HISTOCOMPATIBILITY-LINKED IMMUNE RESPONSE GENE
FUNCTION IN GUINEA PIGS

SPECIFIC INHIBITION OF ANTIGEN-INDUCED LYMPHOCYTE
PROLIFERATION BY ALLOANTISERA

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The ability of both mice and guinea pigs to form an immune response to a variety
of distinct antigens has been shown to be under control of a number of autosomal
dominant immune response (IR) genes (1). In guinea pigs, a number of specific IR
genotes have been identified. The prototype is the “PLL gene” (2) which is found in
strain 2 guinea pigs and is absent in strain 13 animals (3). The PLL gene controls
responsiveness to poly-L-lysine (PLL), poly-L-arginine (PLA), a copolymer of L-
glutamic acid and L-lysine (GL), protamine, and the hapten conjugates of these
polypeptides (4). More recently, responsiveness to the linear random copolymer of
L-glutamic acid and L-alanine (GA) (5) and to limiting doses of bovine serum albumin
(BSA) (6) and dinitrophenyl-BSA (DNP-BSA) (7) have also been shown to be under
control of IR genes present in strain 2 animals. Responsiveness to PLL and GA are
linked in strain 2 animals and in outbred animals; however, population studies of
immune responses in outbred animals demonstrate that the linkage is not absolute
and some outbred animals respond to one but not the other suggesting that two distinct
genotes are involved (8). Responsiveness to a random copolymer of L-glutamic acid and
L-tyrosine (GT) (9, 10) and to limiting doses of DNP-guinea pig albumin (11) is
under control of IR genes present in strain 13 animals, but absent in strain 2 animals.

Although the importance of IR genes in the regulation of the immune response has
been amply confirmed, the mechanism of IR gene function has not been elucidated.
Some IR genes are involved in the mechanism of antigenic recognition by the thymus-
derived (T) lymphocyte. Thus an analysis of the PLL immune system in guinea pigs
strongly suggests that the initiation of functions thought to be mediated by the T
lymphocyte (delayed cutaneous hypersensitivity, antigen-induced lymphocyte prolif-
eration in vitro, and carrier function) require the presence of the appropriate IR
genote (12). Furthermore, animals that lack the PLL gene (nonresponders) are capable

Abbreviations used in this paper: B lymphocytes, antibody-forming cell precursors; BSA,
bovine serum albumin; CFA, complete Freund's adjuvant; DNP, dinitrophenyl; GA, a
copolymer of L-glutamic acid and L-alanine; GL, a copolymer of L-glutamic acid and L-lysine;
GT, a copolymer of L-glutamic acid and L-tyrosine; HBSS, Hanks' balanced salt solution;
IR, immune response; PBS, phosphate-buffered saline; PELS, peritoneal exudate lymphocytes;
PHA, phytohemagglutinin; PLA, poly-L-arginine; PLL, poly-L-lysine; PPD, purified protein
derivative of tuberculin; T lymphocytes, thymus-derived lymphocytes.
of recognizing DNP-PLL as a hapten and producing antibodies to DNP-PLL when this molecule is coupled to an immunogenic protein carrier (13). This implies that the population of antibody-forming cell precursors (B lymphocytes) in the nonresponder animal is not deficient in precursors specific for DNP-PLL. In mice strong evidence has been presented that IR gene function is expressed on the T lymphocyte in that the genetically controlled difference in the 7S antibody responses between high and low responder strains immunized with a branched-chain copolymer of tyrosine, glutamic acid, d, l-alanine, and lysine [(T, G)-A--L] is lost in thymectomized irradiated mice restored only with syngeneic bone marrow (14, 15).

Another property of some IR genes of both mice and guinea pigs that may offer an insight into their mechanism of function is their close linkage to genes controlling histocompatibility antigens (16). Thus, in mice the Ir-1 locus has been mapped within the H-2 region (1), while in guinea pigs the closeness of the relationship between the PLL gene and strain 2 histocompatibility antigens is demonstrated by the fact that in 18 (F1 X 13) backcross animals and in 94 random-bred animals the PLL gene has not been dissociated from the genes controlling strain 2 histocompatibility specificity (17, 18).

In order to more fully understand the relationship between IR genes, histocompatibility antigens, and immune recognition, we here examine the effect of specific alloantisera on lymphocyte stimulation induced by antigens under control of IR genes. The population of lymphocytes used in this study, the peritoneal exudate lymphocytes (PELS) of the guinea pig, have a high degree of reactivity in antigen-induced lymphocyte proliferation and are composed almost entirely of thymus-derived cells (19). We will demonstrate that alloantisera can specifically block the activation of T lymphocytes by antigens, the response to which is linked to the presence of histocompatibility types against which the alloantisera are directed.

Materials and Methods

Animals.—Inbred strain 2 and strain 13 guinea pigs were obtained from the Division of Research Services, National Institutes of Health, Bethesda, Md. (2 X 13)F1 animals were obtained by mating strain 2 with strain 13 animals in our own colony.

Antigens.—A copolymer of L-glutamic acid (60%) and L-lysine (40%) (GL) with an average molecular weight of 115,000 was obtained from the Pilot Chemical Division of New England Nuclear Corp., Boston, Mass. DNP4GL was prepared by the reaction of 2,4-dinitrofluorobenzene with GL (20). The subscript refers to the average number of DNP groups per molecule. A copolymer of L-glutamic acid (50%) and L-tyrosine (50%) (GT), mol wt 14,500, was obtained from Miles Laboratories, Kankakee, Ill. Purified protein derivative of tuberculin (PPD) was purchased from Connaught Medical Research Laboratories, Willowdale, Ontario, Canada. Phytohemagglutinin (PHA) was obtained from Wellcome Research Laboratories, Beckenham, England.

Immunization of Guinea Pigs.—Solutions of each antigen in 0.015 M phosphate buffer, pH 7.4, containing 0.15 M NaCl (PBS) were emulsified with an equal volume of complete Freund's adjuvant (CFA) containing 0.5 mg of Mycobacterium tuberculosis/ml (Difco Laboratories, Detroit, Mich.). Strain 2 animals were immunized with 100 µg of DNP-GL divided equally among the four footpads. Strain 13 animals were immunized with 500 µg of GT divided
among the four footpads. F1 animals were immunized simultaneously with 100 ug of DNP-GL and 500 ug of GT; each antigen was administered in one front footpad and one rear footpad.

Preparation of Alloantisera.—Strain 2 animals were immunized in the footpads with a CFA emulsion of a homogenate of lymph node and spleen cells obtained from strain 13 animals. The animals were boosted 2 and 4 wk after primary immunization by an intradermal injection of a homogenate of lymph node and spleen. A strain 13 anti-strain 2 serum was prepared in similar fashion. The sera used in these studies were obtained 8-10 wk after initial immunization. Both pools of antisera exhibited significant cytotoxicity against the lymph node cells of the immunizing strain as determined by 51Cr cytotoxicity testing. All antisera were sterilized by Millipore filtration and heat inactivated at 56°C for 45 min before use.

Absorption of Alloantisera with Gamma Globulin of the Opposite Strain Conjugated to Agarose Beads.—Gamma globulin fractions were prepared from normal strain 2 and normal strain 13 serum by precipitation with 50% saturated ammonium sulfate. Agarose beads (Sepharose 2B; Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) were suspended to twice their packed volume in distilled water at 4°C and activated by the addition of an equal volume of cold water saturated with cyanogen bromide (CNBr). The pH was kept above 11 by the addition of 5 M NaOH. The suspension was then washed free of reaction products and excess CNBr by the addition of a large volume of chilled 0.1 M NaHCO3, pH 8.5. The activated beads were brought to twice their packed volume with this buffer and 1 mg of gamma globulin was added per milliliter of agarose. The suspension was then stirred for 16-20 hr in the cold and unconjugated gamma globulin removed by washing with 10 vol of PBS, 10 vol of 50% acetic acid, and then another 10 vol of PBS. The agarose-gamma globulin conjugate was packed into a small column. The appropriate alloantiserum was then slowly passed through the column.

Preparation of PELS.—Peritoneal exudate lymphocytes (PELS) were obtained according to the method of Rosenstrech, Blake, and Rosenthal (19). In brief, animals were injected intraperitoneally with 25 ml of sterile mineral oil (Marcol 52; Humble Oil and Refining Co., Houston, Tex.) to induce a peritoneal exudate. 3-4 days later the peritoneal cavity was lavaged with 200 ml of Hanks' balanced salt solution (HBSS). Cells were washed three to four times in HBSS to remove residual oil. The lymphocytes were separated from the whole exudate cell population by passage over a column of viscose rayon. The population of cells after column purification was composed of 80% lymphocytes, 10-15% macrophages, and 5-10% neutrophiles.

In Vitro Assay of Antigen-Induced DNA Synthesis.—PELS (10 X 10^6 cells/ml) were

FIG. 1. Over-all scheme of the experiment.
incubated with 100 μg antigen/ml or 10 μg PHA/ml RPMI-1640 (Grand Island Biological Co., Grand Island, N. Y.) containing 10% heat-inactivated fetal calf serum (Industrial Biological Laboratories, Rockville, Md.) for 30 min at 37°C; the cells were then washed three times with media to remove excess antigen and then cultured at a concentration of 2 × 10⁶ cells/ml in RPMI-1640 supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), and L-glutamine (300 μg/ml) and either 10% normal guinea pig serum or 10% of the allogeneic serum. 1.0-ml cultures were incubated in triplicate in plastic 12 × 75 mm tubes (Falcon Plastics, Oxnard, Calif.) at 37°C for 72 hr in a humid atmosphere of 5% CO₂ in air. 4 hr before harvesting, 2.0 μCi of tritiated thymidine (thymidine-³H, 6.7 Ci/mM; New England Nuclear Corp.) were added to each culture. The amount of thymidine-³H incorporated into cellular DNA was measured by the Millipore filter method of Robbins, Burk, and Levis (21); radioactivity was counted in a Beckman liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.). The over-all scheme of the experiment is shown in Fig. 1.

**RESULTS**

**Inhibition of Antigen-Induced DNA Synthesis: Studies in Strain 2 and Strain 13 Animals.**—PELS from strain 2 animals were incubated with DNP-GL, PPD, or PHA for 30 min, washed, and then cultured in the presence of 10% normal guinea pig serum for 72 hr; this procedure leads to a significant stimulation of thymidine incorporation (Table 1). However, when aliquots of these same cells were cultured in the presence of the 13 anti-2 serum, the proliferative response to DNP-GL was markedly inhibited and the responses to PPD and PHA were inhibited to a lesser extent. A parallel result is seen in cultured

| Stimulant | Normal | 13 Anti-2 |
|-----------|--------|-----------|
|            |        |           |
| Strain 2 animals |        |           |
| 0          | 546*   | 489       |
| DNP-GL     | 13,409 (0.35)† | 380 (0.00) |
| PPD        | 31,917 (0.86) | 2,383 (0.12) |
| PHA        | 37,152 (1.00) | 15,979 (1.00) |
| Strain 13 animals |        |           |
| 0          | 466    | 599       |
| GT         | 3,612 (0.11) | 320 (0.00) |
| PPD        | 14,807 (0.50) | 387 (0.00) |
| PHA        | 29,001 (1.00) | 10,934 (1.00) |

*Results are expressed as counts per minute per tube (cpm); each value is the mean of three determinations.
†Number in parentheses is the fraction (Fx) of PHA response

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Fx = \frac{cpm \text{ with antigen} - cpm \text{ with no antigen}}{cpm \text{ with PHA} - cpm \text{ with no antigen}}
\]
PELS obtained from strain 13 animals; the response to GT is markedly inhibited by the 2 anti-13 serum as is the response to PPD. A summary of the results, three experiments in strain 13 animals and 3 experiments in strain 2 animals, is shown in Table II. Because in all cases the proliferative response to PHA was less inhibited than the response to antigen the results are expressed as the ratio of response to antigen to the response induced by PHA. In strain 2 animals, marked inhibition of the proliferative response to DNP-GL was seen and the PPD response was inhibited to a lesser extent. In strain 13 animals both the GT and PPD responses were markedly inhibited, and in these experiments the PPD response was inhibited to a greater extent than the GT response.

The effect of the alloantisera on the proliferative response of PELS in culture was also studied by culturing the PELS with a fixed concentration of antigen for the entire 72 hr culture period either in the presence of normal guinea pig serum or the alloantisera. In these experiments inhibition could not be demonstrated as regularly as when antigen was present only during the initial antigen “pulse” period.

All the experiments described above were performed with the alloantisera present for the entire 72 hr culture period. In order to evaluate whether the alloantisera could exert their inhibitory effect during the short period of time

| Stimulant | Normal (Fx PHA response*) | 13 Anti-13 (Fx PHA response) | Suppression |
|-----------|---------------------------|-------------------------------|-------------|
| Strain 2 animals |                           |                               |             |
| DNP-GL    | 0.63 ± 0.14§              | 0.08 ± 0.05                   | 87          |
| PPD       | 0.94 ± 0.08               | 0.46 ± 0.18                   | 51          |
| PHA       | 1.00                      | 1.00                          |             |
| Strain 13 animals |                       |                               |             |
| GT(GT)    | 0.21 ± 0.05               | 0.07 ± 0.04                   | 67          |
| PPD       | 0.72 ± 0.14               | 0.11 ± 0.11                   | 85          |
| PHA       | 1.00                      | 1.00                          |             |

* See Table I for calculation.

‡ % Suppression = 100 \[\frac{\text{Fx PHA response with alloantisera}}{\text{Fx PHA response with normal serum}}\].

§ Each number is the mean ± SE of three determinations.
the lymphocytes were incubated with antigen, the cells were pulsed with antigen either in the presence of normal guinea pig serum or the alloantisera; after three washes the cells were cultured in the presence of normal guinea pig serum or the alloantisera. The results of a typical experiment utilizing PELS from strain 13 animals are shown in Table III. When the cells are incubated with antigen in the presence of the 2 anti-13 alloantisera and then cultured in normal guinea pig serum, no inhibition of antigen-induced lymphocyte prolif-

TABLE III

| Stimulant | Pulse Culture | Serum |
|-----------|--------------|-------|
| 0         | Normal       | Normal |
| GT        | 8,045        | 6,693 |
| PPD       | 23,869       | 35,070 |
| PHA       | 34,643       | 28,168 |

* Results are expressed as counts per minute per tube; each value is the mean of three determinations.

**TABLE IV**

Inhibition of Lymphocyte Proliferation in F1 Animals

| Stimulant | Serum |
|-----------|-------|
| 0         | Normal |
| DNP-GL    | 68,361 (1.21) |
| GT        | 17,070 (0.25) |
| PPD       | 20,049 (0.31) |
| PHA       | 56,887 (1.00) |

* Results are expressed as counts per minute per tube; each value is the mean of three determinants.

† Fraction of PHA response.
and GT were immunized simultaneously with these antigens. The PELS from these animals were incubated with antigen and then cultured in the presence of either the 13 anti-2 serum or the 2 anti-13 serum. The results of a typical experiment are shown in Table IV. The 13 anti-2 serum markedly inhibited the response to DNP-GL, but only slightly inhibited the response to GT. The 2 anti-13 serum inhibited the response to GT while only slightly inhibiting the response to DNP-GL. The PPD and PHA responses of F1 cells were depressed to a much lesser extent than had been observed in cultures derived from the parental 2 or 13 animals. Furthermore, as can be seen from Table IV, when the two antisera were combined and added to the cultures, marked depression of the PPD response as well as inhibition of both the DNP-GL

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**Fig. 2.** Inhibition of antigen-induced proliferation of F1 PELS by alloantisera. The results are expressed as the fraction of the PHA response. Each bar represents the arithmetic mean of five experiments ± 1 SEM. The response to DNP-GL in the presence of anti-2 serum was significantly less than in the presence of normal serum ($P < 0.001$) or in the presence of anti-13 serum ($P < 0.001$). The response to GT in anti-13 serum was significantly less than the response in the presence of normal ($P < 0.01$) or in the presence of anti-2 serum ($P < 0.01$). No statistically significant depression of the PPD response was seen.
and GT responses was seen. The results of five experiments in F1 animals are illustrated in Fig. 2. Each bar represents the ratio of the response induced by antigen to the response induced by PHA. When analyzed in this fashion, it can be clearly seen that the 13 anti-2 serum specifically suppresses the response to DNP-GL, but does not impair the response to GT. The 2 anti-13 serum suppresses the response to GT, but not that to DNP-GL. The 13 anti-2 serum also produced some apparent suppression of the response to PPD, although this was not statistically significant.

**TABLE V**

| Stimulant | Serum | Normal | 2 Anti-13 | 2 Anti-13 absorbed with 13 γ-globulin |
|-----------|-------|--------|-----------|--------------------------------------|
| Normal    | 2 Anti-13 absorbed with 13 γ-globulin |
| 0         | 842†   | 1,364  | 569       |
| DNP-GL    | 60,531 | 41,413 | 41,716    |
| GT        | 3,492  | 1,743  | 1,556     |
| PPD       | 72,469 | 45,309 | 44,940    |
| PHA       | 79,582 | 51,544 | 45,116    |

| 2 Anti-13 serum absorbed with 13 gamma globulin |
| Normal | 13 Anti-2 absorbed with 2 γ-globulin |
| 0      | 1,053  | 615  | 948   |
| DNP-GL | 28,569 | 1,028| 731   |
| GT     | 3,506  | 3,129| 6,098 |
| PPD    | 60,826 | 17,899| 38,981|
| PHA    | 12,226 | 19,064| 19,980|

* Both experiments were performed in F1 animals.
† Results are expressed as counts per minute per tube; each value is the mean of three determinations.

Effect of Absorption of the Alloantisera with Gamma Globulin of the Opposite Strain:—In order to rule out the possibility that the inhibition produced by the alloantisera could be secondary to anti-immunoglobulin allotype antibodies which might also have been present in the alloantisera, absorptions of the alloantisera with immunoglobulin were performed. The 13 anti-2 serum was absorbed with strain 2 gamma globulin coupled to agarose beads, while the 2 anti-13 serum was absorbed with 13 gamma globulin coupled to agarose. Table V shows the results of experiments performed in F1 animals with the absorbed antisera. After absorption with gamma globulin of the opposite strain, no decrease of the inhibitory activity of either of the alloantisera is seen.
DISCUSSION

The data presented demonstrate that alloantiserum which are primarily directed against histocompatibility antigens on the cell surface produce marked depression of the in vitro proliferative response to antigen by peritoneal exudate lymphocytes, an enriched population of highly reactive T lymphocytes. The studies in strain 2 and 13 animals do not demonstrate any apparent specificity in this inhibition of T lymphocyte function. Thus, the responses to antigens which are known to be under genetic control (DNP-GL in strain 2 animals and GT in strain 13 animals) and to an antigen which is not presently known to be under genetic control, PPD, are inhibited to a similar degree. One might conclude from these observations that the alloantisera coat the surface of the T lymphocyte and nonspecifically block the T cell receptor for antigen. Results similar to these have been reported in the human by Ceppelini et al. (22) and by Buckley, Schiff, and Amos (23). However, the experiments reported here utilizing cells from the F1 animals clearly demonstrate the specificity of the inhibition. The alloantisera markedly inhibit the response which is linked to the histocompatibility antigens against which the serum is directed. Thus, the anti-2 serum inhibits the response to DNP-GL, but not that to GT; the anti-13 serum inhibits the GT response, but not the DNP-GL response. The inhibition of the PPD and PHA responses in the F1 animals is slight compared to that observed in parental animals.

The inhibition of the PPD response in cultures of cells from strain 2 and strain 13 animals can be reinterpreted in the light of the data from the F1 experiments. In cultures from these parental animals the PPD response was inhibited to a considerably greater degree than the PHA response. This is consistent with a requirement for a recognition step controlled by histocompatibility-linked IR gene products in the activation of T lymphocytes by any antigen. Antigens such as DNP-GL or GT, the response to which is linked to the 2 or 13 histocompatibility complex, respectively, would represent substances for which IR genes associated with only the strain 2 or strain 13 alleles are specific; on the other hand, complex antigens such as PPD might be recognized by both the strain 2- and strain 13-associated IR gene products. Thus, in the parental strain, alloantisera block all available IR gene products and thus prevent activation by PPD. In the F1, an individual alloantiserum would block only half the IR gene products and the remaining ones would be sufficient for antigen recognition. When both alloantisera are used with F1 cells, the PPD response is blocked to the extent seen in the parental animal as would be expected from this line of reasoning.

Although the antisera are heat inactivated before use in tissue culture, it is possible that the inhibition seen could be secondary to killing of the responding cell population during the 3 day culture period, perhaps by complement components which are synthesized in vitro. This explanation seems unlikely in
view of the results of experiments in F1 animals. Studies of the distribution of alloantigens in humans (24) and mice (25) do not reveal evidence of allelic exclusion of the expression of histocompatibility genes. If this is also true for the guinea pig, then cytotoxic reactions mediated by alloantisera could hardly explain the specific suppression of antigen-stimulated proliferation noted in the F1 animals.

Another possible mechanism for the action of the alloantisera which must be considered is that the sera contain antibodies capable of interacting directly with the stimulating antigen. In order to rule this out, 13 anti-2 serum was passed over a DNP-GL-agarose bead column. This serum retained full inhibitory activity. Moreover, Bluestein et al. (9) have shown that the 2 anti-13 serum does not bind GT in a Farr assay.

The experiments in which the alloantisera have been absorbed with gamma globulin of the opposite strain and yet retain their inhibitory activity rule out the possibility that these sera produce their effect by blocking cell surface immunoglobulins which are similar to those found in the serum. Moreover attempts to demonstrate immunoglobulin allotype differences between strain 2 and strain 13 guinea pigs have failed (26). However, these studies do not exclude the possibility that the antisera are directed against a cell-associated immunoglobulin which is not present in the serum.

The steps involved in the in vitro proliferative response to antigen by sensitized lymphocytes have not been clearly defined. A number of studies have demonstrated that the response of lymphocytes to antigen requires the presence of macrophages. In both humans (27, 28) and guinea pigs (29) removal of macrophages from lymphocyte populations by passage through adherence columns significantly reduces the thymidine-3H incorporation of antigen-stimulated lymphocyte cultures. In addition, one can enhance the transformation of purified lymphocyte populations by the addition of macrophages. Seeger and Oppenheim (29) have shown that when macrophages were preincubated with antigen for a 1/2- to 6-hr period and then washed, they were able to stimulate the transformation of immune lymph node cell populations; this transformation could be produced equally well by antigen-pulsed macrophages obtained from immune or nonimmune animals. Rosenstreich, Waldron, and Rosenthal (30) have extended these observations to the peritoneal exudate lymphocyte population and have also shown that effective stimulation of PELS by antigen using the antigen pulse wash technique requires the presence of macrophages. These studies suggest that the initial step in the in vitro proliferative response to antigen is interaction of antigen with macrophages followed by macrophage-lymphocyte interaction by as yet unknown mechanisms. In the present experiments, the alloantisera inhibited the in vitro proliferative response only when present during the culture period and not during the initial incubation with antigen. Thus, it is likely that the alloantisera inhibit antigen-induced proliferation by blocking the recognition of macrophage
bound or processed antigen by the T lymphocyte rather than by blocking the initial uptake of antigen by macrophages.

The major conclusions which can be drawn from these observations are that immune response genes produce a cell surface-associated product and that this product plays a role in the mechanism of antigen recognition by the T lymphocyte. Alternative mechanisms, such as that suggested by Jerne (31), based upon the operation of IR genes during ontogenesis, fail to explain the specific suppression by alloantisera observed in the F1 lymphocytes.

Alloantisera might block the process of antigenic recognition by a number of different mechanisms. One possibility is that the immune response genes may be identical with histocompatibility genes and the product of the histocompatibility genes functions on the cell surface in the process of immune recognition. However, data demonstrating that IR genes are separate from D and K region genes of the H-2 system in the mouse (32) suggest that the IR gene product is not any of the now recognized histocompatibility antigens. Alternatively, the IR gene product may represent a separate and distinct set of antigen recognition substances presumably present on the surface of the T lymphocyte. Alloantisera could block the function of these IR gene-controlled substances by reacting with histocompatibility antigens which are physically close to the IR gene product on the cell surface. A precedent for the linkage of determinants both on the chromosome and on the cell surface is available in the mouse in the case of the H-2 and TL determinants (33). It is also possible that the alloantisera may contain antibodies directly reactive with and capable of directly blocking the specific IR gene products which are, in this instance, different from specific histocompatibility antigens.

The relationship between the proposed IR gene-controlled antigen recognition substance on the T lymphocyte and a receptor composed of immunoglobulin can at present only be a subject for speculation. Although a number of studies (34, 35) have demonstrated small amounts of immunoglobulin determinants on mouse T lymphocytes by a variety of sensitive methods, these findings have not been confirmed by all investigators (36, 37). The functional role of classical immunoglobulin molecules on the surface of the T lymphocyte has proven even more difficult to evaluate. Certain T cell-mediated functions such as the in vitro proliferative response to antigen (38), the production of graft-versus-host disease (39, 40), and the binding of antigen by T lymphocytes (41) can be inhibited by anti-immunoglobulin reagents. Again, not all of these experimental observations have been confirmed and the issue remains controversial (42). The IR gene product could represent a primordial V region immunoglobulin molecule expressed on the surface of the T lymphocyte. Alternatively, the IR gene product could represent a distinct set of molecules unrelated to immunoglobulin and functioning as the prime antigen-binding receptor of the T lymphocyte. Finally, the IR gene product may represent a nonclonally distributed substance found on the surface of T cells capable of
interacting with antigen with a relatively low degree of energy and specificity and acting in an auxiliary fashion with the prime antigen-binding receptor of the T lymphocyte. An analysis of the mechanism by which alloantisera block the activation of T lymphocytes should lead to an understanding of the nature of the IR gene product and its relation to the antigen-binding receptor of the T lymphocyte.

SUMMARY

A number of autosomal dominant immune response (IR) genes have been identified in both mice and guinea pigs. These IR genes have been shown to be linked to the major histocompatibility antigens of the species and to be functionally expressed primarily in T lymphocytes. In order to more fully understand the relationship between IR genes, histocompatibility antigens, and immune recognition, the effect of specific alloantisera on lymphocyte stimulation induced by antigens under control of IR genes was examined. Using lymphocytes from strain 2 or strain 13 animals, the in vitro proliferative responses both to antigens which are known to be under genetic control (DNP-GL in strain 2 guinea pigs and GT in strain 13 guinea pigs) and to an antigen which is not known to be under genetic control (PPD) were inhibited to a similar degree and to a much greater extent than the response to phytohemagglutinin. However, when cells from F1 (2 X 13) animals are used, the alloantisera markedly inhibit only the response which is linked to the histocompatibility antigens against which the serum is directed. Thus, the anti-2 serum inhibited the response to DNP-GL but not to GT; the anti-13 serum inhibited the response to GT but did not affect DNP-GL response. The inhibitory activity of the alloantisera could not be removed by absorption with gamma globulin of the opposite strain. It can be concluded from these observations that immune response genes produce a cell surface-associated product and that this product plays a role in the mechanism of antigen recognition by the T lymphocyte. The mechanisms by which alloantisera block this process of antigenic recognition is not resolved nor is the relationship between the IR gene product and the antigen-binding receptor of the T lymphocyte. The approach described here offers a powerful tool for the resolution of these problems.

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