MAPPING OF THE IMMUNE RESPONSE GENES IN THE
MAJOR HISTOCOMPATIBILITY COMPLEX OF THE
RHESUS MONKEY*

BY MARTIN E. DORF, HANS BALNERJ AND BARUJ BENACERRAF

(From the Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115 and the
Primate Center, TNO, Rijswijk, Z. H., The Netherlands)

In several mammalian species, a chromosomal region has been identified containing
a number of closely linked genetic systems which have a major influence
on histocompatibility and immunological responsiveness. In mice, the major
histocompatibility complex (MHC) has been shown to include at least two
systems coding for the classical serologically defined (SD) transplantation antigens (SD loci), one or more systems determining lymphocyte-activating determinants (Lad) which govern reactivity in mixed lymphocyte cultures, a distinct
segment (the S region) which controls the quantitative level of the Ss serum
protein and the presence of a sex-limited antigen (Slp), and finally, systems
which control the capacity to recognize and respond to a large number of
thymus-dependent antigens (Ir loci).*

In the mouse, all the histocompatibility linked Ir genes investigated map
within the H-2 complex between the K and S regions (1–3). The Ir genes are
clearly separable from the K and Ss-Slp genes which code for distinct molecules
themselves (4–6). Moreover, in mice there is evidence that genes controlling the
response to different antigens are distinct from each other and map separately

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† Member of the Biology Division of Euratom.

Abbreviations used in this paper: ABC-33, antigen-binding capacity; GA, copolymer of L-
glutamic acid with L-alanine; GAT, copolymer of L-glutamic acid-O'-L-alanine-3°-L-tyrosine; GBG,
glycine-rich betaglycoprotein; GL, copolymer of L-glutamic acid with L-lysine; GT, copolymer of L-
glutamic acid with L-tyrosine; Lad, lymphocyte-activating determinants; MHC, major histocom-
patibility complexes; MLR, mixed lymphocyte reactivity; PBS, phosphate-buffered saline; SD,
serologically defined.

2 The genetic nomenclature referring to the regions of the rhesus monkey’s MHC complex
remains tentative. For consistency with previous published reports, we have tentatively design-
nated the chromosomal region containing the genes coding for the two segregant series of
serological determinants of RhL-A collectively as SD. The gene(s) or genetic region coding for
mixed lymphocyte reactivity are termed Lad (for lymphocyte-activating determinants), and the
immune response genes controlling responsiveness to the GA and DNP-GL copolymers as Ir-GA
and Ir-GL, respectively. The locus coding for glycine-rich betaglycoprotein (GBG) of the alternate
complement pathway (properdin factor B) is termed Bf. This operational system of nomenclature
may require future revision once international conventions describing the nomenclatures for
MHC of all species are formulated.
The region in the murine H-2 complex where the Ir genes map has recently been termed the I region (8). Additional studies in the mouse on the activities controlled by the I region have shown that genes in this region also control mixed lymphocyte and graft-vs.-host reactivity (9-11), T- and B-cell cooperative interactions (12, 13), and a series of alloantigens (collectively termed Ia for I-region-associated antigens) of restricted tissue distribution (1, 14, 15).

Genetic analysis of the MHC of other species may provide additional insights into the interrelationships of the multiple-linked genetic systems of the MHC. Rhesus monkeys, a species phylogenetically close to man, are the only outbred species for which extensive data regarding SD, Lad, and Ir genes are available (16-20). Initial experiments to demonstrate genetic control of immune responses in rhesus monkeys were started by one of us (Dr. H. Balner) in collaboration with Doctors M. Sela, E. Mozes, H. McDevitt, and J. van Rood. They studied the humoral response to the synthetic polypeptide poly-L-(tyrosine, glutamic acid)-poly-DL-alanine--poly-L-lysine, one of the antigens initially used to identify the Ir-1 locus of mice (4). After it was shown that unrelated animals could be either high, intermediate, or low responders, members of several rhesus families were immunized. Those preliminary studies (19, 20) suggested that the capacity to respond seemed to correlate with the inheritance of a single parental RhL-A haplotype. These initial experiments were continued using the random linear copolymer of L-glutamic acid and L-alanine or L-lysine which had been employed primarily to demonstrate histocompatibility linked Ir genes in guinea pigs (21) to identify and map Ir genes in the rhesus monkey. In a previous report (22), we presented preliminary data indicating that the immune response genes to these linear copolymers are also linked to the the RhL-A system. The present paper details the genetic mapping of the immune response genes within the RhL-A complex.

Materials and Methods

Animals. The related and unrelated animals used in these experiments are part of the animal colony maintained at The Primate Center, TNO, Rijswijk, The Netherlands. Monkeys were typed for the serologically defined RhL-A determinants. The related monkeys used in the current studies were recently serotyped and genotyped for SD antigens as well as for reactivity in mixed lymphocyte cultures. This analysis has been presented elsewhere (18, 23).

Immunization. The copolymers of L-glutamic acid with L-alanine (G"A"e) or L-lysine (G"sL"e) were synthesized to order by Pilot Chemicals, Inc., Watertown, Mass. Superscripts refer to the molar amino acid ratios. Preparations of DNP-GL were prepared as previously described (24). The subscript refers to the average number of moles of dinitrophenyl groups per mole of GL.

Preliminary studies had shown that distinct humoral responses to these antigens required the administration of complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.). Our standard immunization procedure consisted of deep intramuscular injections of 100 μg of GA and 50 μg DNP-GL in complete Freund's adjuvant given in separate sites on day 0, followed by intradermal skin tests with both substances on day 21 (in doses of 10 and 50 μg/antigen given in 0.1 ml/injection). Serum samples were usually collected on days 0, 21, and 28. Sera were stored at −20°C until assayed. In some cases, another intradermal boost was given after day 28 and serum taken a week later to confirm the initial findings.

Pilot studies suggested that the copolymer of L-glutamic acid and L-tyrosine (G"T"e) could also be used to identify Ir genes in monkeys. However, additional experiments indicated that most monkeys were weak responders to GT, and the differences between individuals were not sufficiently large for meaningful comparisons.
Antigen-Binding Assay. The humoral response to GA was measured by an antigen-binding assay employing the cross-reactive random linear terpolymer of L-glutamic acid⁴⁻L-alanine⁴⁻L-tyrosine⁶⁻ (GAT) purchased from Pilot Chemicals, Inc. (25). GAT was iodinated by the chloramine-T method (26) with carrier-free ¹²⁵I (New England Nuclear, Boston, Mass.) and separated from inorganic iodide by passage over 0.5 x 25 cm columns of Sephadex G-25F (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.). The radioiodinated ligand was diluted with phosphate-buffered saline (PBS) containing 1% normal rhesus monkey serum to a concentration of about 2 x 10⁻⁸ M for use and had sp act between 0.2 and 4 Ci/g. Serum samples were diluted 1:5 with PBS for assay. In order to maintain a constant serum concentration, further serial fivefold dilutions were made in PBS containing 20% normal rhesus serum. To 25 μl of diluted serum in each well of Linbro V plates were added 20 μl of radiolabeled ligand solution; the plates were mixed and incubated for at least 60 min at 4°C. Precipitation of the GAT antibody complexes was achieved with 50 μl of either rabbit anti rhesus globulin or with 80% saturated ammonium sulfate (pH adjusted to 7.4 with ammonia). In a similar fashion [³H]DNP-e-amino-N-caproic acid was used to measure DNP-GL responses as described in detail elsewhere (27). The DNP Farr assays employed 95% saturated ammonium sulfate. 1-2 h after the addition of the precipitating agent, the plates were centrifuged at 800 g for 20 min at 4°C and 50 μl of supernate from each well were counted in a Packard gamma counter (Packard Instrument Co., Inc., Downers Grove, Ill.) or a Beckman scintillation spectrometer (Beckman Instruments, Inc., Fullerton, Calif.), respectively. The dilution at which 33% of the radiolabeled ligand was bound was determined by interpolation and the antigen-binding capacity (ABC-33) per milliliter undiluted serum calculated.

Testing of Cellular Reactivity. 3 wk after the primary immunization with GA and DNP-GL in complete Freund's adjuvant, all monkeys were skin tested with 10 and 50 μg of soluble antigen. Owing to the thickness and dark coloration of the rhesus skin, induration was noted only after careful inspections at both 24 and 48 h. Most of the skin test reactions were rather weak and difficult to read.

Secondary lymphocyte responses to GA and DNP-GL in vitro were also attempted, usually a week after skin testing. However, these assays were abandoned when it became clear that the technique (28) used did not provide sufficiently reproducible results.

Results

Ir Studies in Unrelated Monkeys. We have previously reported on our initial experiments which were undertaken to identify appropriate antigens for use in analyzing the immune response genes in the rhesus monkey (22). Consequently, we selected the two antigens GA and DNP-GL, described in the Materials and Methods, for further study. The primary immune response to GA and the secondary immune response to DNP-GL were previously shown to be under dominant histocompatibility linked Ir-gene control in the rhesus monkey (22, 29). To determine whether there was linkage disequilibrium between the Ir genes and any of the SD alleles, we immunized 74 unrelated monkeys and the parents of selected families with the synthetic polypeptides, GA and/or DNP-GL. Fig. 1 summarized the distribution of immune responses to these antigens.

After primary immunization with DNP-GL, only very low levels of DNP-specific antibody were detected in any of the unrelated monkeys (data not shown). Therefore, secondary anti-DNP antibody levels measured 7 days after skin testing with 60 μg of DNP-GL were used to analyze immune response patterns. Approximately 10% (7/74) of the monkeys made no detectable anti-DNP humoral response; these monkeys were classified as GL nonresponders. Secondary levels of anti-DNP antibody were detected in the remaining 90% (67/74) of the immunized monkeys. Seven of these animals showed low serum levels of DNP-specific antibody (between 0.1 and 10 pmol of DNP binding/ml serum). We have
FIG. 1. Distribution of immune response to DNP-GL and GA in unrelated rhesus monkeys. Left panel: Distribution of secondary DNP-GL responses among 74 unrelated monkeys immunized with 50 μg DNP-GL in complete Freund's adjuvant and boosted intradermally with 60 μg of antigen. Sera collected 7 days after secondary immunization were tested from antigen-binding activity using the DNP-ε-amino-N-caproic acid (EACA) ligand. Results are expressed in picomoles of DNP-EACA bound per milliliter of undiluted serum, based on the serum dilution binding 33% of the antigen. Right panel: Distribution of primary GA responses among 65 unrelated monkeys immunized with 50 μg GA in complete Freund's adjuvant. Sera collected 21 days after immunization were tested for GA-binding activity using the GAT ligand. Results are expressed in picomoles of GAT bound per milliliter of undiluted serum, based on the serum dilution binding 33% of the antigen.

considered the latter group of animals as low responders, while the remaining monkeys were classified as high responders (80% of total).

The distribution of GA responses in our laboratory population of rhesus monkeys is illustrated in Fig. 1. 14 of 65 unrelated monkeys failed to make detectable levels of anti-GA antibody after primary immunization with the GA polypeptide. Again, a few animals (7/65) could be considered low responders (with ABC-33 values between 0.1 and 10 pmol/ml) while approximately 70% of the population were classified as high responders (with ABC-33 values greater than 10 pmol/ml).

The 74 unrelated monkeys in this study have been serotyped for 21 RhL-A SD specificities. Conventional 2 × 2 and chi-square analyses (30) were used to compare the immune response pattern to either antigen with the serologically defined histocompatibility antigens. There were no statistically significant (P <
0.05) associations of immune responsiveness with any of the 21 RhL-A specificities.

Additional searches for evidence of linkage disequilibrium between the Ir genes with other marker genes coded for in the RhL-A complex utilized two highly selected populations of unrelated monkeys. These monkeys were preselected on the basis of: (a) sharing genes of the major Lad locus (as evidenced by less than threefold activation in unilateral mixed lymphocyte reactions) or (b) sharing four known SD specificities. The resulting group of 15 monkeys were selected from many different lots of animals shipped at various times over a period of several years. Thus, it seems rather unlikely that any of the monkeys are closely related. These monkeys were tested for their ability to respond to GA and/or DNP-GL. Pairs of RhL-A-matched animals were also compared for skin graft survival times of 1 cm full thickness skin grafts exchanged between them. Low mixed lymphocyte reactivity (MLR) (less than threefold stimulation compared to controls) was observed in 8 of 15 pairwise combinations of RhL-A-matched animals. Table I depicts the eight combinations (sharing one, two, or three RhL-A [SD] antigens; homozygosity for some combinations cannot be excluded), where a negative or low MLR response was observed. In two pairs, the MLR was negative in both directions (pairs 2337/2464 and 2463/2394); in the four remaining examples, low or no mixed lymphocyte responses were noted in one direction only. It is important to note that in six of the eight combinations shown in Table I, there is identity among the known SD antigens of the first segregant series. In contrast, only three of eight pairs shared all known determinants of the second segregant series (although only one specificity could be detected in each case). Thus, it appears that there is an association presumably due to linkage disequilibrium between the Lad and SD loci. However, sharing of Lad determinants did not necessarily lead to similar responses to GA or DNP-GL.

Table II shows groups of two and three animals which were "full-house identical" for four known RhL-A specificities, SD antigens, but showed distinctly positive MLR reactions in the eight possible combinations. Identity or compatibility for major Lad genes was therefore unlikely. Also, the immune response pattern to GA and DNP-GL of the SD compatible monkeys were not identical. There were no statistically significant (P < 0.05) correlations between the immune response patterns to either or both synthetic polymers with RhL-A compatibility.

Among this highly selected group of phenotypically RhL-A-matched monkeys, 6 of 15 pairs demonstrated prolonged skin graft survival (≥11.5 days). As reported elsewhere (31), there were statistically significant correlations between skin graft survival times and identity for the serologically defined RhL-A specificities, however, there were no correlations of prolonged graft survival and immune responsiveness to GA and/or DNP-GL.

**Correlation of Cellular and Humoral Immunity.** 3 wk after the primary immunization with GA and DNP-GL in complete Freund's adjuvant, all but one monkey illustrated in Fig. 1 were skin tested with 10 and 50 μg of soluble antigen. Induration was noted only after careful inspections at both 24 and 48 h. Although most of the skin test reactions were rather weak and difficult to read,
**Table I**

Comparisons of Immune Responsiveness with RhL-A (SD) Antigens and Skin Graft Survival in Ld Matched Unrelated Monkeys

| Responder (R) | Donor (D) | RhL-A phenotype | Immune Response* | Unilateral MLR† | Skin graft survival‡ |
|--------------|-----------|-----------------|------------------|-----------------|----------------------|
|              |           | SD₁ | SD₂ | GA | DNP-GL | R→Dₘ | days |
| 960          | 2453      | 9   | 6   | 11  | 25  | −   | −   | −   | 11.5 |
| 2337         | 2464      | 6   | 10  | 13  | −   | +   | +   | −   | 11.5 |
| 2464         | 2337      | 6   | 23  | 13  | −   | +   | −   | −   | 9.5 |
| 2463         | 2394      | 6   | 10  | 13  | 26  | −   | +   | −   | 9.5 |
| 2394         | 2463      | 6   | 10  | 13  | −   | −   | +   | −   | 8.5 |
| 2368         | 2412      | 6   | 10  | 2   | −   | +   | +   | −   | 10 |
| 2345         | 2407      | 10  | −   | 13  | 17  | NT§ | +   | −   | 10 |
| 2414         | 2407      | 10  | −   | 11  | 26  | +   | +   | −   | 10 |

* Immune responses to GA or DNP-GL are indicated as positive (+) when ABA-33 values were >10 pmol/ml.
† Unilateral mixed lymphocyte responses using mitomycin C-treated donor cells. Positive responses (+) indicate stimulation ratios >3. All monkeys in the control group demonstrated greater than three-fold stimulation ratios.
‡ Mean survival time of first set grafts. Control grafts survived 9.4 days with a standard deviation of 1.2 days.
§ NT, not tested.

There was a correlation of skin test and humoral immunity (Table III). Nearly all animals which displayed skin test reactivity also produced high levels of specific antibody (>40 pmol/ml). However, many monkeys made good humoral responses without evidence of delayed reactivity in the skin test assay.

Testing the in vitro cellular responses to these synthetic copolymers has not been particularly successful. Stimulation of in vitro lymphocyte proliferative responses with these antigens was erratic. However, recent studies suggest that technical modifications may make this technique a valuable tool in the study of the genetic control of immune responsiveness.
Table II
Comparisons of Immune Responsiveness with MLR and Skin Graft Survival in Phenotypically RhL-A Matched Unrelated Monkeys

| Responder (R) | Donor (D) | RhL-A phenotype | Immune response | Unilateral MLR$^R \rightarrow D_m$ survival$^\S$ |
|--------------|-----------|-----------------|----------------|-----------------------------------------------|
|              |           | SD$_1$ | SD$_2$ | GA | DNP-GL | days | |
| 1062         | 2398      | 9     | 6     | 11 | 2     | +    | -   | +   | 30-35 |
| 2453         | 1062      | 9     | 6     | 11 | 2     | +    | +   | -   | 11   |
| 2398         | 2453      | 9     | 6     | 11 | 2     | +    | +   | +   | 11   |
| 2463         | 2496      | 6     | 10    | 13 | 26   | -    | +   | -   | 10.5 |
| 2496         | 2463      | 6     | 10    | 13 | 26   | NT$^K$ | +  | -   | 16   |

Unmatched controls

2-4 Antigen mismatches

70% + 80% + + 9.4 ± 1.2

For footnotes, see Table I.

Table III
Correlation of Cellular and Humoral Immunity

| GA          | DNP-GL       |
|-------------|--------------|
| Antibody    | Antibody     |
| (ABC-33)    | (ABC-33)     |
| Skin test   | Skin test    |
| +           | +            |

>40          | >40          |
14           | 17           |
24           | 35           |
0            | 0            |
<40          | <40          |
3            | 0            |
22           | 21           |

$^\chi^2 = 4.72$  $^\chi^2 = 8.94$

$^P = 0.03$     $^P = 0.003$

* 2 × 2 contingency tables demonstrate the correlation of antibody level, expressed in pmol/ml, with skin test reactivity. Chi-square and probability values are indicated for each antigen.

Ir Studies in Related Monkeys. Members of several rhesus families were also immunized with the antigens DNP-GL and GA. Tables IV–VII summarize segregation of the humoral responses to these antigens among the 57 progeny.
from 21 female and 3 male parents. It is important to note that these data represent only the informative genetic information in which segregation for immune responsiveness to either antigen can be observed. In approximately 50% of the families studied (including the offspring of a fourth male), all offspring were high responders to these antigens. These findings are consistent with the high frequency of responders found in unrelated monkeys and do not contradict any of our genetic hypotheses. With a few exceptions, to be discussed below, the capacity to respond to DNP-GL and GA was always inherited with a particular parental haplotype. Linkage of the immune response patterns to DNP-GL and GA with the RhL-A histocompatibility complex was confirmed (P < 0.005) by the statistical method of Buckley et al. (32).

**Responses to DNP-GL.** Table IV shows the humoral response to DNP-GL in the informative families of father 381. 11 offspring (siblings and half siblings AK, GM, V, JJ, AV, BU, CZ, BJ, BL, CU, and GS) were either non or low responders to antigen DNP-GL, each of these monkeys carried the paternal b,RhL-A haplotype (Table IV). In addition, all 10 monkeys carrying the paternal A,RhL-A haplotype demonstrated high levels of DNP-specific antibody. It is important to note the reactions of monkeys GO and BJ (families 584 and 594, respectively), which seem to contradict the hypothesis of close linkage of the immune response system with the MHC. However, mixed lymphocyte culture data have demonstrated that both monkeys GO and V, as well as FU and BJ, who are genotypically identical for the RhL-A serological determinants, were mutually stimulatory in bilateral and unilateral mixed leukocyte cultures. Thus, recombination within the RhL-A complex between the SD and Lad loci were assumed (18, 23). Incidentally, all "aberrant" results from intersibling mixed lymphocyte reactions have been confirmed in three or more tests on different days. Therefore, the simplest assumption from these data is that the immune response gene(s) to DNP-GL segregate with the particular Lad locus controlling mixed lymphocyte activation. However, a third example of recombination between serologically defined RhL-A antigens and the major Lad was noted in monkey CZ, who consistently demonstrated mutually positive mixed lymphocyte reactions with each of three serologically identical siblings (JJ, AV, and BU). Although monkey CZ very likely also represents an example of recombination between the Lad and SD regions of RhL-A, its immune response gene(s) controlling responsiveness to the DNP-GL conjugate did not segregate with the genes controlling the Lad.

Table V depicts data for families sired by father 598. The results also show segregation for DNP-GL responsiveness among the offspring of several females. All seven DNP-GL low- and nonresponder offspring inherited the paternal b2,RhL-A haplotype, while the three high responder offspring all inherited the paternal A2,RhL-A chromosome. The inheritance of maternal responses was also in agreement with linkage to the RhL-A complex.

**Responses to GA.** It is important to note that the families immunized with DNP-GL were also immunized with GA. This enabled us to study the inheritance of the Ir-GL and Ir-GA genes in the same rhesus families. This approach permitted identification of animals in which a recombination event occurred between distinct Ir genes.
| Parents and offspring * | RhL-A genotype † | Antibody response (ABC-33) § |
|------------------------|------------------|----------------------------|
| δ 381 (1)              | A₁ b₁            | 159                        |
| ♀ 432 (1)              | C₁ d₁            | 266                        |
| N                      | A₁ C₁            | 125                        |
| CP                     | A₁ d₁            | 178                        |
| FN                     | A₁ C₁            | 1,126                      |
| AK                     | b₁ d₁            | 53                         |
| GM                     | b₁ d₁            | 5                          |
| ♀ 584 (3)              | c₃ d₃            | 17                         |
| V                      | b₁ d₁            | <0.1                       |
| JJ                     | b₁ c₃            | 36                         |
| AV                     | b₁ c₃            | 16                         |
| BU                     | b₁ c₃            | 3                          |
| CZ                     | b₁ c₃ (a₃c₅)     | 9                          |
| GO                     | b₁ d₃ (a₃d₃)     | 131                        |
| ♀ 594 (4)              | c₄ D₄            | 77                         |
| YY                     | A₁ D₄            | 7,188                      |
| BJ                     | b₁ D₄(b₁c₄)      | 5                          |
| FU                     | b₁ D₄            | 193                        |
| ♀ 852 (5)              | c₅ d₅            | 28                         |
| AH                     | A₁ c₅            | 91                         |
| DN                     | A₁ c₅            | 101                        |
| EV                     | A₁ d₅            | 214                        |
| BL                     | b₁ d₅            | 8                          |
| ♀ 324 (6)              | c₆ D₆            | 3,182                      |
| CU                     | b₁ c₆            | <0.1                       |
| EM                     | A₁ c₆            | 886                        |
| ♀ 1646 (8)             | c₆ D₆            | 3,678                      |
| FM                     | A₁ c₆            | 519                         |
| GS                     | b₁ c₆            | <0.1                       |
| ♀ 1472 (10)            | c₁₀ d₁₀          | <0.1                       |
| EQ                     | A₁ c₁₀           | 774                         |

* Parents are indicated with numerical symbols; offspring with letters. Parents have a "rank order" (in parentheses) which corresponds to subscripts used in parental haplotypes shown in the second column.
† Paternal RhL-A haplotypes are indicated with a or b (a single father for all offspring); maternal haplotypes with c and d (different for each family). Capital letters indicate haplotypes controlling responsiveness to appropriate antigen. Genotype according to MLR data indicated in parentheses and underlined only when "aberrant" (see text).
§ Antigen-binding capacity for undiluted serum expressed as picomoles of ligand bound per milliliter of serum. DNP-GL responses are based on secondary bleedings taken approximately 7 days after skin testing, while GA humoral responses are based on primary sera taken 3 wk after initial immunizations.
†† Indicates animal has died and/or no data available. Assignment of "reactive" RhL-A haplotype (when given) is based on data obtained from other parents and offspring.
Preliminary studies reported elsewhere (22) demonstrated that maximal differentiation between high and low responses to the copolymer GA were observed after primary immunization. Since the levels of GA antibody in low responders after secondary immunization were generally greater than those after an initial injection of GA, the relative differences between the responses of siblings were frequently of lesser magnitude.

Table VI summarizes the primary humoral responses to the synthetic copolymer GA among the progeny of father 600 who was mated with four genetically informative females. Monkey 600, a very old animal in failing health, gave a deceptively weak primary GA response. However, after a secondary challenge, father 600 gave a good secondary response (139 pmol/ml, not indicated in Table VI). In the first family mothered by GA-responder female 597, one monkey, CG, failed to respond to GA. Monkey CG inherited the paternal a4 and maternal d2 nonresponder haplotypes. The response of monkey DW can be attributed to the inheritance of the maternal C1 haplotype. The very high primary response of monkey AB was unexpected, in view of the moderate response by RhL-A-identical sibling, BF.

In the second family, three RhL-A-identical offspring carrying the paternal a4 haplotype (AC, BI, and CR) born to female 669 made no detectable primary response to antigen GA, while their full siblings EL and FS carrying different RhL-A genotypes demonstrated an intermediate strength primary GA response. This finding is consistent with the hypothesis that genes linked to the paternal B4 and maternal C2 RhL-A genotypes code for GA responsiveness, while the a4 and d2 haplotypes are associated with nonresponsiveness. The results with

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**Table V**

*Humoral Response of Related Rhesus Monkeys to the Antigen DNP-GL*

| Parents and offspring* | RhL-A genotype† | Antibody response (ABC-33)$ |
|------------------------|-----------------|-----------------------------|
| 596 (2)                | A2 b2           | 40                          |
| 730 (2)                | c2 d2           | <0.1                        |
| FF                     | b2 d2           | 21                          |
| DA                     | A2 d2           | 183                         |
| 834 (4)                | c4            | 1,758                       |
| UU                     | b2 c4           | 39                          |
| BD                     | b2 c4           | 0.3                         |
| EK                     | b2 c4           | <0.1                        |
| CS                     | A2 D4           | >9,000                       |
| 434 (5)                | c5 d5           | 22                          |
| K                      | b5 c5           | 0.5                         |
| 833 (8)                | c6 d6           | 185                         |
| CT                     | A2 c6           | >9,000                       |
| 1355 (10)              | c10 d10         | 16                          |

* For footnotes, see Table IV.
mother 426 and her low responder offspring EE also support this genetic interpretation. However, the antibody responses of the two low responder offspring (XX and BG) from female 429 cannot be explained without evoking recombination or technical error. There is no evidence for recombination from mixed lymphocyte culture studies. Monkeys XX and BG are mutually stimulatory in one way mixed lymphocyte culture, as would be expected from their RhL-A genotypes. The secondary responses of female 429 (83 pmol/ml) and her offspring XX (82 pmol/ml) and BG (72 pmol/ml) suggest that all members of this family are intermediate responders.

The family presented in Table VII illustrates the anti-GA responses of male 598 and his 19 progeny by 9 different females. Seven GA low or nonresponder offspring (Z, BM, CS, EP, BK, CT, and EU) shared the paternal $RhL$-A haplotype, designated $a_2$. The high response of monkey KK who also inherited the $a_2$ haplotype can be attributed to inheritance of the $D_4$ chromosome from female 728. 10 of 11 monkeys carrying the allelic paternal $RhL$-A haplotype, termed $B_2$, were high responders to the GA copolymer. Special attention should be paid to the reactions of monkey EK. The latter animal inherited the $B_2 RhL$-A haplotype yet failed to make a detectable primary immune response to the antigen GA. After secondary challenge, negligible levels of anti-GA antibody were observed. Monkey EK appears to be genotypically identical for the $RhL$-A complex with full siblings UU and BD who share the same RhL-A serological specificities, Lad, and DNP-GL immune response genes (refer to Table IV). Thus, if confirmed, monkey EK represents the first documented case of recombination in which the GA immune response gene was inherited from one paternal
haplotype while the genes coding for the major Lad, the DNP-GL response, and the RhL-A antigens are derived from another RhL-A haplotype. This permits tentative mapping of the various genes in the RhL-A complex as discussed below.

Discussion

The rhesus monkey immune response genes have been identified by using a series of synthetic polypeptide antigens which present the immune system with determinants of very limited structural heterogeneity. After rigorous immunization of unrelated monkeys with either GA or DNP-GL in complete Freund's adjuvant, a bimodal distribution was noted in the antibody levels to each antigen (Fig. 1). Some monkeys mounted either nondetectable or low level humoral responses. In contrast, the majority of animals produced high levels of

### Table VII

Humoral Response of Related Rhesus Monkeys to the Antigen GA

| Parents and offspring | RhL-A genotype | Antibody Response |
|-----------------------|----------------|-------------------|
|                       | (ABC-33)†      |                   |
| **GA**                |                |                   |
| **Z**                 |                |                   |
| 598 (2)               | a₂ B₃ c₁ d₁    | †                   |
| 589 (1)               | a₂ c₁          | 0.1                |
| BP                    | B₃ c₁          | 1,023              |
| FL                    | B₃ d₁          | 300                |
| 832 (3)               | C₃ d₃          | 81                 |
| AD                    | B₃ d₃          | 148                |
| BM                    | a₂ d₃          | 26                 |
| DS                    | B₃ C₃          | 170                |
| 834 (4)               | c₁ d₄          | 41                 |
| UU                    | B₃ c₁          | 4,410              |
| BD                    | B₃ c₁          | <9,000             |
| EK                    | B₃ c₁          | <0.1               |
| CS                    | a₂ d₄          | 47                 |
| 434 (5)               | c₁ d₅          | †                   |
| K                     | B₃ c₅          | <9,000             |
| 728 (6)               | c₄ D₄          | 462                |
| KK                    | a₂ c₄ D₄       | 2801               |
| AS                    | B₃ D₄          | <9,000             |
| EP                    | a₂ c₅          | 0.2                |
| 306 (7)               | C₇ D₇          | 118                |
| SS                    | B₃ c₇          | 3,484              |
| BK                    | a₂ c₇          | 5.7                |
| 833 (8)               | c₈ d₈          | <0.1               |
| CT                    | a₂ c₈          | <0.1               |
| 1114 (9)              | c₉ d₉          | 6.6                |
| EU                    | a₂ c₉          | †                   |
| 1355 (10)             | c₁₀ d₁₀        | 3,056              |

* For footnotes, see Table IV.
specific antibodies. In addition, there was a good correlation between the cellular (delayed skin reactions) and humoral reactivity to each antigen (Table III). In a population of 74 unrelated monkeys, there were no statistically significant associations of immune responsiveness to either GA or DNP-GL with any of the 21 serologically defined SD specificities. In addition, among a highly selected group of 15 pairs of unrelated monkeys including 7 pairs which shared all four serologically defined RhL-A specificities and 8 pairs which shared common Lad, there were no correlations between immune responsiveness and identity of SD antigens, Lad, or skin graft survival times (Tables I and II). Thus, in this highly selected population of unrelated monkeys from which we might expect evidence of linkage disequilibrium, there was no evidence for genetic disequilibrium between the Ir genes and the other marker genes of the RhL-A complex.

Analysis of the GA and DNP-GL antibody response patterns of 21 families sired by 3 male monkeys demonstrated linkage of immune responsiveness to the RhL-A complex. Of 33 informative progeny from 12 rhesus families, 30 monkeys demonstrated linkage of the Ir-GL gene with the RhL-A complex. The remaining 3 monkeys all represented well-documented examples of recombination between the SD and Lad loci of the RhL-A complex. In 2 of the 3 animals (GO and BJ), the immune response genes segregated with the Lad loci (Table IV), while in the other monkey (CZ), responsiveness was associated with the inheritance of the SD and Lad chromosomal regions. This permits provisional mapping of the Ir-GL gene in the area between the SD and Lad chromosomal regions (Fig. 2).

Among 31 informative progeny for GA responsiveness, 29 animals demonstrated that inheritance of immunological responsiveness to the synthetic polymer GA was linked to the RhL-A haplotype. Two animals, monkeys XX and EK, were apparent exceptions to the linkage of specific immunological responsiveness with the classical RhL-A serologically defined specificities. Of the latter two monkeys, one animal (XX) may have carried a recombinant RhL-A chromosome, however, no RhL-A-identical siblings were available to test this hypothesis. Monkey EK had two RhL-A-identical siblings and there was no evidence of recombination between the SD and Lad loci of the RhL-A complex in any of the three siblings. The provisional mapping of the Ir-GA gene outside the SD and Lad loci, but linked to the RhL-A complex, is therefore based on the

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**Fig. 2.** Mapping of the Ir-GL gene. Arrows indicate the position of crossing over for monkeys CZ, GO, and BJ positioning the Ir-GL locus between the Lad and SD loci. The position of the first and second SD loci in relation to the Lad, genes is not yet certain. See text for details.
immune response pattern of monkey EK who is most likely a recombinant between \textit{Ir-GA} gene and the other marker genes of the \textit{RhL-A} complex. The available data did not permit precise localization of the \textit{Ir-GA} genes within the \textit{RhL-A} complex. Fig. 3 depicts the \textit{Ir-GA} genes, however, as discussed elsewhere (29), the data do not rule out localization on the opposite side of the complex.

Fig. 3 illustrates our present concept of the genetic fine structure of the \textit{RhL-A} complex. This gene complex contains at least six marker genes including two \textit{SD} loci, two \textit{Ir} genes, a locus coding for the major \textit{Lad}, and the \textit{Bf} locus which codes for the serum protein, glycine-rich betaglycoprotein (GBG) related to the complement system. Ziegler et al. (35) have recently demonstrated the linkage of the structural genes for GBG of the rhesus monkey to the \textit{RhL-A} complex. These investigators have positioned the \textit{Bf} locus outside the \textit{SD} loci and tentatively outside the \textit{Lad}, and \textit{Ir-GL} genes. Our provisional localization of the \textit{Ir-GA} gene in the same region as the \textit{Lad}, and \textit{Ir-GL} genes is primarily based on data from other species which make this hypothesis intuitively preferable. Thus, in guinea pigs there is a high degree of linkage disequilibrium between the \textit{Ir-GA} and \textit{Ir-GL} genes which suggests that these genes are closely linked to each other (34). In addition, in the mouse where several \textit{Ir} genes have been precisely mapped, all histocompatibility linked \textit{Ir} genes are closely linked to each other and all map within the \textit{I} region (1-3).

An additional report has described a series of restricted tissue alloantigens coded for in the \textit{RhL-A} complex (31). These alloantigens are primarily present on B lymphocytes and most probably represent the primate analogue of the murine \textit{Ia} antigens. The genes coding for the monkey \textit{Ia} antigens are probably localized in the region of the \textit{Lad} genes (31). However, the precise relationship between the genes controlling the \textit{Ia} specificities and the genes coding for the \textit{Lad} or the \textit{Ir} genes is currently under investigation. Based on the analogies to

\textbf{Fig. 3.} Tentative genetic fine structure of the \textit{RhL-A} gene complex. Proposed linear arrangement of genes comprising the \textit{RhL-A} chromosomal regions and a listing of marker genes associated with each region are indicated. See text for details.
the murine I region, we have tentatively designated the chromosomal region controlling the Ir genes, Lad, and Ia alloantigens as the I region of the RhL-A complex (Fig. 3).

Histocompatibility linked immune response genes have been demonstrated in several species. We have chosen to examine the genetic mapping of Ir genes in the rhesus monkey, a species which is phylogenetically closely related to man and whose MHC, RhL-A, has been thoroughly investigated. The rhesus monkey RhL-A complex has several features in common with the human HL-A system (Fig. 4). The RhL-A and HL-A complexes code for at least two segregant series of alloantigens which are present on most, if not all, nucleated cells. These alloantigens are coded for by closely linked SD loci (33, 36). Closely linked to the SD genes lies another chromosomal region which controls the major Lad (Lad loci) responsible for activation of T lymphocytes in the MLR (23, 37, 38). In contrast to the mouse, the major Lad loci lie outside of the SD region in both primate species (Fig. 4 and references 18 and 37–39). Similar mapping of SD vs. Lad regions hold true for the canine histocompatibility complex (40). In addition, the Bf genes coding for factor B of the properdin system are also linked to the RhL-A and HL-A complex although the precise localization of the Bf locus is only tentative (35, 41, 42). A final point of similarity between the human and monkey histocompatibility systems is the localization of genes outside the SD region in the area of the Lad genes which code for a restricted class of alloantigens found primarily on B lymphocytes (31, 43). This class of molecules is probably analogous to the Ia system of mice (1, 44, 45). This report further characterizes the genetic fine structure of the rhesus monkey MHC by provisionally mapping Ir genes in the same region as the genes coding for the major Lad and the postulated rhesus Ia antigens. In view of the multiple similarities between the human and rhesus monkey MHC, one is tempted to predict that at least some of the human Ir genes will map in the same relative positions within the histocompatibility complex as those described for the monkey.

As further illustrated in Fig. 4, there are striking similarities among the genes associated with the MHC of the mouse and those of the two reviewed primate species. These similarities even extend to the analogy of genes in the murine S region, which are associated with the level of serum complement activity (46) and perhaps additional genes controlling murine C3 and C4 levels (47, 48) with the HL-A-linked genes which regulate C2 deficiency (49) or the Bf loci which determine polymorphisms in properdin factor B (GBG) of the alternate complement pathway (35, 41, 42). An initial report (42), which has not been confirmed, demonstrated close linkage of the genes controlling GBG polymorphism with the Lad regions in certain human families. In addition, the rhesus Bf locus has been provisionally mapped outside the SD, Ir-GL, and Lad loci (35).

The order of genes in the H-2 complex differs from that found in monkey, dog, and man (Fig. 4). A chromosomal inversion or an intrachromosomal translocation may be responsible for the different linear arrangement of genes observed in the mouse. The genetic distance measured in terms of recombination units also differs dramatically between the mouse (in which the K and D regions are separated by less than 0.4 centiMorgans [50]) and the other species in which the distance between the outermost markers of the histocompatibility gene complex
are approximately 6 centiMorgans apart (35). From the present study using a laboratory population of rhesus monkeys, we estimate the total distance spanning all six marker genes to be on the order of 5 centiMorgans. However, it must be emphasized that we have used a population of monkeys which were preselected for a high incidence of recombination within the RhL-A complex, thus, this may be an overestimate.

The data from the mouse have established that at least two closely linked genetic regions separable by recombination can control specific immune respon-
siveness (6, 7). In this report we have described one example of such a recombinant event in monkey EK, in which crossing over occurred between the Ir gene controlling immune responsiveness to the copolymer GA and the Ir gene governing responsiveness to DNP-GL. If the genetic distance across the I region of the RhL-A complex is as large as suggested by the four recombinant animals found among the 57 siblings tested, we would predict additional examples of crossing over between specific Ir genes to be found among the remaining large rhesus families of the Rijswijk colony once they are tested.

The fine structure mapping of the rhesus MHC may provide an insight into the order of genes in the human HL-A complex. Indeed, some of the weak associations of serologically defined HL-A specificities with various human diseases may be attributed to human Ir genes which are positioned outside the SD loci near the Lad genes. If the tentative RhL-A mapping presented in this report represents an accurate model for the fine structure mapping of the HL-A complex, we may expect some disease susceptibilities to demonstrate loose genetic disequilibrium with the serologically defined HL-A specificities and a somewhat better correlation with the human Lad genetic markers. The data of Jersild et al. (51) for multiple sclerosis and Blumenthal et al. (52) for ragweed pollinosis support this contention. Although some of the reports describing human Ir genes have been the target of severe criticism (53), nonetheless, the similarities are striking. The genetic forces which have preserved the close linkage of the many marker genes contained within the MHC of several species are unknown.

Summary

Interest in the Ir genes of rhesus monkeys stems from their phylogenetic relationship to man and the extensive data already available on the major histocompatibility complex of the monkey. At least two independent dominant H-linked Ir genes have been identified in the rhesus. These genes control the ability of monkeys to respond to the random linear copolymer of glutamyl alanine (GA), or the dinitrophenyl conjugate of glutamyl lysine (DNP-GL). These synthetic polymers can elicit weak delayed-type skin reactions and strong humoral responses in some monkeys. In a series of unrelated monkeys phenotyped for the serologically defined RhL-A specificities of both segregant series, there were no correlations between any RhL-A specificity and responder status to the GA or DNP-GL polymers. However, segregation analysis of 21 rhesus families sired by 3 fathers indicated the capacity of the offspring to form antibodies was associated with genes coded for in the RhL-A complex. In three monkeys, verified recombination within the RhL-A complex between the genes coding for the serologically defined determinants (SD loci) and the gene(s) controlling the lymphocyte-activating determinants (Lad loci) responsible for mixed lymphocyte reactivity was established. In two of these monkeys the immune response genes controlling the DNP-GL response segregated with the Lad genes, while in the third case the Ir-GL gene segregated with the SD loci, tentatively localizing the Ir-GL gene between the SD and Lad loci. In addition, we have shown that genetically distinct genes control responsiveness to DNP-GL and GA. These genes were separated by recombination, thus one monkey
inherited the Lad, Ir-GL, and SD loci from one paternal haplotype and by crossing over inherited the gene controlling GA responsiveness from the other paternal haplotype. The fine structure mapping of the RhL-A gene complex is compared with the H-2 and HL-A gene complexes. Several striking similarities were noted.

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References
1. Shreffler, D. C., and C. S. David. 1975. The H-2 major histocompatibility complex and the I immune response region: genetic variation, function and organization. Adv. Immunol. 20:125.
2. Benacerraf, B., and M. E. Dorf. 1974. Genetic control of specific immune responses. Prog. Immunol. 2:181.
3. Benacerraf, B., and D. H. Katz. 1975. The nature and function of histocompatibility-linked immune response genes. In Immunogenetics and Immunodeficiency. B. Benacerraf, editor. Medical and Technical Publishers, London, England. In press.
4. McDevitt, H. O., B. D. Deak, D. C. Shreffler, J. Klein, J. H. Stimpfling, and G. D. Snell. 1972. Genetic control of the immune response. Mapping of the Ir-1 locus. J. Exp. Med. 135:1259.
5. Dunham, E. K., M. E. Dorf, D. C. Shreffler, and B. Benacerraf. 1973. Mapping of H-2-linked genes governing respectively the immune responses to a glutamic acid-alanine-tyrosine copolymer and to limiting doses of ovalbumin. J. Immunol. 111:1621.
6. Lieberman, R., W. E. Paul, W. Humphrey, and J. H. Stimpfling. 1972. H-2-linked immune response (Ir) genes. Independent loci for Ir-IgG and Ir-IgA genes. J. Exp. Med. 136:1231
7. Dorf, M. E., F. Lilly, and B. Benacerraf. 1974. Characterization of a new intra H-2 recombinant. Separation of the Ir-RE and Ir-GLT genes. J. Exp. Med. 140:859.
8. Klein, J., F. H. Bach, F. Festenstein, H. O. McDevitt, D. C. Shreffler, G. D. Snell, and J. H. Stimpfling. 1974. Genetic nomenclature for the H-2 complex of the mouse. Immunogenetics. 1:184.
9. Bach, F. H., M. B. Widmer, M. Segall, M. L. Bach, and J. Klein. 1972. Genetic and immunological complexity of major histocompatibility regions. Science (Wash. D. C.). 176:1024.
10. Meo, T., J. Vives, V. Migiano, and D. C. Shreffler. 1973. A major role for the Ir-1 region of the mouse H-2 complex in the mixed leukocyte reaction Transplant. Proc. 5:277.
11. Klein, J., and J. M. Park. 1973. Graft-versus-host reaction across different regions of the H-2 complex of the mouse. J. Exp. Med. 137:1213.
12. Katz, D. H., M. Graves, M. E. Dorf, H. DiMuzio, and B. Benacerraf. 1975. Cell interactions between histoincompatible T and B lymphocytes. VII. Cooperative responses between lymphocytes are controlled by genes in the I region of the H-2 complex. J. Exp. Med. 141:263.
13. Dorf, M. E., D. H. Katz, M. Graves, H. DiMuzio, and B. Benacerraf. 1975. Cell interactions between histoincompatible T and B lymphocytes. VIII. In vivo cooperative responses between lymphocytes are controlled by genes in the K-end of the H-2 complex. J. Immunol 114:1717.
14. Hauptfeld, V., D. Klein, and J. Klein. 1973. Serological identification of an Ir region product. Science (Wash. D. C.). 181:167.
15. Sachs, D. H., and J. L. Cone. 1973. A mouse B cell alloantigen determined by gene(s) linked to the major histocompatibility complex. J. Exp. Med. 138:1289.
16. Balner, H., B. W. Gabb, H. Dersjant, W. van Vreeswijk, and J. J. van Rood. 1971. Major histocompatibility locus of rhesus monkeys (RhL-A). Nat. New Biol. 230:177.
17. Rogentine, G. N., Jr., C. B. Merritt, L. A. Vaal, E. B. Ellis, and C. C. Darrow III. 1972. Rhesus lymphocyte alloantigens. II. Serologic, genetic and chemical characteristics. Transplant. Proc. 4:21.
18. Balner, H., W. van Vreeswijk, M. L. de Groot, and J. D’Amaro. 1974. The major histocompatibility complex of rhesus monkeys: IV. Serologic identification of several new antigens of both series of RhL-A. Transplant. Proc. 6:111.
19. Balner, H. 1973. Current knowledge of the histocompatibility complex of rhesus monkeys. Transplant. Rev. 15:50.
20. Balner, H., M. E. Dorf, M. L. de Groot, and B. Benacerraf. 1973. The histocompatibility complex of rhesus monkeys. III. Evidence for a major MLR locus and histocompatibility-linked Ir genes. Transplant. Proc. 5:1555.
21. Bluestein, H. G., I. Green, and B. Benacerraf. 1971. Specific immune response genes of the guinea pig. I. Dominant genetic control of immune responsiveness to copolymers of L-glutamic acid and L-alanine and L-glutamic acid and L-tyrosine. J. Exp. Med. 134:458.
22. Dorf, M. E., H. Balner, M. L. de Groot, and B. Benacerraf. 1974. Histocompatibility-linked immune response genes in the rhesus monkey. Transplant. Proc. 6:119.
23. Balner, H., and E. K. Toth. 1973. The histocompatibility complex of rhesus monkeys. II. A major locus controlling reactivity in mixed lymphocyte cultures. Tissue Antigens. 3:273.
24. Benacerraf, B., and B. B. Levine. 1962. Immunological specificity of the delayed and immediate hypersensitivity reactions. J. Exp. Med. 113:1023.
25. Dorf, M. E., E. K. Dunham, J. P. Johnson, and B. Benacerraf. 1973. Genetic control of the immune response. The effect on non-H-2 linked genes on antibody production. J. Immunol. 112:1329.
26. Greenwood, F. C., W. M. Hunter, and J. S. Glover. 1963. The preparation of 131I-labelled human growth hormone of high specific radioactivity. Biochem. J. 89:114.
27. Katz, D. H., W. E. Paul, E. A. Goold, and B. Benacerraf. 1970. Carrier function in anti-hapten immune responses. I. Enhancement of primary and secondary anti-hapten antibody responses by carrier preimmunization. J. Exp. Med. 132:261.
28. Du Bois, M. J. G. J., D. R. Huismans, P. Th. A. Schellekens, and V. P. Eijswogel. 1973. Investigation and standardization of the conditions for microlymphocyte cultures. Tissue Antigens. 3:402.
29. Dorf, M. E., H. Balner, and B. Benacerraf. 1975. The major histocompatibility complex of rhesus monkeys (RhL-A). VI. Mapping of RhL-linked immune response genes. Transpl. Proc. 7:21.
30. Van Rood, J. J., and A. van Leeuwen. 1963. Leukocyte grouping: a method and its application. J. Clin. Invest. 42:1382.
31. Balner, H., and W. van Vreeswijk. 1975. The major histocompatibility complex of rhesus monkeys (RhL-A). V. Attempts at serological identification of MLR determinants and postulation of an I region of the RhL-A complex. Transplant. Proc. 7:13.
32. Buckley, C. E., F. C. Dorsey, R. B. Corley, W. B. Ralph, M. A. Woodbury, and D. B. Amos. 1973. HL-A-linked human immune-response genes. Proc. Natl. Acad. Sci. U. S. A. 70:2157.
33. Balner, H. et al. 1972. Joint report of first histocompatibility workshop on primates. Transplant. Proc. 4:141.
34. Bluestein, H. G., I. Green, and B. Benacerraf. 1971. Specific immune response genes
of the guinea pig. II. Relationship between the poly-L-lysine gene and the genes controlling immune responsiveness to copolymers of L-glutamic acid and L-alanine and L-glutamic acid and L-tyrosine in random-bred Hartley guinea pigs. J. Exp. Med. 134:471.

35. Ziegler, J. B., C. A. Alper, and H. Balner. 1975. Properdin factor B and histocompatibility genes linked in the rhesus monkey. Nature (Lond.). 254:609.

36. Allen, F., D. B. Amos, R. Batchelor, W. Bodmer, R. Ceppellini, J. Dausset, C. Engelfriet, M. Jeannet, F. Kismeyer-Nielsen, P. Morris, R. Payne, P. Terasaki, J. J. van Rood, R. Walford, C. Zmijewski, E. Albert, P. Mattiuz, M. R. Mickey, and A. Piazza. 1970. Joint report of fourth international histocompatibility workshop. Histocompatibility Testing, Munksgaard, A/S, Copenhagen, Denmark. 18.

37. Yunis, E. J., and D. B. Amos. 1971. HL-A mixed leukocyte reaction (MLR) and hypersensitivity delayed reaction (HDR). Three closely linked genetic systems relevant to transplantation. Proc. Natl. Acad. Sci. U. S. A. 68:3031.

38. Eijsvoogel, V. P., M. J. G. J. du Bois, C. J. M. Melief, M. L. de Groot-kooij, C. Koning, J. J. van Rood, A. van Leeuwen, E. du Toit, and P. Th. A. Schellekens. 1972. Position of a locus determining mixed lymphocyte reaction (MLR), distinct from the known HL-A loci and its relation to cell-mediated lympholysis (CML). Histocompatibility Testing. (Munksgaard, A/S, Copenhagen, Denmark. 501.

39. Bach, F. H., M. L. Bach, P. M. Sondel, and G. Sundharadas. 1972. Genetic control of mixed leukocyte culture reactivity. Transplant. Rev. 12:30.

40. Vriesendorp, H. M., et al. 1973. Joint report of first international workshop on canine immunogenetics. Tissue Antigens. 3:145.

41. Allen, F. H. 1974. Linkage of HL-A and GBG. Vox Sang. 27:382.

42. Rittner, C., H. Grosse-Wilde, B. Rittner, B. Netzel, S. Scholz, H. Lorenz, and E. D. Albert. 1975. Linkage group HL-A-MLC-BF (properdin factor B). The site of BF locus at the immunogenetic linkage group on chromosome 6. Humangenetik. 2:in press.

43. Van Rood, J., A. van Leeuwen, J. J. Keuning, and A. Termijtelen. 1975. Serotyping for MLC. III. Family and population studies with an MLC inhibiting serum Pl. Transplant. Proc. 7:31.

44. Hämerling, G. J., B. D. Deak, G. Mauve, U. Hämerling, and H. O. McDevitt. 1974. B lymphocyte alloantigens controlled by the I region of the major histocompatibility complex in mice. Immunogenetics. 1:88.

45. Unanue, E. R., M. E. Dorf, C. S. David, and B. Benacerraf. 1974. The presence of I region associated antigens on B cells in molecules distinct from Ig and H-2K and H-2D. Proc. Natl. Acad. Sci. U. S. A. 71:5014.

46. Demant, P., J. Capkova, E. Hinzova, and B. Voracova. 1973. The role of the histocompatibility-2-linked Se-Slp region in the control of mouse complement. Proc. Natl. Acad. Sci. U. S. A. 70:863.

47. Ferreira, A., and V. Nussenzweig. 1975. Genetic control of complement (C3) levels and the development of the lymphoid system. Fed. Proc. 34:979. (Abstr.)

48. Goldman, M. B., and J. N. Goldman. 1975. Relationship of levels of early components of complement to the H-2 complex of mice. Fed. Proc. 34:979. (Abstr.)

49. Fu, S. W., H. G. Kunkel, H. P. Brusman, F. H. Allen, Jr., and M. Fotino. 1974. Evidence for linkage between HL-A histocompatibility genes and those involved in the synthesis of the second component of complement. J. Exp. Med. 140:1108.

50. Shreffler, D. C., and J. Klein. 1970. Genetic organization and gene action of mouse H-2 region. Transplant. Proc. 2:5.

51. Jersild, C., G. S. Hansen, A. Svejgaard, T. Fog, M. Thomsen, and B. DuPont. 1973. Histocompatibility determinants in multiple sclerosis with special reference to clinical course. Lancet. 7840:1221.
52. Blumenthal, M. N., D. B. Amos, H. Noreen, N. R. Mendell, and E. J. Yunis. 1974. Genetic mapping of Ir locus in man: linkage to second locus of HL-A. *Science (Wash. D. C.)*. 184:1301.

53. Bias, W. B., and D. G. Marsh. 1975. HL-A linked antigen E immune response genes: an unproved hypothesis. *Science, (Wash. D. C.)*. 188:375.