ALLOANTISERUM-INDUCED INHIBITION OF MIGRATION
INHIBITION FACTOR PRODUCTION IN IMMUNE
RESPONSE GENE-CONTROLLED IMMUNE SYSTEMS

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A number of immune response (Ir) genes which control the capacity of individual animals to respond to specific antigens have been described in guinea pigs, mice, rats, and man (1–3). These Ir genes are linked to the major histocompatibility gene complex in each species. It is likely that products of Ir genes are involved in the process of antigen recognition by thymus-derived (T) lymphocytes in at least some genetic systems. It has previously been shown that antigen-specific stimulation of proliferation by guinea pig T lymphocytes could be blocked by incorporating alloantisera in the culture medium (4–6). These alloantisera were raised by the immunization of strain 2 guinea pigs with strain 13 lymphoid cells (anti-13 sera) or by the immunization of strain 13 guinea pigs with strain 2 lymphoid cells (anti-2 serum). The capacity of alloantisera to inhibit T-lymphocyte proliferative responses was specific in that the stimulation of (2 × 13)F1 cells by antigens, responsiveness to which was linked to strain 2 alloantigens, was blocked by anti-2 sera but not by anti-13 sera. Similarly, the activation of F1 lymphocytes by antigens, responsiveness to which is controlled by 13-linked Ir genes, was blocked by anti-13 sera but not anti-2 sera.

We concluded from these studies that alloantisera directly or indirectly block a T-lymphocyte surface structure coded for by an Ir gene and that this structure plays a role in antigen recognition by the T lymphocyte or in specific activation of T lymphocytes. Indeed, we suggested that the Ir-gene product

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Abbreviations used in this paper: BSA, bovine serum albumin; CFA, complete Freund's adjuvant; DNP-GL, DNP derivative of the copolymer of l-glutamic acid and l-lysine; GA, copolymer of l-glutamic acid and l-alanine; GPA, guinea pig albumin; GT, copolymer of l-glutamic acid and l-tyrosine; Ir, immune response gene; MIF, migration inhibition factor; NGPS, normal guinea pig serum; PEC, peritoneal exudate cell(s); SK-SD, streptokinase-streptodornase.

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merits serious consideration as the antigen-binding receptor of the T lymphocyte (5).

It is now recognized that T lymphocytes mediate a wide variety of immune phenomena including lymphokine production, specific cytotoxicity, mixed lymphocyte responsiveness, and helper function, as well as the ability to proliferate in vitro in response to antigen (7). As suggestive evidence indicates that some of these functions may be performed by specialized subsets of T lymphocytes (8–10), it is of importance to evaluate the capacity of alloantisera to specifically inhibit T-cell functions other than the in vitro proliferative response to antigen as measured by thymidine incorporation. In this report, we demonstrate that the ability to produce migration inhibition factor (MIF) in response to the DNP derivative of the copolymer of L-glutamic acid and L-lysine (DNP-GL) and to the copolymer of L-glutamic acid and L-tyrosine (GT), and to two other antigens, is controlled by histocompatibility linked Ir genes and that the production of MIF is specifically blocked by alloantisera. These results indicate that Ir gene-controlled antigen recognition is not limited to the activation of a single T-cell function.

**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 bred in our own colony.

*Antigens.*—Bovine serum albumin (BSA) and a copolymer of L-glutamic acid (50%) and L-tyrosine (50%) (GT, mol wt 14,500) were obtained from Miles Laboratories, Inc., Miles Research Div., Kankakee, Ill. A copolymer of L-glutamic acid (60%) and L-lysine (40%) (GL, mol wt 115,000) was obtained from the Pilot Chemical Div., Inc., New England Nuclear, Boston, Mass. Guinea pig albumin (GPA) was prepared from strain 13 guinea pig serum by the method of Schwert (11). DNP3-GL and DNP24-GPA were prepared as previously described (12). The subscripts refer to the average number of DNP groups per molecule.

*Immunization of Guinea Pigs.*—Solutions of each antigen were emulsified with an equal volume of complete Freund's adjuvant (CFA) containing 0.5 mg of Mycobacterium butyricum/ml (Difco Laboratories, Detroit, Mich.). Animals were immunized with either 100 µg of DNP-GL, 500 µg of GT, 1 µg of DNP-GPA, or 1 µg BSA.

*Alloantisera.*—A strain 2 antisera strain 13 serum and a strain 13 antisera strain 2 serum were prepared by reciprocal immunization with lymph node and spleen cells as previously described (13). The sera were sterilized by Millipore filtration and were then heat inactivated at 56°C for 45 min.

*Cell Collection and Purification.*—The techniques for the collection of the peritoneal exudate cells (PEC) and the methods for the purification of the peritoneal exudate lymphocytes have been described in detail (4, 14). The PEC population is composed of 70–80% macrophages, 10–20% lymphocytes, and 10–20% polymorphonuclear leukocytes. After adherence column purification, the peritoneal exudate lymphocyte population is composed of 75–85% lymphocytes and 10–20% macrophages.

*Assay for MIF.*—

**Direct:** PEC were suspended at a concentration of 60 X 10⁶/ml in RPMI-1640 (Grand Island Biological Co., Grand Island, N. Y.) containing 15% heat-inactivated normal guinea pig serum (NGPS) and 25 mM Hepes buffer. The cells were drawn up in 50-µl capillary tubes (Scientific Products Div., American Hospital Supply Corp., Evanston, Ill.), one end of which
was sealed by flaming; the tubes were then spun at 130 g for 5 min, cut at the cell-fluid inter-
phase and fixed to the bottom of 16-mm tissue culture trays (multi-well trays, Linbro Chemical Co., New Haven, Conn.) with sterile silicone grease (Dow Corning Corp., Midland, Mich.). The chambers were then filled with 1 ml RPMI-1640 supplemented with 15% NGPS and 25 mM Hepes buffer. Antigen (100 μg/ml) was then added and the chambers incubated at 37°C for 18-24 h. The areas of migration were projected in a Nikon Profile Projector (Nippon Kogaku, Tokyo, Japan), traced, and measured with a planimeter. The areas of migration of four to six capillaries (two to three chambers) were averaged and the results expressed as the migration index:

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\text{Migration index} = \frac{\text{mean area of migration with antigen}}{\text{mean area of migration without antigen}} \times 100.
\]

**Mixed:** Peritoneal exudate lymphocytes were resuspended in RPMI-1640 containing 15% NGPS and incubated for 30 min at 37°C with the appropriate antigen (100 μg/ml). The cells were then washed four times with media and added to indicator PEC which were prepared from animals immunized with CFA alone. This mixed population was placed in capillary tubes as above. The final concentration of added lymphocytes in the mixtures was 5-10%. When alloantisera (1:20 final dilution) was added to the incubation chamber, the indicator PEC were obtained from the strain which does not possess the histocompatibility antigens against which the alloantisera was directed (i.e., when 13-anti-2 serum was used strain 13 PEC were the indicator cells). The use of this procedure avoided inhibition of migration caused by interaction of alloantisera with PEC.

**Indirect:** Lymphocytes (15 \times 10^6/ml) were suspended in RPMI-1640 containing 10% NGPS and incubated with the appropriate antigen (100 μg/ml) for 30 min at 37°C. The cells were then washed four times with media and incubated at a concentration of 5 \times 10^6/ml in RPMI containing 10% NGPS with or without alloantisera (1:20 final dilution) for 18 h at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. The lymphocytes were then removed by centrifugation and the supernate supplemented with 10% fresh NGPS and added to chambers containing PEC from animals immunized with CFA alone. In experiments using alloantisera, the indicator PEC were obtained from animals that lacked the histocompatibility antigen against which the alloantisera were directed. The inhibition of migration was determined as described above.

**RESULTS**

**Genetic Control of Stimulation of MIF Production.—** As summarized in Table I, strain 2 guinea pigs mount immune responses to DNP-GL and to low doses (1 μg) of BSA while strain 13 guinea pigs are unresponsive to these antigens. On the other hand, strain 13 guinea pigs respond to GT and to low doses (1 μg) of DNP-GPA; strain 2 guinea pigs fail to respond to these antigens. (2 × 13)F_1 animals respond to all four. Backcross analysis has shown that the \(Ir\) genes controlling responses to each of these antigens are linked to the locus controlling either strain 2 or strain 13 alloantigens. The data upon which these statements are based include delayed skin reactions, the stimulation of DNA synthesis in vitro, and antibody production (15–18).

Before proceeding to an evaluation of the effect of alloantisera on the stimulation of MIF production, it was necessary to verify that the genetic control of the response to each of these antigens also extended to the control of MIF production. Initially, the capacity of DNP-GL, BSA, GT, and DNP-GPA to in-
hibit the migration of PEC from unimmunized guinea pigs was studied to provide control values for the results obtained with cells from immune animals. As shown in Table II no significant inhibition of migration of PEC obtained from nonimmune animals was noted with the exception of two experiments

TABLE I

Linkage of Histocompatibility (H) Type and Immune Responsiveness in Inbred and F1 Guinea Pigs

| Strain | H type | Ir type |
|--------|--------|---------|
| 2      | 2, 2   | DNP-GL⁺  |
|        |        | BSA (low dose)⁺ |
|        |        | GT⁻      |
| 13     | 13, 13 | DNP-GPA (low dose)⁻ |
|        |        | DNP-GL⁻  |
| (2 X 13)F1 | 2, 13   | DNP-GL⁺  |
|        |        | BSA (low dose)⁺ |
|        |        | GT⁺      |
|        |        | DNP-GPA (low dose)⁺ |

TABLE II

Migration of PEC from Guinea Pigs Not Immunized to Test Antigen

| Exp. | Strain 2 | Strain 13 |
|------|----------|-----------|
|      | BSA      | DNP-GL    | 0      | DNP-GPA | GT       |
| 1    | 100 ± 3  | 100 ± 3   | 100 ± 5| 100 ± 5|
| 2    | 100 ± 13 | 98 ± 18   | 100 ± 4| 82 ± 6* |
| 3    | 100 ± 8  | 113 ± 12  | 100 ± 5| 110 ± 4|
| 4    | 100 ± 13 | 136 ± 10  | 100 ± 8| 109 ± 14|
| 5    | 100 ± 14 | 114 ± 17  | 100 ± 11| 160 ± 10|
| 7    | 100 ± 30 | 64 ± 14*  | 100 ± 2|

PEC from nonimmunized strain 2 and strain 13 guinea pigs were incubated with no antigen, BSA (100 µg/ml), DNP-GL (100 µg), DNP-GPA (100 µg), or GT (100 µg) and the migration of these cells from a capillary tube measured. The migration of cells exposed to no antigen is accorded a mean value of 100 and the migration of antigen exposed cells is reported as the fraction of the migration of no antigen cells multiplied by 100.

* Values are significantly lower (P < 0.05 by t test) than the no antigen value.

PEC from strain 2 animals which had been immunized with DNP-GL or with 1 µg of BSA were inhibited in their migration by the immunizing antigen in four of four experiments each (Table III). On the other hand, PEC from
TABLE III

| Exp. | Immunizing antigen | Strain 2 | Strain 13 |
|------|--------------------|----------|----------|
|      | DNP-GL; GT         | 100±17 11±5* | 146±43 100±3 95±2 34±5* |
| 2    | DNP-GL; GT         | 100±21 38±8* | 208±17 100±14 68±15* 22±7* |
| 3    | DNP-GL; GT         | 100±17 17±1* | 82±3* 100±5 111±5 26±2* |
| 4    | DNP-GL; GT         | 100±17 13±2* | 77±26 100±9 155±6 59±2* |
| 0    | BSA; DNP-GPA       | 100±13 60±3* | 130±6 100±11 79±17 50±9* |
| 6    | BSA; DNP-GPA       | 100±14 36±6* | 73±13 |
| 7    | BSA                | 100±8 48±3* | 100±5 105±8 |
| 8    | BSA                | 100±4 38±4* | 100±8 73±5* |
| 9    | DNP-GPA            | 100±9 119±11 | 100±9 31±7* |
| 10   | DNP-GPA            | 100±3 146±12 | 100±3 73±4* |

PEC from strain 2 and strain 13 guinea pigs immunized with DNP-GL and GT or with 1 μg of BSA and 1 μg of DNP-GPA were incubated with the antigens used for inhibition and migration measured.

* Values are significantly lower (P < 0.05) than the no antigen control.

strain 13 animals immunized with DNP-GL were not inhibited in their migration by DNP-GL in three of four experiments and demonstrated barely significant inhibition in the fourth. Similarly, PEC from strain 13 guinea pigs immunized with 1 μg of BSA were not inhibited by BSA in two of three experiments and displayed only marginal inhibition in the third experiment. PEC from strain 13 guinea pigs immunized to DNP-GPA or GT were inhibited in their migration by the immunizing antigen in all experiments, while PEC from strain 2 guinea pigs immunized with DNP-GPA or GT were inhibited in only one of eight experiments. These results demonstrate that the genetic control previously established for responsiveness to these antigens on the basis of other tests of T-cell function also extends to the stimulation of the production of MIF.

Alloantiserum-Induced Inhibition of MIF Production.—We next turned to an evaluation of the capacity of alloantisera to inhibit the stimulation of MIF production by antigens, the response to which is under the control of specific Ir genes. In order to perform these experiments, it is necessary that the migrating cell populations not bear antigens against which the alloantisera are directed because preliminary experiments demonstrated that the alloantisera inhibit the migration of cells with which they interact. Consequently, we used either the "indirect" procedure in which supernates of lymphocyte cultures were added to PEC of appropriate genotype or the "mixed" procedure in which immune lymphocytes were mixed with antigen and then added to nonimmune PEC of the appropriate genotype. In both procedures, the peritoneal exudate lympho-
cytes were incubated with antigen for 30–60 min at 37°C, washed, and then cultured in the presence or absence of alloantisera.

As shown in Table IV, in five of six experiments using immune strain 2 peritoneal exudate lymphocytes DNP-GL caused inhibition of migration in the presence of NGPS, but failed to do so in the presence of anti-2 serum. In one experiment (exp. 2), inhibition of migration was observed in the presence of anti-2 serum, but the degree of inhibition was much less than was observed in the presence of normal serum. Anti-13 serum did not interfere with the ability of DNP-GL to stimulate MIF production by strain 2 lymphocytes (exp. 7 and 8). Similarly, anti-13 serum blocked the capacity of GT to stimulate MIF production by strain 13 lymphocytes in three of three experiments while anti-2 serum had no effect (Table V). These experiments establish that alloantisera with primary specificity for histocompatibility antigens present on lymphocytes prevent those cells from producing MIF upon exposure to antigens to which they are normally responsive.

In order to demonstrate that the inhibition of MIF production involves specific blockade of an Ir gene-controlled response rather than a generalized inhibition of T-lymphocyte function, we repeated these experiments using cells from (2 × 13)F1 guinea pigs immunized with and capable of responding to DNP-GL and a low dose of DNP-GPA. Responses to both GT and low doses of DNP-GPA are controlled by Ir genes linked to strain 13 histocompatibility genes, but the magnitude of the in vitro response to DNP-GPA is generally greater than that to GT; we therefore used DNP-GPA for these F1 experiments.

| Exp. Method | Serum | NGPS | DNP-GL | DNP-GL | DNP-GL |
|-------------|-------|------|--------|--------|--------|
|             |       | 0    | 0      | 0      |        |
| 1           | ID    | 100 ± 3 | 76 ± 2 | 100 ± 2 | 120 ± 4 |
| 2           | ID    | 100 ± 2 | 61 ± 4* | 100 ± 6 | 82 ± 3* |
| 3           | M     | 100 ± 5 | 65 ± 3* | 100 ± 7 | 114 ± 8 |
| 4           | M     | 100 ± 11 | 30 ± 6* | 100 ± 7 | 114 ± 10 |
| 5           | M     | 100 ± 15 | 8 ± 12* | 100 ± 21 | 91 ± 2 |
| 6           | ID    | 100 ± 5 | 11 ± 8* | 100 ± 9 | 100 ± 9 |
| 7           | ID    | 100 ± 2 | 51 ± 4* |        | 100 ± 3 | 51 ± 4* |
| 8           | D     | 100 ± 6 | 33 ± 1* |        | 100 ± 0 | 36 ± 1* |

* Values are significantly lower (P < 0.05) than no antigen control.

The effect of alloantisera on MIF production was determined by the direct (D), mixed (M), or indirect (ID) MIF assay. Cells from strain 2 guinea pigs immunized with DNP-GL were exposed to no antigen or DNP-GL and cultured with heat-inactivated NGPS, anti-2 serum, or anti-13 serum.

TABLE IV

**Inhibition of Antigen-Stimulated MIF Production by Alloantisera**

| Exp. Method | Serum | NGPS | DNP-GL | DNP-GL | DNP-GL |
|-------------|-------|------|--------|--------|--------|
|             |       | 0    | 0      | 0      |        |
| 1           | ID    | 100 ± 3 | 76 ± 2 | 100 ± 2 | 120 ± 4 |
| 2           | ID    | 100 ± 2 | 61 ± 4* | 100 ± 6 | 82 ± 3* |
| 3           | M     | 100 ± 5 | 65 ± 3* | 100 ± 7 | 114 ± 8 |
| 4           | M     | 100 ± 11 | 30 ± 6* | 100 ± 7 | 114 ± 10 |
| 5           | M     | 100 ± 15 | 8 ± 12* | 100 ± 21 | 91 ± 2 |
| 6           | ID    | 100 ± 5 | 11 ± 8* | 100 ± 9 | 100 ± 9 |
| 7           | ID    | 100 ± 2 | 51 ± 4* |        | 100 ± 3 | 51 ± 4* |
| 8           | D     | 100 ± 6 | 33 ± 1* |        | 100 ± 0 | 36 ± 1* |
F₁ lymphocytes were incubated with either media alone, DNP-GL, or DNP-GPA for 30 min at 37°C, washed, and then cultured for 18-24 h in the presence of NGPS, anti-2 serum, or anti-13 serum. The supernates of these cultures were then tested for MIF activity with nonimmune strain 13 PEC when anti-2 serum was used, while strain 2 PEC were used to test supernates from cultures incubated with anti-13 serum. Anti-2 serum blocked the capacity of DNP-GL to stimulate MIF production by F₁ cells but had little effect on the stimulation of MIF production of these cells by DNP-GPA (Table VI). On the other hand, anti-13 serum blocked the production of MIF by F₁ cells in response to DNP-GPA but did not effect the response to DNP-GL. These results demonstrate that the capacity of alloantisera to block MIF production is related to a specific interference with Ir-gene-product function rather than to generalized inactivation of the cell.

### DISCUSSION

It has been previously shown that anti-2 and anti-13 alloantisera block the activation of in vitro T lymphocyte DNA synthesis by antigens the response to which is controlled by major histocompatibility complex-linked Ir genes. Several lines of evidence suggest that histocompatibility linked Ir genes of guinea pigs principally function in T lymphocytes. However, T lymphocytes are known to mediate a wide variety of immunologic functions and evidence suggests that some of these functions are performed by distinct subclasses of T lymphocytes. In this report, we have presented data demonstrating that a second major T-lymphocyte function, the production of MIF, can also be specifically blocked by alloantisera.

Initially, it was necessary to establish that the in vitro production of MIF...
Table VI

The Effect of Alloantisera on MIF Production by (2 X 13)F1 Lymphocytes

| Strain of indicator macrophages | Antigen | Exp. 1 NGPS | Exp. 1 Anti-2 | Exp. 2 NGPS | Exp. 2 Anti-2 |
|---------------------------------|---------|-------------|--------------|-------------|--------------|
| 13                              | --      | 100.0±5     | 100.0± 5     | 100.0±8.6   | 100.0±10.5   |
| 13 DNP-GL                       | 39.6±2.7* | 91.4± 4.7   | 38.7±3.2*    | 108.3±13    |
| 13 DNP-GPA                      | 51.4±3*  | 51.4± 3.6*  | 33.9±3*      | 65.0± 5.6*  |

* P < 0.001; † P < 0.025

Effect of alloantisera on MIF production by cells from (2 X 13)F1 guinea pigs which had been immunized with DNP-GL and 1 μg of DNP-GPA. MIF from cultures exposed to anti-2 serum was assayed on PEC from nonimmune strain 13 animals; MIF from cultures exposed to anti-13 serum was assayed on PEC from nonimmune strain 2 animals.

* Values are significantly lower (P < 0.05) than no antigen controls.

Paired t-tests were used to calculate the difference in migration in the presence of alloantisera vs. the differences in the presence of NGPS using a four sample t test.
responsiveness is the same as that seen when antibody production, delayed skin hypersensitivity, or in vitro stimulation of DNA synthesis are studied. Thus, animals that lack a given \textit{Ir} gene fail to produce MIF upon exposure to antigens, the response to which is controlled by that \textit{Ir} gene. As MIF production is a major in vitro correlate of delayed hypersensitivity, this result further supports the concept that specific T-cell activation does not occur in nonresponder guinea pigs.

In order to determine whether alloantisera specifically inhibited the production of MIF, it was necessary to use a system in which direct inhibition of migration by alloantisera could be avoided. This is necessary because in the presence of anti-2 serum, PEC from either strain 2 or \((2 \times 13)F_1\) guinea pigs fail to migrate out of capillary tubes. Similarly, anti-13 serum prevents migration on the part of either 13 or \((2 \times 13)F_1\) PEC. The mechanism of this inhibition is uncertain although a microagglutination reaction is a possibility in view of the fact that the 2 and 13 antigens have recently been shown to be on macrophage surfaces.\(^2\) Our approach to testing the effect of alloantisera on production of MIF involved an assay of MIF utilizing a population of cells which lacked the antigen against which the alloantisera were directed. Thus, when the effect of anti-2 serum on responsiveness of 2, 13, or \(F_1\) cells was tested, the MIF preparation was evaluated with strain 13 PEC. Similarly, when anti-13 serum was used, strain 2 PEC comprised the migrating population. By using this procedure, we could clearly demonstrate that anti-2 serum prevented MIF production by \((2 \times 13)F_1\) cells in response to DNP-GL but not DNP-GPA, while an anti-13 serum inhibited MIF production in response to DNP-GPA but not DNP-GL. The specificity of this inhibition conforms to the genetic control of responsiveness. Responsiveness to DNP-GL depends upon a 2-linked \textit{Ir} gene while responsiveness to DNP-GPA depends mainly upon a 13-linked \textit{Ir} gene. Thus, both MIF production and DNA synthesis in response to antigen stimulation in \textit{Ir} gene-controlled systems are inhibited by the alloantiserum which is directed at products of histocompatibility genes linked to the controlling \textit{Ir} gene.

Although both antigen-induced DNA synthesis and the production of MIF appear to be largely mediated by T lymphocytes, there is data to suggest that, in humans, the populations of cells which produce MIF may be different from those which are responsible for DNA synthesis in response to antigen. Thus, when lymphocytes from humans sensitive to streptokinase-streptodornase (SK-SD) are stimulated with SK-SD and then exposed to bromodeoxyuridine and light, the capacity of these cells to proliferate in response to a further exposure to SK-SD is lost (21). However, in such cultures, SK-SD restimulation still causes production of normal amounts of MIF, suggesting that the MIF-producing cell is distinct from the majority of the proliferating cells. Dissociations between lymphocyte proliferation and the production of MIF have been

\[^2\] Shevach, E. M., F. Finkelman, and I. Green. Unpublished observations.
observed in guinea pigs immunized with tobacco mosaic virus protein (22), the encephalitogenic protein derived from bovine spinal cord myelin (23), and a carbohydrate preparation from culture filtrates of Mycobacterium (24). Animals immunized with these antigens develop a positive delayed skin reaction and their lymphocytes produce MIF in response to antigen in vitro, but lymphocyte proliferation is not observed. On the other hand, a number of reports have shown that lymphocytes from anergic patients with sarcoidosis (25) and chronic mucocutaneous candidiasis (26) fail to produce MIF, but do proliferate normally in response to antigen. Although none of these studies offer definitive proof for the existence of two functionally distinct classes of T cells, they strongly suggest that such classes exist. Our results demonstrating that alloantisera inhibit both antigen-stimulated DNA synthesis and MIF production in genetically controlled systems indicate that the functions potentially ascribable to these two classes of T cells can be specifically blocked by alloantisera. Furthermore, recent studies on the inhibition of responsiveness in the mixed leukocyte culture with the sera from patients with systemic lupus erythematosus (27) and with antisera to $\beta_2$-microglobulin (28) suggest that other T-lymphocyte activities may be inhibited by antisera which recognize nonimmunoglobulin T-lymphocyte membrane components.

The exact mechanism by which alloantisera inhibit T-lymphocyte proliferation or the production of MIF is uncertain. We have recently presented genetic evidence indicating that the target antigen for the inhibitory activity of the anti-2 serum on lymphocyte proliferation is the strain 2 histocompatibility antigen rather than the product of a specific Ir gene (5). In these experiments, we examined the capacity of anti-2 serum to inhibit the function of the product of an Ir gene (the gene controlling responsiveness to a copolymer of L-glutamic-acid and L-alanine, GA) which is normally linked to strain 2 histocompatibility genes, when this gene occurs in an outbred animal lacking strain 2 histocompatibility genes. In the majority of cases, the anti-2 serum was incapable of inhibiting the in vitro proliferative response to GA of T cells derived from such 2-GA$^+$ animals although the sera were quite inhibitory for the GA response of T cells from 2$^+$GA$^+$ outbred animals. Similar results have been obtained by Bluestein (6). These experiments suggest that the inhibition of the GA response by the anti-2 serum is mediated by antibodies to strain 2 histocompatibility antigens rather than by antibodies specific for the product of the GA Ir gene. The mechanism of alloantiserum-induced suppression of Ir-gene function would then most likely be steric interference with the function of the Ir-gene product on the cell surface, rather than by direct binding to it. An interesting possibility suggested by these experimental results is that the products of the 2/13 genes and the products of the Ir genes are phenotypically linked in a single polypeptide chain on the cells surface. In this model the 2/13 antigens would represent a "constant region" while the Ir-gene product would represent a "variable region" of a T-lymphocyte antigen recognition molecule.
Although the controversial issue of the immunoglobulin receptor on T cells has not been resolved, it should be noted that the bulk of the functional data supporting a role for T-cell immunoglobulin of conventional type stems from reports of inhibition of T-cell helper function by anti-immunoglobulin antibodies (29). In general, other T-lymphocyte functions, such as specific in vitro cytolysis have not been inhibitable by anti-immunoglobulin (30). It is therefore conceivable that a major dichotomy in T-lymphocyte antigen recognition mechanisms exists with certain T-cell subpopulations utilizing an \(\text{Ir}\)-gene product mechanism and other populations, such as helper cells, using an immunoglobulin-like receptor. We regard such a proposal as unlikely because of the well established function of \(\text{Ir}\)-gene products in helper cell populations. We would suggest that a single type of antigen recognition system exists for all T cells. It would therefore be of interest to test the capacity of alloantisera to block additional T-lymphocyte functions and to attempt to isolate and characterize the membrane alloantigen for which the alloantisera are specific.

**SUMMARY**

We have previously demonstrated that alloantisera prepared by reciprocal immunization of strain 2 and strain 13 guinea pigs specifically block stimulation of in vitro DNA synthesis in genetically controlled systems. In order to determine whether this blockade extends to other T-lymphocyte functions, we examined the effect of alloantisera on the production of migration inhibition factor (MIF). (2×13)\(F_1\) guinea pigs were immunized with a DNP derivative of the copolymer of L-glutamic acid and L-lysine (DNP-GL) and with DNP guinea pig albumin (GPA). The response to the former is controlled by a 2-linked \(\text{Ir}\) gene while that to the latter is mainly controlled by a 13-linked \(\text{Ir}\) gene. MIF production was assayed by an indirect procedure in which the migrating cell population lacked the histocompatibility antigen against which the alloantisera was directed. Our results showed that anti-2 serum blocked MIF production by \(F_1\) cells in response to DNP-GL but not DNP-GPA while anti-13 serum had the opposite effect. These experiments show that expression of a second major T-cell function is specifically blocked by alloantisera and suggest that \(\text{Ir}\)-gene products may act as antigen recognition substances on more than one type of T cell.

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