strain 13 BPO-BGG) sera, were equilibrated with Phadebas RAST buffer solution, pH 7.4 (Pharmacia Fine Chemicals, Ltd.), containing 2 ml Tween 20/100 ml buffer for 2 h before use. 100 μl of Sepharose (100 mg wet weight) were incubated with 5 μg of ¹²⁵I-labeled antigen (Ig fractions of strain 2 and strain 13 anti-BPO-BGG were labeled with iodine-125 according to the method of Hunter and Greenwood (6), in 100 μl RAST buffer solution in the presence of PBS or unlabeled antigen (5 μg in 100 μl PBS) for 16 h at room temperature. The Sepharose was then washed 10 times with 0.9% NaCl (containing 5 ml Tween 20/liter) and the radioactivity bound to the Sepharose beads determined in a LKB 8000 gamma counter (LKB Produkter, Stockholm, Sweden).

**Sephadex Chromatography.** 2 ml of antiserum (a strain 2 BPO-BGG and a strain 13 BPO-BGG) were chromatographed on Sephadex G-200 (90 x 2.6 cm) which had been equilibrated with TBS. The peak tubes from the 19S, 7S, and 4S regions were tested for their capacity to inhibit antigen-induced lymphocyte proliferation.

**Results**

**Relationship between Histocompatibility Type and Responsiveness to Antigens.** We have previously shown (1) that strain 13 and (2 x 13)F₁ guinea pigs respond to low doses of ASAN, BPO-BGG, and (T,G)-A--L (as assessed by delayed skin reactivity and in vitro lymphocyte stimulation), whereas strain 2 animals fail to respond. However upon stronger and more prolonged immunogenic stimuli [i.e. immunization with 10- to 20-fold higher doses of ASAN, BPO-BGG, and (T,G)-A--L than that required for strain 13 animals] strain 2 animals can also be sensitized to these three antigens. In the present study we immunized strain 2 and strain 13 animals with high doses of ASAN, BPO-BGG, and (T,G)-A--L to ensure that all strain 2 animals would give strong delayed skin responses when tested with these antigens 3 wk after immunization. In addition, all strain 2 animals responded to DNP-GL but not to GT, whereas strain 13 guinea pigs showed strong delayed skin reactivity to GT but failed to react to DNP-GL; (2 x 13)F₁ responded to both GT and DNP-GL. Responsiveness to DNP-GL and GT is known to be linked to the strain 2 and strain 13 haplotype, respectively (7, 8).

**Inhibition of Lymphocyte Stimulation by Anti-Idiotype Antisera Raised in Strain 2 and Strain 13 Guinea Pigs.** As described in the Material and Methods and Fig. 1, antisera were raised in strain 2 and 13 guinea pigs using as antigen anti-BPO-BGG antibodies from strain 2 or 13, purified by immunoadsorption on polyacrylamide columns bearing BPO-BGG. The following four antisera were investigated for their ability to inhibit antigen-induced lymphocyte proliferation: a strain 2 BPO-BGG, a strain 13 BPO-BGG, strain 2 antistrain 13 anti-BPO-BGG, and strain 13 antistrain 2 anti-BPO-BGG. The first two antisera,
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**FIG. 1.** Scheme for the production of anti-idiotypic sera in strain 2 and strain 13 guinea pigs.

**TABLE I**

Inhibition of Lymphocyte Proliferation by Anti-R<sub>PBO-BGG</sub> and Anti-Idiotypic Sera in Primed High Dose-Immunized (2 × 13)<sub>F</sub><sub>1</sub> Lymph Node Cells

| Guinea pig serum added* | Stimulant | Normal | 13 Anti-2 | 2 Anti-13 | Anti-R<sub>PBO-BGG</sub> | a Strain 2 | A Strain 13 | Strain 2 | Strain 13 |
|-------------------------|-----------|--------|-----------|-----------|--------------------------|------------|------------|----------|----------|
|                         |           |        |           |           |                          |            |            |          |          |
| NIL                     | 4983      | 378    | 508       | 389       | 462                      | 728        | 499        | 600      |
| PHA                     | 48,632    | 42,422 | 46,984    | 39,806    | 41,182                   | 38,980     | 42,506     | 43,087   |
| DNP-GL                  | 34,472    | 30,826 | 34,820    | 33,432    | 34,820                   | 30,800     | 36,087     | 30,627   |
| GT                      | 30,466    | 29,842 | 30,826    | 30,800    | 29,842                   | 30,800     | 36,087     | 29,783   |
| ASP-OVA                 | 40,863    | 35,678 | 37,874    | 34,343    | 35,874                   | 38,874     | 39,975     | 37,008   |
| BPO-BGG                 | 39,508    | 36,444 | 34,444    | 33,432    | 35,444                   | 33,432     | 32,857     | 35,626   |
| (T,G)-A--L              | 36,462    | 32,432 | 34,444    | 33,432    | 35,444                   | 33,432     | 32,857     | 35,626   |

*Final concentration of serum in culture was 1%.
† Results are expressed as mean counts per minute per culture. Each result represents the mean of three cultures; significantly depressed results are underlined.

presumed to be directed against strain 2 and strain 13 anti-idiotypes, were designated as a. None of the four sera appeared to contain lymphocytotoxic antibodies to currently known alloantigens coded for by the B and I regions of the guinea pig MHC, as determined by the ³¹Cr-release assay on strain 13 and strain 2 lymphocytes (results not shown). Table I illustrates that primed (2 × 13)<sub>F</sub><sub>1</sub> lymph node cells from high dose-immunized guinea pigs pulsed with PHA, DNP-GL, GT, ASP-OVA, BPO-BGG, or (T,G)-A--L and then cultured in the presence of normal guinea pig serum for 72 h are stimulated as measured by tritiated thymidine incorporation into their DNA. When a portion of these cells is cultivated in the presence of 13 anti-2 serum there is a marked inhibition of the DNP-GL-induced stimulation while the responses of cells pulsed with the other antigens are unaffected (1). In the presence of 2 anti-13 serum the GT
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TABLE II

Comparison of the Effect of Anti-Idiotypic and Anti-\(R_{\text{BPO-BGG}}\) Sera on the In Vitro Proliferative Response of Lymph Node Cells from High Dose- and Low Dose-Immunized \((2 \times 13)F_1\) Guinea Pigs

| Stimulant        | Guinea pig serum added* | Normal | \(\alpha\) Strain 2 BPO-BGG | \(\alpha\) Strain 13 BPO-BGG | Anti-\(R_{\text{BPO-BGG}}\) |
|------------------|-------------------------|--------|-----------------------------|-----------------------------|-----------------------------|
| High dose immunized |                         |        |                             |                             |                             |
| NIL              | 262*                    | 459    | 424                         | 448                         |                             |
| PHA              | 102,949                 | 111,869| 78,975                      | 126,043                     |                             |
| ASP-OVA          | 100,783                 | 99,831 | 93,031                      | 129,701                     |                             |
| BPO-BGG          | 90,423                  | 284    | 215                         | 624                         |                             |
| \((T,G)-A--L\)    | 100,772                 | 108,916| 102,487                     | 109,064                     |                             |
| Low dose immunized |                         |        |                             |                             |                             |
| NIL              | 235                     | 289    | 293                         | 284                         |                             |
| PHA              | 121,606                 | 140,059| 134,868                     | 119,632                     |                             |
| ASP-OVA          | 113,101                 | 130,974| 87,193                      | 71,286                      |                             |
| BPO-BGG          | 82,592                  | 111,350| 350                         | 310                         |                             |
| \((T,G)-A--L\)    | 100,200                 | 100,927| 100,670                     | 98,501                      |                             |

* See footnote to Table I.

The response is suppressed as well as the proliferative responses of cells stimulated with ASP-OVA, BPO-BGG, and \((T,G)-A--L\) (7, 9). By contrast, anti-\(R_{\text{BPO-BGG}}\) serum (raised by immunizing with lymphoid cells among \((2 \times 13)F_2\) hybrid guinea pigs), \(\alpha\) strain 2 BPO-BGG as well as \(\alpha\) strain 13 BPO-BGG sera totally suppress the proliferative response only of BPO-BGG-pulsed cells but do not affect the response to any of the other antigens. On the other hand, antisera raised in strain 2 and strain 13 guinea pigs against anti-BPO-BGG antibody of the allogeneic strain (i.e., strain 2 antistrain 13 anti-BPO-BGG and strain 13 antistrain 2 anti-BPO-BGG) failed to interfere with the in vitro reactivity induced by any of the antigens.

In contrast to the situation with \(2 \times 13\) lymphoid cells from high dose-immunized animals, cells from low dose-immunized animals can only be inhibited by \(\alpha\) strain 13 BPO-BGG serum but not by \(\alpha\) strain 2 BPO-BGG (Table II) suggesting that high dose immunization is required to induce the synthesis of the appropriate strain 2-specific idiotype recognition structures on the membrane of primed \((2 \times 13)F_1\) cells.

The \textit{Failure of \(\alpha\) Strain 2 BPO-BGG and \(\alpha\) Strain 13 BPO-BGG Sera to Suppress Lymphocyte Activation of Cells Derived from the Allogeneic Strain}. To investigate the possibility that the \(\alpha\) strain 2 BPO-BGG and \(\alpha\) strain 13 BPO-BGG sera may be directed against strain-specific idiotypes, the capacity of these antisera to inhibit T-cell activation in the syngeneic and allogeneic strain was studied. Furthermore, to ensure that the stimulation observed in this system was due to tritiated thymidine incorporation into the DNA of T cells, the influence of the antisera on PEL (which are composed almost entirely of thymus-derived cells [10]) from primed strain 2 and strain 13 guinea pigs was also investigated.

The data in Table III demonstrate that anti-\(R_{\text{BPO-BGG}}\) fails to inhibit BPO-
**TABLE III**

*Inhibition of T-Cell Proliferation by Anti-Idiotypic Sera in Primed Strain 2 PEL Capable of Responding to ASP-OVA, BOP-BGG, and (T,G)-A--L*

| Stimulant          | Guinea pig serum added* |
|--------------------|-------------------------|
|                    | Normal  | Anti-\(\text{R}_{\text{BPO-BGG}}\)  | \(\text{a Strain 2 BPO-BGG}\) | \(\text{a Strain 13 BPO-BGG}\) |
| **Strain 2 BPO-BGG** | NIL     | 161*    | 152           | 94                   | 151          | 118          | 151          |
| **PHA**            | 108,031 | 98,772  | 94,818        | 64,024              | 86,111       | 104,037      |
| **ASP-OVA**        | 54,826  | 69,037  | 50,933        | 57,068              | 74,859       | 86,818       |
| **BPO-BGG**        | 110,851 | 75,105  | 140           | 70,402              | 154          | 86,468       |
| **(T,G)-A--L**     | 101,148 | 68,934  | 79,059        | 77,889              | 90,542       | 96,934       |

* See footnote to Table I.

BGG-induced T-cell proliferation of strain 2 cells which are capable of responding to all three antigens. Antiserum \(a\) strain 13 BPO-BGG also does not suppress the in vitro BPO-BGG response. By contrast, the corresponding antiserum \(a\) strain 2 BPO-BGG specifically suppresses T-cell activation of BPO-BGG-pulsed immune strain 2 cells. In addition, immune BPO-BGG\(^+\) cells from strain 2 guinea pigs are capable of absorbing the inhibitory activity from \(a\) strain 2 BPO-BGG serum, while immune BPO-BGG\(^+\) cells from strain 13 animals fail to do so. By contrast, cells from nonimmunized animals failed to absorb the inhibitory activity from the two strain-specific antisera (results not shown).

Conversely, BPO-BGG-pulsed PEL from primed strain 13 guinea pigs can only be inhibited by an antiserum against anti-BPO-BGG raised in the isogeneic strain (\(a\) strain 13 BPO-BGG) but not by \(a\) strain 2 BPO-BGG, raised in the allogeneic strain (Table IV). In addition, anti-\(\text{R}_{\text{BPO-BGG}}\) can also suppress the BPO-BGG response of strain 13 but not of strain 2 cells. It is clear, therefore, that the antisera (\(a\) strain 2 BPO-BGG and \(a\) strain 13 BPO-BGG) must interact with some gene product(s) characteristic of the isogeneic strain in order to suppress antigen-induced T-cell proliferation in vitro. It seems likely that the antisera are directed against membrane-associated strain-specific immunoglobulin idiotypes which may function as T-cell antigen receptors.

The **Inhibitory Activity of \(a\) Strain 13 BPO-BGG Can Only Be Absorbed by BPO-BGG\(^+\) Cells.** In addition to strain specificity, the anti-idiotypic serum, raised in a strain 13 combination against "receptors" for BPO-BGG, is specific for BPO-BGG-pulsed cells and does not suppress either ASP-OVA- or (T,G)-A--L-induced lymphocyte proliferation (Tables I, III, and IV). The data in Table V lend further support to the specificity of the antiserum: inhibitory activity can only be removed by absorption with cells from strain 13 guinea pigs which respond to low doses of BPO-BGG and not by cells from the occasional strain 13 animals which fail to respond to BPO-BGG but give strong responses to ASAN and (T,G)-A--L.

The **Inhibitory Activity of the Anti-Idiotypic Sera is Associated with the 19S Fraction.** 2 ml of \(a\) strain 13 BPO-BGG serum were chromatographed on
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TABLE IV

Inhibition of T-Cell Proliferation by Anti-Rspo-~GG and Anti-Idiotypic Sera in Primed Strain 13 PEL Capable of Responding to ASP-OVA, BPO-BGG, and (T,G)-A--L

| Guinea pig serum added* | Stimulant         | Normal | Anti-Rspo-BGG  | â Strain 2 BPO-BGG | â Strain 13 BPO-BGG absorbed with strain 13 cells | â Strain 13 BPO-BGG absorbed with strain 2 cells |
|-------------------------|-------------------|--------|----------------|--------------------|-----------------------------------------------|-----------------------------------------------|
|                         | Stimulant         |        |                |                    |                                               |                                               |
|                         | NIL               | 555*   | 830            | 1,156              | 778                                           | 356                                           | 690                                           |
|                         | PHA               | 113,442| 89,423         | 107,980            | 65,000                                        | 94,673                                        | 96,527                                        |
|                         | ASP-OVA           | 97,673 | 81,353         | 89,218             | 86,124                                        | 79,419                                        | 77,379                                        |
|                         | BPO-BGG           | 93,525 | 1,129          | 87,926             | 1,348                                         | 80,687                                        | 916                                           |
|                         | (T,G)-A--L        | 105,458| 68,904         | 96,712             | 86,771                                        | 86,084                                        | 91,979                                        |

* See footnote to Table I.

TABLE V

The Absorption of Inhibitory Activity from â Strain 13 BPO-BGG Serum with BPO-BGG+ Cells

| Guinea pig serum added* | Stimulant | NIL | PHA | ASP-OVA | BPO-BGG | (T,G)-A--L |
|-------------------------|-----------|-----|-----|---------|---------|------------|
|                         | Normal    | 1,042* | 168,539 | 140,443 | 127,165 | 181,877 |
|                         | â Strain 13 BPO-BGG | 1,214 | 177,565 | 139,221 | 1,098 | 176,504 |
|                         | â Strain 13 BPO-BGG absorbed with ASAN- BPO-BGG- (T,G)-A--L cells | 1,039 | 148,632 | 199,962 | 1,296 | 188,419 |
|                         | â Strain 13 BPO-BGG absorbed with ASAN- BPO-BGG+ (T,G)-A--L+ cells | 1,157 | 236,255 | 212,217 | 167,704 | 216,369 |
|                         | â Strain 13 BPO-BGG absorbed with ASAN- BPO-BGG+ (T,G)-A--L+ cells | 1,155 | 191,444 | 195,746 | 1,164 | 192,056 |

* See footnote to Table I.

Sephadex G-200, and the fractions eluted with TBS. The peak tubes from the 19S, 7S, and 4S fractions were tested for their capacity to inhibit BPO-BGG-induced lymphocyte proliferation.

The antigen-pulsed cells were incubated with 1% normal guinea pig serum and 9% heat-inactivated fetal calf serum together with either 0.1 ml of TBS or 0.1 ml of the appropriate 19S, 7S, or 4S Sephadex G200 column fractions. The absorbances at 278 nm of the peak tubes of the various fractions were as follows: 1.1 (19S); 0.7 (7S); and 1.0 (4S).

The data in Table VI show that the undiluted 19S fraction of â strain 13 BPO-BGG totally inhibits the in vitro BPO-BGG response but does not affect (T,G)-A--L-induced in vitro proliferation. The 7S and 4S fraction, on the other hand, suppress BPO-BGG reactivity by only 30%. At a 1/20 dilution only the 19S fraction inhibits the in vitro BPO-BGG response. It appears, therefore, that the inhibitory activity of the anti-idiotypic serum is primarily associated with the 19S fraction. Similar data were obtained with â strain 2 BPO-BGG which had been chromatographed on Sephadex G-200 (results not shown).

The Relative Affinity of Strain 2 and Strain 13 Anti-Idiotype Sera for Isogenic and Allogeneic Anti-BPO-BGG. The antisera raised in strain 2 and strain
TABLE VI
The Inhibition of BPO-BGG-Induced Proliferation of Primed Strain 13 Lymph Node Cells by the 19S Fraction of a Strain 13 BPO-BGG Serum

| Stimulant     | Serum fraction | Fraction dilution | Inhibition* | %  |
|---------------|----------------|-------------------|-------------|----|
| BPO-BGG       | 19S            | None              | 100         |    |
|               | 19S            | 1/20              | 72          |    |
| (T,G)-A--L    | 19S            | None              | 0           |    |
|               | 19S            | 1/20              | 0           |    |
| BPO-BGG       | 7S             | None              | 34          |    |
|               | 7S             | 1/20              | 0           |    |
| (T,G)-A--L    | 7S             | None              | 0           |    |
|               | 7S             | 1/20              | 0           |    |
| BPO-BGG       | 4S             | None              | 32          |    |
|               | 4S             | 1/20              | 0           |    |
| (T,G)-A--L    | 4S             | None              | 10          |    |
|               | 4S             | 1/20              | 3           |    |

* The % inhibition was calculated as follows: 1 - [(cpm in presence of serum fraction)/cpm in presence of serum diluent (TBS)] × 100; see Materials and Methods.

13 guinea pigs, which had been immunized with either strain 2 or strain 13 anti-BPO-BGG, failed to form precipitating antibodies as detected by double diffusion in 1% Ionagar no. 2 (Difco Laboratories) even when the antisera were concentrated up to 10-fold and tested against a wide range of antigen (anti-BPO-BGG) concentrations. Furthermore, none of the antisera formed a precipitin line with BPO-BGG suggesting that no more than 0.1 µg of BPO-BGG (a quantity of BPO-BGG which when injected in CFA induces precipitating antibodies after 14 days) could have been eluted from the antigen immunoadsorbent column during the preparation of anti-BPO-BGG. Table VII illustrates that strain 2 and strain 13 anti-idiotypic sera bind isogeneic anti-BPO-BGG preferentially to the allogeneic antibody. Furthermore, the binding of isogeneic ¹²⁵I-labeled anti-BPO-BGG, on the other hand, by the anti-idiotypic serum immunoadsorbents can be inhibited by "cold" isogeneic anti-BPO-BGG to a slightly greater extent than by the allogeneic antibody. By contrast, Sepharose alone or Sepharose bearing 500 µg of normal guinea pig Ig under these conditions, bind about 7,500 and 12,000 cpm of labeled antibody, respectively.

Does the Interaction of a Strain 2 and a Strain 13 BPO-BGG Sera with BPO-BGG-Sensitive Cells Result in the Liberation of Cytotoxic Factors or Immune Complexes which could Inhibit the Proliferation of Cells Sensitive to Another Antigen?  The inhibition of antigen-induced lymphocyte proliferation by anti-idiotypic antisera might be visualized as a specific blindfolding of antigen receptors on T cells. However, it is also conceivable that the interaction of anti-idiotypic antibodies with receptors might lead to the release of cytotoxic factors and/or immune complexes capable of nonspecifically blocking lymphocyte prolif-
TABLE VII
The Relative Affinity of Strain 2 and Strain 13 Anti-Idiotypic Sera for Isogeneic and Allogeneic Anti-BPO-BGG

| Immunoadsorbent Incubated with: | In the presence of: | Net cpm bound by immunoadsorbent |
|---------------------------------|---------------------|---------------------------------|
| Sepharose-a strain 2 BPO-BGG    | PBS                 | 20,500                           |
| 125I-strain 2 anti-BPO-BGG      | Strain 13 anti-BPO-BGG, 5 µg | 16,836                           |
| 125I-strain 2 anti-BPO-BGG      | Strain 2 anti-BPO-BGG, 5 µg | 7,500                            |
| 125I-strain 13 anti-BPO-BGG     | PBS                 | 10,024                           |
| 125I-strain 13 anti-BPO-BGG     | Strain 2 anti-BPO-BGG, 5 µg | 6,641                            |
| 125I-strain 13 anti-BPO-BGG     | Strain 13 anti-BPO-BGG, 5 µg | 9,789                            |
| Sepharose-a strain 13 BPO-BGG   | PBS                 | 5,810                            |
| 125I-strain 2 anti-BPO-BGG      | Strain 13 anti-BPO-BGG, 5 µg | 3,802                            |
| 125I-strain 2 anti-BPO-BGG      | Strain 2 anti-BPO-BGG, 5 µg | 5,686                            |
| 125I-strain 13 anti-BPO-BGG     | PBS                 | 15,031                           |
| 125I-strain 13 anti-BPO-BGG     | Strain 2 anti-BPO-BGG, 5 µg | 12,514                           |
| 125I-strain 13 anti-BPO-BGG     | Strain 13 anti-BPO-BGG, 5 µg | 7,075                            |

* Sepharose-a strain 2 and a strain 13 immunoadsorbents (100 mg wet weight of gel bearing 500 µg of protein, suspended in 100 µl PBS) were incubated with 5 µg of radiolabeled antigen (1g fractions of strain 2 and strain 13 anti-BPO-BGG) representing 5 × 10^6 cpm of labeled protein, in the presence of PBS or unlabeled antigen (5 µg). The radioactivity bound to the Sepharose immunoadsorbent was estimated as described in the Materials and Methods. Results are expressed as counts per minute of triplicate determinations.

† Net counts per minute are given by counts per minute of 125I-labeled anti-BPO-BGG bound by Sepharose anti-idiotypic sera – counts per minute bound by Sepharose alone.

Discussion
There is a growing body of evidence that anti-idiotypic antibodies can specifically regulate a number of immunological phenomena such as the suppression (11–13) or enhancement (14, 15) of antibody production and the specific inhibition of graft-vs.-host (16, 17) and mixed leukocyte culture reactivity (18). In most instances anti-idiotypic antibodies were raised by allo- (17) or heteroimmunization (14) though the production of autoanti-idiotypic antibodies has also been reported in rabbits (19). The data presented in this paper clearly demonstrate that anti-idiotypic antibodies raised syngeneically in strain 2 and 13 guinea pigs against anti-BPO-BGG can specifically and markedly suppress in vitro lympho-
The Inhibition by Anti-Idiotypic Sera of High Dose-Immunized (2 × 13)F₁, Lymph Node Cells Which Have Been Pulsed Simultaneously with BPO-BGG and (T,G)-A--L

| Stimulant             | Guinea pig serum added* |
|-----------------------|-------------------------|
|                       | Normal 13 Anti-2 2 Anti-13 á Strain 2 BPO-BGG 13 BPO-BGG |
| NIL                   | 173 183 205 267 235 |
| PHA                   | 26,921 27,657 26,413 24,600 24,975 |
| BPO-BGG               | 14,002 14,494 28 354 290 |
| (T,G)-A--L            | 11,767 11,897 250 11,612 11,409 |
| BPO-BGG + (T,G)-A--L  | 20,507 20,993 254 11,834 8,838 |

* See footnote to Table I.

cyte proliferation of cells from syngeneic guinea pigs. It is striking that in our system only the syngeneic strain produced an antiserum capable of inhibiting antigen-induced lymphocyte proliferation in that same strain, whereas alloimmunization was ineffective. No evidence was obtained that anti-idiotype or even antiallotype antibodies were produced by immunizing strain 2 animals with anti-BPO-BGG antibodies from strain 13 and vice versa. This would suggest, that strain 2 and 13 (which are closely related) may also share the same immunoglobulin allotypes. On the other hand, our findings also raise the interesting notion that strain 2 and strain 13 guinea pigs are apparently high responders to their own idiotypes but not to the idiotypes of the allogeneic strain. This is consistent with the hypothesis that autoanti-idiotypes play an important role in the regulation of the immune response (20). We have previously described the preparation of antisera which can specifically block antigen-induced lymphocyte proliferation (1). These antisera were raised by immunizing with lymphoid cells from various (2 × 13)F₁ hybrid guinea pigs in which the responsiveness to low doses of ASAN, BPO-BGG, and (T,G)-A--L segregated independently of each other. One of these anti-R sera (anti-R₈P₉B₈G₉G) closely resembles the strain 13 anti-idiotypic serum which has been raised by immunizing strain 13 guinea pigs with immunoadsorbent-purified anti-BPO-BGG antibody. The inhibitory activity of both sera, raised by these two different immunization procedures, can be absorbed by lymphoid cells from immunized strain 13 guinea pigs but not by cells from immunized strain 2 animals. The failure of nonimmunized strain 2 and strain 13 cells to absorb inhibitory activity suggests that immunization with BPO-BGG is necessary to stimulate the proliferation of sufficient clones of cells bearing specific receptors or recognition structures for BPO-BGG. This view would also be consistent with the finding that antigen-specific receptors for phosphorylcholine on the surface of murine spleen cells are demonstrable on the cells of immunized but not of unimmunized animals (21).

The anti-R₈P₈B₈G₉G serum and the strain 13 anti-idiotype serum can specifically inhibit BPO-BGG-induced T-cell stimulation and do not interfere with the in vitro proliferation of ASP-OVA- and (T,G)-A--L-pulsed lymphoid cells. They seem therefore to interact specifically with BPO-BGG-specific lymphocyte receptors. It appears also that these antisera are not directed against gene products.
which are linked to or part of the 2 or the 13 MHC. Firstly, the antisera were raised either between 2 × 13 siblings (anti-R\textsubscript{BPO-BGG}) or between inbred strain 13 guinea pigs which should possess identical MHC. Secondly, neither antiserum inhibits the DNP-GL or GT response in (2 × 13)F\textsubscript{1} animals. The immune response to DNP-GL and GT is controlled by autosomal \textit{Ir} genes linked to the 2 and 13 MHC, respectively (7, 8). It view of the similarities in activity, specificity, and adsorption properties it is likely that anti-R\textsubscript{BPO-BGG} and a strain 13 BPO-BGG are both directed against the same set of recognition structures for BPO-BGG on the cell membrane of strain 13 lymphocytes. Whether these recognition structures are represented to the same extent on T or B lymphocytes will have to await immunofluorescence and autoradiographic studies. Preliminary studies indicate that PEL are more efficient than lymph node cells in absorbing inhibitory activity from the anti-idiotypic sera suggesting that these idiootype receptors may be expressed to a greater extent on T rather than on B cells. The observation that the anti-idiotypic antisera inhibited antigen-induced proliferation of a purified T-cell population suggests the presence on T cells of receptors with similar idiotypic specificities to those present on circulating immunoglobulins. This would confirm the findings of Binz and Wigzell obtained with transplantation antigens (17).

The inhibition of the BPO-BGG-induced response seems to involve an interaction between the antiserum and the appropriate strain-specific idiootype receptors on immune cells, i.e., a kind of blindfolding. The fact that inhibition occurs even after brief pulse with antigen but requires the continuous presence of the antibody during culture (1) is compatible with this interpretation. Furthermore, the failure of the BPO-BGG anti-idiotypic sera to completely inhibit a sensitive cell population which had been pulsed simultaneously with both BPO-BGG and (T,G)-A--L, suggests that the inhibition of BPO-BGG-sensitive cells is largely specific and that cytotoxic cell products or antigen-antibody complexes which may be released by BPO-BGG-sensitive cells are not able to suppress nonspecifically (T,G)-A--L-induced cell proliferation. It may be wondered whether, in addition to anti-idiotypic antibodies, the antisera raised by immunization with purified anti-BPO-BGG antibodies may also contain anti-BPO-BGG antibodies. This could theoretically be the case if BPO-BGG or immune complexes had been eluted from the immunoabsorbent column. However, if the animals, in addition to free anti-BPO-BGG antibody, had been immunized with a small amount of eluted BPO-BGG-antibody complexes, it is difficult to see how the "contaminating" anti-BPO-BGG in the anti-idiotype sera could account for the specific inhibition of BPO-BGG-induced lymphocyte activation. Firstly, no such precipitating anti-BPO-BGG antibodies were detected in the anti-idiotype sera. Secondly, a strain 13 BPO-BGG totally inhibits the BPO-BGG response at a final concentration of 1:100, whereas a precipitating anti-BPO-BGG antiserum fails to do so at that concentration (data not shown). Anti-BPO-BGG even at a final concentration of 1:50 has virtually no effect on the PHA and antigen responses in vitro while a strain 13 BPO-BGG at the same concentration totally abolishes antigen responsiveness. Finally, the possible presence of anti-BPO-BGG antibodies could not explain the strain-specific inhibitory effect of the anti-idiotypic antisera. These observations strongly suggest therefore that the anti-BPO-BGG
antibodies in the anti-idiotypic sera do not contribute to the inhibitory activity of these sera.

The ability of both strain 2 and strain 13 anti-idiotypic sera to suppress BPO-BGG responsiveness of primed (2 × 13)F₁ cells (Table I) could at first glance suggest that, unlike the situation with immunoglobulin allotypes which frequently appear to display allelic exclusion (22, 23), both allelic forms of the BPO-BGG-specific immunoglobulin idiotypes could be expressed on F₁ lymphoid cells. However, this possibility holds for primed (2 × 13)F₁ cells immunized with high doses of antigen (Table II). By contrast, (2 × 13)F₁ cells primed with low doses of antigens are only inhibited by the α strain 13 BPO-BGG antiserum which also inhibits immunized strain 13 (Table IV) cells but not high dose-immunized strain 2 cells (Table III). Accordingly, it is likely that strain 2 and 13 produce different sets of antibodies against BPO-BGG with different idiotypic determinants. Inhibition of lymphocyte proliferation by anti-idiotypic antibodies is therefore likely to involve different clones of immune lymphoid cells. Experiments on the heterogeneity of anti-BPO-BGG antibodies are in progress.

Preliminary evidence suggests that the inhibitory activity of the anti-idotype sera is predominantly associated with the 19S fraction. It would appear therefore that these antisera (even when obtained from animals 4 mo after immunization; results not shown) are IgM-like and in this respect perhaps similar to the anti-idiotypic antibody raised in Lewis × Brown Norway F₁ rats immunized with Lewis anti-BN alloantibody (reference 23 and F. W. Fitch, personal communication). Our anti-idotype sera appear therefore to differ from the anti-idiotypic antibodies of Eichmann (14) produced in guinea pigs against the A/J antibodies specific for the Group A streptococcal carbohydrate. The IgG₁ fraction from the guinea pig anti-idiotypic serum injected into normal A/J mice can induce both T- and B-cell memory against the Group A streptococcal carbohydrate and prime both T and B lymphocytes for a secondary immune response to this antigen (24). On the other hand, guinea pig IgG₂ can suppress in vivo antibody production to streptococcal carbohydrate in mice presumably by the elimination of specific precursor cells (14).

Obviously, the production and specificity of anti-idiotypic antisera against the individual's own idiotypes (possibly functioning as antigen receptors) may have important implications for antigen recognition and the regulation of the immune response in vitro and possible also in vivo. Studies on the in vivo effects of these sera are currently in progress.

**Summary**

The in vitro T-cell proliferation induced by penicilloylated bovine IgG (BPO-BGG) in sensitized strain 2 and strain 13 guinea pigs could be specifically blocked by strain-specific antisera presumably directed against cell membrane-associated immunoglobulin idiotypes. The anti-idiotypic antisera were prepared in strain 2 and strain 13 guinea pigs against immunoabsorbent purified anti-BPO-BGG antibodies which had been raised in strain 2 and strain 13 animals. Strain 13 antistrain 13 anti-BPO-BGG (α strain 13 BPO-BGG) suppressed the in vitro BPO-BGG response of cells from immunized strain 13 animals but did not inhibit the response of cells from immune strain 2 animals. Conversely, the
corresponding antiserum raised in a strain 2 combination (a strain 2 BPO-BGG) only inhibited the in vitro BPO-BGG response of strain 2 cells. Furthermore, the inhibitory activity of the antiserum could only be absorbed by immune cells from the syngeneic strain. The activity of the a strain 13 BPO-BGG serum was highly specific; the inhibitory activity could only be absorbed by BPO-BGG-sensitive strain 13 cells. The inhibitory activity of the anti-idiotypic sera was predominantly associated with the 19S fraction. The data suggest that immune cells and in particular T lymphocytes from strain 2 and strain 13 guinea pigs possess strain-specific recognition structures for BPO-BGG with the same idiotypes as the corresponding strain-specific immunoglobulins. Furthermore, the production of such inhibitory anti-idiotypic sera was restricted to syngeneic combinations, which suggests a potential role of autoanti-idiotypic antibodies in the regulation of the immune response. The anti-idiotypic antisera used here are apparently directed against gene products not associated with the strain 2 or strain 13 major histocompatibility complex.

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