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

By MARTIN E. DORF, HANS BALNER,‡ 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)[1] 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).[2]

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

* This investigation was supported by grant AI-09920 from the National Institutes of Health, U. S. Public Health Service; the Dutch Government; and contract no. 6243-22/001 from the Commission of the European Communities, Luxembourg.

‡ Member of the Biology Division of Euratom.

[1] *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^{60}-L-alanine^{30}-L-tyrosine^{10}; 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 histocompatibility 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 designated 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.

(6, 7). 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 ($G^{60}A^{40}$) or L-lysine ($G^{50}L^{50}$) were synthesized to order by Pilot Chemicals, Inc., Watertown, Mass. Superscripts refer to the molar amino acid ratios. Preparations of DNP_7-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_7-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 ($G^{50}T^{50}$) 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 acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT) purchased from Pilot Chemicals, Inc. (25). GAT was iodinated by the chloramine-T method (26) with carrier-free ^{125}I (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 2×10^{-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 [^{3}H]DNP-ϵ-amino-*N*-caproic acid was used to measure DNP$_T$-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

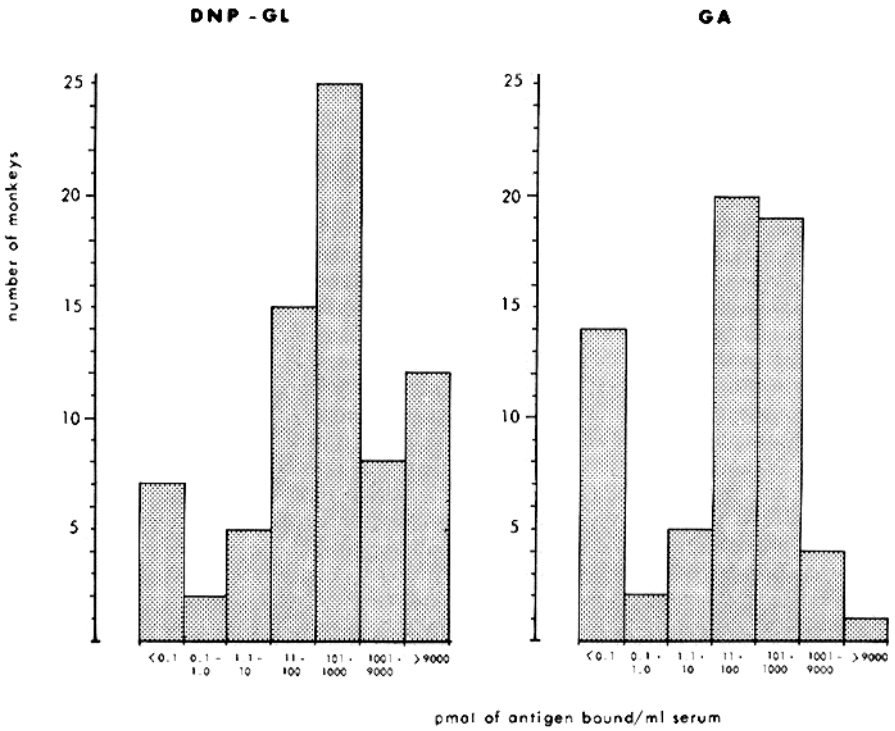

FIG. 1. Distribution of immune response to DNP-GL and GA in unrelated rhesus monkeys. *Left panel:* Distribution of secondary DNP-GL responses among 74 unrelated monkeys immunized with 50 μg DNP-GL in complete Freund's adjuvant and boosted intradermally with 60 μg of antigen. Sera collected 7 days after 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 $\times$ 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 ($\geq$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 Lad Matched Unrelated Monkeys

Responder (R)	Donor (D)	RhL-A phenotype		Immune Response*		Unilateral MLR‡ $R \rightarrow D_m$	Skin graft survival§
		SD_1	SD_2	GA	DNP-GL		
							days
960		9 6	11 25	−	−	−	11.5
	2453	9 6	11 2	+	+		
2337		6 10	13 —	+	+	−	11.5
	2464	6 23	13 —	+	−		
2464		6 23	13 —	+	−	−	9.5
	2337	6 10	13 —	+	+		
2463		6 10	13 26	−	+	−	9.5
	2394	6 10	13 —	−	+		
2394		6 10	13 —	−	+	−	8.5
	2463	6 10	13 26	−	+		
2368		6 10	2 —	+	+	−	10
	2412	6 10	2 —	−	+		
2345		10 —	13 17	NT‖	+	−	10
	2407	10 —	2 26	+	+		
2414		10 —	11 26	+	+	−	10
	2407	10 —	2 26	+	+		

* 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‡ $R \rightarrow D_m$	Skin graft survival§
		SD_1		SD_2		GA	DNP-GL		
									days
1062		9	6	11	2	+	−		
	2398	9	6	11	2	+	+	+	30-35
2453		9	6	11	2	+	+		
	1062	9	6	11	2	+	−	+	11
	2398	9	6	11	2	+	+	+	11
2398		9	6	11	2	+	+		
	1062	9	6	11	2	+	−	+	13
	2453	9	6	11	2	+	+	+	10.5
2463		6	10	13	26	−	+		
	2496	6	10	13	26	NT¶	+	+	16
2496		6	10	13	26	NT¶	+		
	2463	6	10	13	26	−	+	+	11.5
Unmatched controls		2-4 Antigen mismatches				70% + 30% −	80% + 20% −	+	9.4 ± 1.2

For footnotes, see Table I.

TABLE III

*Correlation of Cellular and Humoral Immunity**

GA			DNP-GL		
Antibody (ABC-33)	Skin test		Antibody (ABC-33)	Skin test	
	+	−		+	−
>40	14	24	>40	17	35
<40	3	22	<40	0	21
	$\chi^2 = 4.72$ $P = 0.03$			$\chi^2 = 8.94$ $P = 0.003$	

* 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_1 *RhL-A* haplotype (Table IV). In addition, all 10 monkeys carrying the paternal A_1 *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). Incidently, 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 b_2 *RhL-A* haplotype, while the three high responder offspring all inherited the paternal A_2 *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.

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

Parents and offspring*	RhL-A genotype‡	Antibody response (ABC-33)§
♂381 (1)	A_1 b_1	159
♀ 432 (1)	C_1 d_1	266
N	A_1 C_1	125
CP	A_1 d_1	178
FN	A_1 C_1	1,126
AK	b_1 d_1	53
GM	b_1 d_1	5
♀ 584 (3)	c_3 d_3	17
V	b_1 d_3	<0.1
JJ	b_1 c_3	36
AV	b_1 c_3	16
BU	b_1 c_3	3
CZ	b_1 c_3 ($\underline{a_1}c_3$)	9
GO	b_1 d_3 ($\underline{a_1}d_3$)	131
♀ 594 (4)	c_4 D_4	†‖
YY	A_1 D_4	7,188
BJ	b_1 D_4($b_1\underline{c_4}$)	5
FU	b_1 D_4	193
♀ 852 (5)	c_5 d_5	28
AH	A_1 c_5	91
DN	A_1 c_5	101
EV	A_1 d_5	214
BL	b_1 d_5	8
♀ 324 (6)	c_6 D_6	3,182
CU	b_1 c_6	<0.1
EM	A_1 c_6	886
♀ 1646 (8)	c_8 D_8	3,678
FM	A_1 c_8	519
GS	b_1 c_8	<0.1
♀ 1472 (10)	c_{10} d_{10}	<0.1
EQ	A_1 c_{10}	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.

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

Parents and offspring*	RhL-A genotype‡	Antibody response (ABC-33)§
♂ 598 (2)	A_2 b_2	‖
♀ 730 (2)	c_2 d_2	4.0
FF	b_2 d_2	<0.1
ZZ	b_2 c_2	21
DA	A_2 d_2	183
♀ 834 (4)	c_4 D_4	1,758
UU	b_2 c_4	39
BD	b_2 c_4	0.3
EK	b_2 c_4	<0.1
CS	A_2 D_4	>9,000
♀ 434 (5)	c_5 d_5	‖
K	b_2 c_5	22
♀ 833 (8)	c_8 d_8	0.5
CT	A_2 c_8	185
♀ 1355 (10)	c_{10} d_{10}	‖
EG	b_2 c_{10}	16

* For footnotes, see Table IV.

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 a_4 and maternal d_1 nonresponder haplotypes. The response of monkey DW can be attributed to the inheritance of the maternal C_1 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 a_4 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 B_4 and maternal C_2 *RhL-A* genotypes code for GA responsiveness, while the a_4 and d_2 haplotypes are associated with nonresponsiveness. The results with

TABLE VI
*Humoral Response of Related Rhesus Monkeys to the Antigen GA**

Parents and offspring*	RhL-A genotype‡	Antibody response (ABC-33)§
♂ 600 (4)	a_4 B_4	19
♀ 597 (1)	C_1 d_1	89
AB	B_4 C_1	1,190
BF	B_4 C_1	49
DW	a_4 C_1	95
CG	a_4 d_1	<0.1
♀ 669 (2)	C_2 d_2	46
AC	a_4 d_2	<0.1
BI	a_4 d_2	<0.1
CR	a_4 d_2	<0.1
EL	B_4 C_2	61
FS	B_4 C_2	38
♀ 429 (4)	c_4 d_4	<0.1
XX	B_4 c_4	5
BG	a_4 c_4	28
♀ 426 (5)	c_5 d_5	‖
EE	a_4 c_5	5.7

* 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_6 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

TABLE VII
*Humoral Response of Related Rhesus Monkeys to the Antigen GA**

Parents and offspring*	RhL-A genotype‡			Antibody Response (ABC-33)§
♂ 598 (2)	a_2	B_2		‡‖
♀ 589 (1)		c_1	d_1	‡‖
Z	a_2	c_1		0.1
BP		B_2 c_1		1,023
FL		B_2	d_1	300
♀ 832 (3)		C_3	d_3	81
AD		B_2	d_3	148
BM	a_2		d_3	26
DS		B_2 C_3		170
♀ 834 (4)		c_4	d_4	41
UU		B_2 c_4		4,410
BD		B_2 c_4		<9,000
EK		B_2 c_4		<0.1
CS	a_2		d_4	47
♀ 434 (5)		c_5	d_5	‡‖
K		B_2 c_5		<9,000
♀ 728 (6)		c_6	D_6	462
KK	a_2		D_6	2801
AS		B_2	D_6	<9,000
EP	a_2	c_6		0.2
♀ 306 (7)		c_7	D_7	118
SS		B_2 c_7		3,484
BK	a_2	c_7		5.7
♀ 833 (8)		c_8	d_8	<0.1
CT	a_2	c_8		<0.1
♀ 1114 (9)		c_9	d_9	
EU	a_2	c_9		6.6
♀ 1355 (10)		c_{10}	d_{10}	‡‖
EG		B_2 c_{10}		3,056

* For footnotes, see Table IV.

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

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

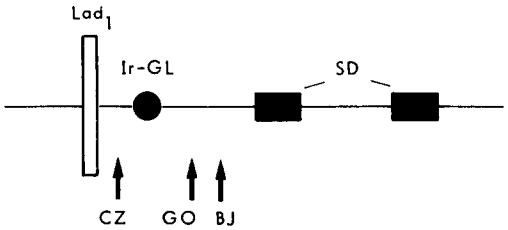

FIG. 2. Mapping of the *Ir-GL* gene. *Arrows* indicate the position of crossing over for monkeys CZ, GO, and BJ positioning the *Ir-GL* locus between the *Lad* and *SD* loci. The position of the first and second *SD* loci in relation to the Lad_1 genes is not yet certain. See text for details.

immune response pattern of monkey EK who is most likely a recombinant between *Ir-GA* gene and the other marker genes of the *RhL-A* complex. The available data did not permit precise localization of the *Ir-GA* genes within the *RhL-A* complex. Fig. 3 depicts the *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 *RhL-A* complex. This gene complex contains at least six marker genes including two *SD* loci, two Ir genes, a locus coding for the major *Lad*, and the *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 *RhL-A* complex. These investigators have positioned the *Bf* locus outside the *SD* loci and tentatively outside the Lad_1 and *Ir-GL* genes. Our provisional localization of the *Ir-GA* gene in the same region as the Lad_1 and *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 *Ir-GA* and *Ir-GL* genes which suggests that these genes are closely linked to each other (34). In addition, in the mouse where several Ir genes have been precisely mapped, all histocompatibility linked Ir genes are closely linked to each other and all map within the *I* region (1-3).

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

FIG. 3. Tentative genetic fine structure of the *RhL-A* gene complex. Proposed linear arrangement of genes comprising the *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_1 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

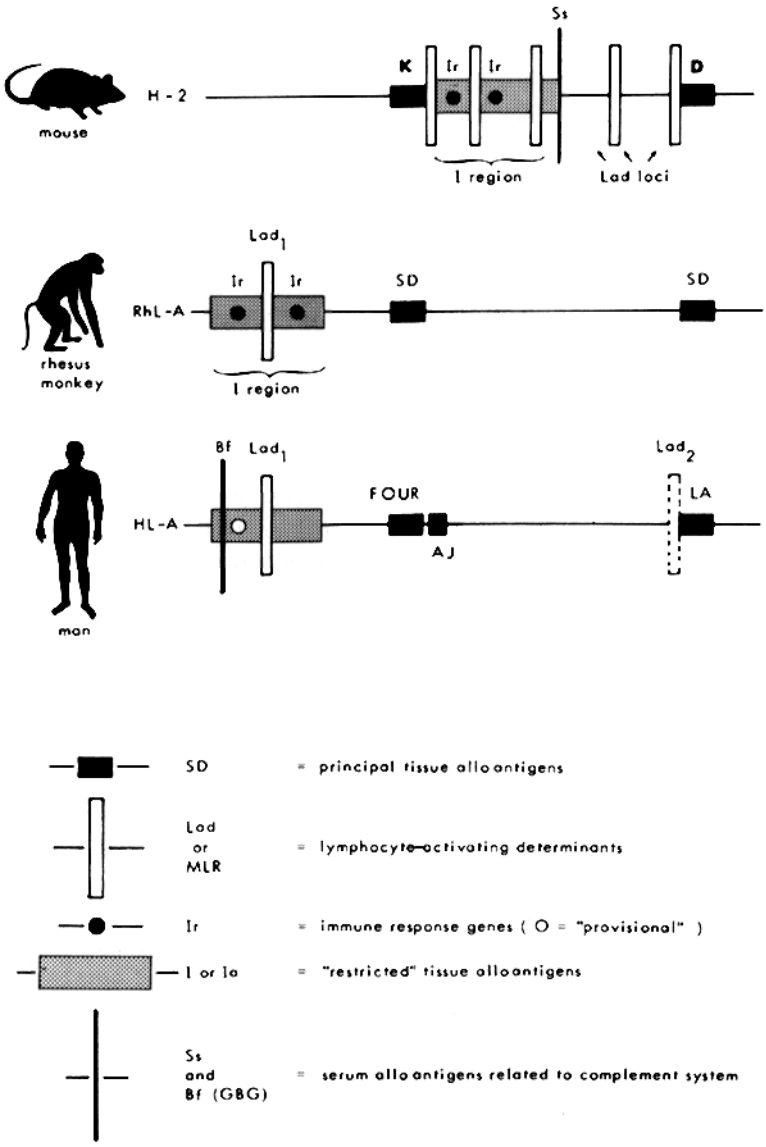

FIG. 4. Comparison of the MHC of several mammalian species. Refer to Figs. 2 and 3 for explanation of the tentative mapping of *RhL-A*.

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 pollenosis 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.

Received for publication 28 April 1975.

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. Miggiano, 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**:377.
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 alloantogens. 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 indentification of several new antigens of both series of RhL-A. *Transplant. Proc.* **6**:111.
19. Balner, H. 1973. Current knowledge of the histocompatibiltiy 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.* **115**: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 ^{131}I-labelled human growth hormone of high specific radioactivity. *Biochem. J.* 89:114.
27. Katz, D. H., W. E. Paul, E. A. Goidl, 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. Eijsvoogel. 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-kooy, 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. Hämmerling, G. J., B. D. Deak, G. Mauve, U. Hämmerling, and H. O. McDevitt. 1974. B lymphocyte alloantigens controlled by the *I* region of the major histocompatibility complex in mice. *Immunogenetics.* 1:68.
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. Démant, P., J. Capkova, E. Hinzova, and B. Voracova. 1973. The role of the histocompatibility-2-linked Ss-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 of 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.