HISTOCOMPATIBILITY ANTIGENS AND GENETIC
CONTROL OF THE IMMUNE RESPONSE IN GUINEA PIGS
IV. Specific Inhibition of Lymphocyte Proliferation by Auto-Anti-
Idiotypic Antibodies*

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In the course of raising high affinity antibodies to penicilloylated bovine Ig
(BPO-BGG) in strain 2 guinea pigs by repeated antigenic stimulation over a
period of 9 mo, it was observed that one animal from a group of seven no longer
gave a delayed hypersensitivity skin response when challenged with BPO-BGG.
Further investigation revealed that the serum of this animal (*305) had no
detectable antibodies to BPO-BGG but possibly possessed strain-specific anti-
idiotypic antibodies which specifically suppressed BPO-BGG-induced T-cell pro-
liferation in vitro. The inhibitory activity which was associated with the serum
of animal *305 was functionally similar to the strain 2 anti-idiotypic antibodies
(à strain 2 BPO-BGG) raised by immunizing strain 2 guinea pigs with immuno-
adsorbent column-purified strain 2 BPO-BGG (1). This communication com-
pares the in vitro inhibitory effect of serum *305 with that of à strain 2 BPO-
BGG antiserum.

Materials and Methods

Animals. Guinea pigs of strain 2, 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 as described elsewhere (2).

Antigens and Immunization of Guinea Pigs. The immunization procedures have previously,
been described in detail (1, 2). Briefly, 5 mg aspirin anhydride (Chemische Fabrik Aubing,
München, Germany) were injected intradermally in 0.1 ml dimethyl sulfoxide on days 0, 4, and 8.
BPO-BGG (100 ~g) and multichain copolymer poly-L-(Tyr, Glu)-poly-dL-Ala-poly-L-Lys [(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, 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, strain 2 and (2 × 13)F,
animals were also immunized with a dinitrophenylated copolymer of L-glutamic acid (60%) and L-
lysine (40%) (DNP, GL). 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, III.). Solutions of DNP, GL and GT
in 0.01 M PBS, pH 7.4, were emulsified with an equal volume of CFA. Animals were immunized
with 100 ~g of DNP, GL or 500 ~g of GT subcutaneously in multiple sites in the nuchal skin and
thigh.

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

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site was measured 24 h after testing with a caliper (Schnelltaster; Kroeplin, Schüchten, Germany). Delayed skin reactivity to BPO-BGG was assessed by the intradermal injection into the shaved flank of 5 and 50 μg BPO-BGG in 100 μl of PBS (0.01 M, pH 7.4).

Production of Antisera. Strain 13 anti-strain 2 serum was prepared by immunizing strain 13 guinea pigs with a homogenate of strain 13 lymph node and spleen cells as previously described (3). Antisera against strain 2 and strain 13 anti-BPO-BGG were prepared as previously described (2). The immune anti-BPO-BGG antibodies were then used as immunogens for the sensitization of strain 2 and strain 13 guinea pigs (1).

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Serum *305 was obtained from a strain 2 guinea pig (*305) which had been immunized with BPO-BGG according to the immunization schedule outlined in Table I. All animals (a total of seven) were bled (6–8 ml blood) at about 2 wk intervals and their serum used for the preparation of anti-BPO-BGG antibody (2). At the end of day 284 one strain 2 animal out of a group of seven no longer gave a delayed hypersensitivity skin response when challenged with 50 μg BPO-BGG. The sera of all animals were tested for their ability to inhibit BPO-BGG-induced T-cell proliferation in vitro.

Absorption of Antisera. Antisera were mixed with an equal volume of packed lymph node and spleen cells (approximately 10⁷ 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.

Antibody Determinations. Antibodies directed against the penicilloyl (BPO) group were determined by the neutralization of penicillin-coated T4 bacteriophages as previously described (4). The passive hemagglutination technique was used to measure antibodies to bovine Ig (BGG) (5). BGG was bound with carbodiimide to sheep erythrocytes as described by Golub et al. (5).

Cell Preparation

Peritoneal exudate lymphocytes (PEL). Guinea pig PEL were purified by passage through nylon wool columns (2). For the assay of antigen-induced proliferation, lymphocyte suspensions (30 × 10⁶/ml) were incubated with either 100 μg of DNP-GL and GT or 400 μg aspiryl ovalbumin (ASP-OVA), BPO-BGG, and (T, G)-A-L/ml of Medium 199 (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, washed three times with M-199, and then cultured at a cell concentration of 1.25 × 10⁶/ml. 0.2-ml aliquots of the antigen-pulsed PEL (0.25 × 10⁶ cells) were cultured in round-bottom microtiter plates (Cooke Microtiter system; Sterilin LTD, Richmond, Surrey, England) in medium RPMI-1640 (Grand Island Biological Co., Grand Island, N. Y.) supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml), adding either 1% normal guinea pig serum or 1% guinea pig antiserum supplemented with 9% heat-inactivated fetal calf serum to 72 h at 37°C. 18 h before the termination of the cultures, 0.5 μCi of tritiated thymidine (sp act, 5 Ci/mmol; The Radiochemical Centre, Amersham, Great Britain) was added to each well. The cells were harvested with a multiple cell culture harvester (Skatron, Lierbyen, Norway), and the radioactivity was measured in a Beckman liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.). The results are expressed as total counts per minute per culture well.

Results

Repeated immunization with BPO-BGG (Table I) over a period of about 9 mo, resulted in one strain 2 guinea pig (out of a group of seven) becoming unresponsive when challenged with BPO-BGG in vivo. Whereas the other members of the group gave intense 4-h Arthus and 24-h delayed skin reactions to 50 μg of intradermally injected BPO-BGG, animal *305 failed to respond. The skin test was repeated 10 days later with the same results. The serum of animal *305 contained no detectable antibodies to either the BPO group or to the BGG carrier, whereas the sera of the other strain 2 guinea pigs in the group had high antibody titers to both the BPO and the BGG determinants (results not shown). The sera of all animals were then tested for their capacity to inhibit BPO-BGG-
Table I

**Immunization Schedule which Resulted in the Appearance in One Strain 2 Guinea Pig Out of a Group of Seven of Antibodies with BPO-BGG-Specific Anti-Idiotypic Activity**

| Antigen administered | day | 0     | 13    | 31    | 44    | 72    | 91    | 105   | 120   | 163   | 179   | 220   | 239   | 264   |
|----------------------|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| BPO-BGG in CFA (in foot pads) | 500 µg | 500 µg | 10 µg | 100 µg | 100 µg | 100 µg | 100 µg | 100 µg | 100 µg | 100 µg | 50 µg | 50 µg | 50 µg |
| PHA                  | 8742 | 844   | 466   | 709   | 246   | 292   | 412   | 573   | 774   | 56,432 | 67,643 | 67,643 | 81,140 | 120,843 |
| BPO-BGG              | 844  | 466   | 709   | 246   | 292   | 412   | 573   | 774   | 56,432 | 67,643 | 67,643 | 81,140 | 120,843 |
| ASP-OVA              | 90,738 | 347   | 77,723 | 56,432 | 67,643 | 67,643 | 81,140 | 120,843 |

Table II

**Inhibition of T-Cell Proliferation, by Anti-Idiotypic Serum (a Strain 2 BPO-BGG) and Sera from Strain 2 Guinea Pigs Repeatedly Immunized with BPO-BGG, in Primed High Dose-Immunized Strain 2 PEL**

| Stimulant | Guinea pig serum added* | Normal | *301 | *302 | *303 | *304 | *305 | *307 | *308 |
|-----------|-------------------------|--------|------|------|------|------|------|------|------|
| NIL       | 8742                    | 844    | 466  | 709  | 246  | 292  | 412  | 573  | 774  |
| PHA       | 173,386                 | 115,141| 66,963| 77,723| 56,432| 67,643| 67,643| 81,140| 120,843|
| BPO-BGG   | 90,738                  | 347    | 77,723| 56,432| 67,643| 67,643| 81,140| 120,843|
| ASP-OVA   | 135,506                 | 44,254 | 61,885| 47,857| 55,839| 59,370| 49,578| 80,020| 82,735|

* Final concentration of serum in culture was 1%.

Results are expressed as counts per minute per culture. Each result represents the mean of three cultures; significantly depressed results are underlined.

Discussion

A number of recent studies have reported the production of autologous anti-idiotypic antibodies (6-9) and it has been suggested (9) that the in vivo appearance of anti-idiotypic antibodies serves a regulatory function, possibly by controlling the synthesis of a particular antibody elaborated in response to an antigenic stimulus. The phenomenon of idiotype-anti-idiotype interactions, as suggested by Jerne (10) and recently modified by Hoffmann (11), is pictured as a network of interacting variable domains of immunoglobulin molecules. A simi-
lar theory based on the interaction of complementary idiotypes has been put forward by Köhler et al. (12).

The results in this paper indicate that a serum with specific in vitro inhibitory activity may arise in the course of repeated antigenic stimulation. These data are reminiscent of the production of anti-idiotypic antibody in rats repeatedly immunized with alloantigens (9). A striking feature of serum *305 however, is that its activity appears to be directed against strain-specific idiotypes characteristic of strain 2 guinea pigs and in this respect serum *305 is functionally similar to a strain 2 BPO-BGG produced by repeated immunization with syngeneic antibodies. Both serum *305 and a strain 2 BPO-BGG are exquisitely specific in vitro in that they only inhibit BPO-BGG-induced in vitro T-cell proliferation but they do not interfere with the stimulation induced by the other antigens (Table III; reference 1).

The significance of the production of "auto-anti-idiotypic antibodies", specifically reactive with a particular antigen (in one case out of seven), is at present not clear. One possibility is that the production of anti-BPO-BGG may have been suppressed by an excess of complementary (12) anti-idiotypic antibodies with the result that the immune balance in the "suppressed" animal may have been temporarily shifted in the favor of auto-anti-idiotypic antibody production. Further studies with strain 2 guinea pigs using different immunization schedules and monitoring the appearance and disappearance of antibodies and anti-idiotypic antibodies may clarify this point.

In support of the suggestion that the auto-anti-idiotypic antibodies (8, 9),
which sometimes appear in the serum of immunologically unresponsive animals, may serve an immunoregulatory role is the finding that the in vivo administration of a strain 2 BPO-BGG into sensitized strain 2 guinea pigs results in a dramatic but short-lived (about 4 wk) fall in the level of anti-BPO antibodies.¹

It remains to be seen whether further immunization with BPO-BGG can induce unresponsiveness in the remaining responders of the group with the concomitant appearance of “auto-antibody” activity and whether recovery from unresponsiveness results in a decline in the level of BPO-BGG-specific inhibitory activity in the serum of animal *305.

Summary

The in vitro T-cell proliferation induced by penicilloylated bovine IgG (BPO-BGG) in sensitized strain 2 guinea pigs could be specifically blocked by the serum of guinea pig (*305) which had been repeatedly immunized with BPO-BGG over a period of 9 mo. The antibodies which appeared in the serum of this animal (*305) were functionally similar to the strain 2 anti-idiotypic antibodies (a strain 2 BPO-BGG) raised by immunizing strain 2 guinea pigs with immunoadsorbent column-purified BPO-BGG. Animal *305 had no detectable antibodies to BPO-BGG and failed to give a delayed hypersensitivity skin response when challenged with BPO-BGG.

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