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Genetics

Genetic control of major histocompatibility complex-linked immune responses to synthetic polypeptides in man

[human heteropolymer stimulation/*Ir* gene complementation/poly(LHis, LGlu)-poly(DLAla)--poly(LLys)/poly(LTyr, LGlu)-poly(DLAla)--poly(LLys)]

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ABSTRACT Vigorous lymphocyte proliferative response to synthetic polypeptides was observed in cells from 50 normal volunteers. Results indicated that 64% responded to poly(LHis, LGlu)-poly(DLAla)--poly(LLys) [(H, G)-A--L] and 54% to poly(LTyr, LGlu)-poly(DLAla)--poly(LLys) [(T, G)-A--L]. Subjects could be classified into high-, intermediate-, and non-responder phenotypes according to their stimulation indices. Family studies indicated that high responses to these antigens are inherited as histocompatibility antigen gene (*HLA*)-linked dominant traits. Two matings suggested gene complementation in response to (T, G)-A--L and (H, G)-A--L. One, with an intra-*HLA* recombinant offspring, provided evidence localizing the immune response gene(s) controlling lymphocyte proliferation to (T, G)-A--L and (H, G)-A--L, presumably the homologue to *Ir-1* of mouse, closer to the *HLA-B* than to the *HLA-D* region.

The discovery of the major histocompatibility (MHC)-linked immune response (*Ir*) genes for different synthetic polypeptides in rodents, birds, and rhesus monkeys has contributed significantly to our understanding of contemporary immunobiology (1). In man, the existence of *Ir* genes has been implied but never formally proved (2). Nevertheless, it is reasonable to assume the existence of *Ir* genes mapping within the histocompatibility antigen gene (*HLA*) region not only because of the homology between *H-2* and *HLA* but also because of the phylogenetic relationship of the rhesus monkey to man.

Because it is not possible to duplicate the *in vivo* animal experiments in man, we attempted both primary and secondary *in vitro* stimulation with some synthetic polypeptides. We report herein a demonstration of *HLA*-linked genetic control of immune response to poly(LHis, LGlu)-poly(DLAla)--poly(LLys) [(H, G)-A--L] and poly(LTyr, LGlu)-poly(DLAla)--poly(LLys) [(T, G)-A--L] in families.

MATERIALS AND METHODS

Subjects. Human lymphocytes were isolated by a standard Ficoll/Hypaque gradient technique from 50 healthy unrelated volunteers selected to include all known *HLA-A, B, C,* and *D* specificities and members of 10 families. The *HLA-A, B, C* types of all subjects were determined by the standard two-stage microcytotoxicity test. Our laboratory's (3) modification of the two-color fluorescent technique of Van Rood *et al.* (4) was used for the *HLA-DR* typing. *HLA-D* locus alleles were determined by using homozygous typing cells recognizing specificities *Dw1, Dw2, Dw3, Dw4, Dw5, Dw6, Dw7,* and *Dw11*.

Synthetic Antigens. Synthetic polymers of (H, G)-A--L and (T, G)-A--L were obtained from Miles Yeda (Elkhart, IN). To ensure that the antigen preparations were not contaminated with mitogenic material, 12 samples of human cord blood lymphocytes and spleen cells from six unimmunized female mice of strains C57 (BL/6N)(H-2^b), C3H/J(H-2^k), CBA(H-2^k), BALB/c(H-2^d), and DBA/N(H-2^d), known to be nonresponders or high responders to the synthetic polymers, were cocultured *in vitro* with both antigens. Neither antigen induced blast transformation in either human cord blood lymphocytes or the unimmunized mouse spleen cells.

Table 1. Correlation between peak stimulation indices (SI) in duplicate tests with synthetic polymers

Subject	(H, G)-A--L		(T, G)-A--L	
	Test 1	Test 2	Test 1	Test 2
1	10.5	7.0	3.3	3.1
2	1.5	1.7	2.0	1.8
3	5.2	4.1	7.8	10.0
4	4.5	5.4	9.3	8.4
5	2.2	2.1	7.7	6.3
6	1.5	2.0	2.4	2.1
7	1.7	1.1	1.6	1.6
8	2.1	1.8	3.4	4.1
9	4.6	4.0	4.3	4.2
10	3.8	4.3	3.4	5.6
12	3.6	3.7	5.1	4.1
15	2.3	1.6	1.1	1.3
17	1.5	1.8	1.4	1.8
19	3.4	3.9	2.9	3.0
20	1.8	1.9	2.1	1.9
24	1.4	1.9	3.0	3.3
25	1.9	2.4	2.0	2.1
26	1.8	1.6	3.1	3.3
29	1.1	1.3	1.9	1.7
31	3.5	4.5	4.5	3.9
32	1.7	1.5	1.8	2.1
34	7.8	12.6	15.5	12.1
36	9.7	8.2	18.5	17.8
37	10.5	7.1	8.2	6.6
38	1.6	1.6	1.5	2.2
39	14.6	10.0	12.8	14.3
40	29.8	20.0	12.2	11.4
41	0.4	1.4	0.9	1.8
42	8.1	7.7	10.2	8.5
43	2.4	2.3	1.6	2.1
44	1.8	1.6	3.8	3.3
47	1.8	1.9	2.1	1.9
48	1.5	1.3	1.0	2.0
49	2.4	2.3	1.2	1.7
50	0.9	1.0	1.6	1.6
r*	0.94		0.97	

* Correlation coefficient.

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Abbreviations: (H, G)-A--L, poly(L-histidine, L-glutamic acid)-poly(DL-alanine)--poly(L-lysine); (T, G)-A--L, poly(L-tyrosine, L-glutamic acid)-poly(DL-alanine)--poly(L-lysine); *HLA*, gene for histocompatibility antigen; SI, stimulation index; MHC, major histocompatibility complex.

Table 2. Proliferative response of human lymphocytes stimulated with (H, G)-A--L

(H, G)-A--L, μg/well	High responders: Subject no.			Intermediate responders: Subject no.			Nonresponders: Subject no.		
	45	28	2	31	9	22	15	6	21
0	700	830	3,700	2500	4,300	3,300	1400	1700	420
0.5	1300 (1.9)	400 (0.4)	45,000 (12.1)	3600 (1.4)	12,000 (2.9)	2,000 (0.6)	820 (0.6)	2400 (1.4)	190 (0.4)
2	1400 (2.0)	900 (1.0)	*49,000 (13.0)*	3900 (1.5)	13,000 (3.0)	5,500 (1.7)	2500 (1.7)	1500 (0.8)	*740 (1.7)*
4	1000 (1.4)	1400 (1.6)	33,000 (8.8)	5300 (2.0)	14,000 (3.4)	7,900 (2.4)	2300 (1.6)	*2600 (1.5)*	510 (1.2)
10	2500 (3.5)	430 (0.5)	32,000 (8.5)	6000 (2.3)	14,000 (3.4)	7,700 (2.3)	2400 (1.7)	1300 (0.7)	230 (0.5)
20	3400 (4.8)	560 (0.7)	26,000 (7.0)	5400 (2.1)	*20,000 (4.6)*	9,400 (2.8)	2600 (1.8)	800 (0.4)	390 (0.9)
40	*7000 (10.0)*	1400 (1.7)	9,000 (2.4)	5700 (2.2)	6,100 (1.4)	*12,000 (3.7)*	*3400 (2.3)*	560 (0.3)	430 (1.0)
80	6500 (9.3)	*6300 (7.6)*	6,500 (1.7)	*8800 (3.5)*	4,300 (1.0)	12,000 (3.6)	2000 (1.4)	330 (0.2)	530 (1.2)
100	3500 (5.0)	5200 (6.3)	5,600 (1.5)	8000 (3.1)	3,800 (0.9)	3,300 (1.0)	1900 (1.2)	450 (0.3)	300 (0.7)

Values are given as cpm; SI are in parentheses; peak individual responses are italicized.

***In Vitro* Lymphocyte Stimulation.** Serial dilutions of each antigen were cultured in triplicate with 8–10 × 10^4 lymphocytes in 0.2 ml of RPMI 1640 medium with 10% (vol/vol) human AB serum in round-bottom microtiter plates. On day 7, 1 μCi of [^{3}H]thymidine (specific activity 6.7 Ci/mol; 1 Ci = 3.7 × 10^{10} becquerels) was added to each well. The cultures were terminated 16–19 hr after labeling. Results were expressed as stimulation index (SI) according to the following formula:

$$SI = \frac{\text{cpm of culture in the presence of antigen}}{\text{cpm of culture in the absence of antigen}}$$

The SI were used to classify subjects as high responders, intermediate responders, and nonresponders. Each subject was tested with five to seven serial dilutions of each antigen, covering the optimal range of concentration that was determined for each subject in a standardization procedure. The optimal concentration for most subjects tested was between 48 and 96 μg/well for (T, G)-A--L, but was 80 μg/well for (H, G)-A--L. The concentration of antigen eliciting the maximal response varied among individuals tested. Consequently, it was necessary to test cells of each subject with multiple antigen concentrations. The maximal S.I. obtained for each antigen was used to classify a subject as a high responder, an intermediate responder, or a nonresponder.

Secondary cultures were carried out on cells of seven subjects that did not respond to (T, G)-A--L in the primary culture. Lymphocytes (2 × 10^6) were cultured with 96 μg of (T, G)-A--L for 10 days. On day 10, the cells were centrifuged and resuspended in fresh medium to a final concentration of 8–10 × 10^4 cells per ml. The primed cells were then cultured in microtiter plates in the presence of serial dilutions of (T, G)-A--L for an additional 3 days as described for primary cultures.

RESULTS

Immune Response to Synthetic Antigens by Cells of Unrelated Volunteers. Of the 50 unrelated subjects tested, 32 (64%) responded to (H, G)-A--L and 27 (54%) to (T, G)-A--L. S.I. <2.5 were considered as negative, and such cultures were classified as nonresponders. The SI of nonresponders ranged from 0.3 to 2.5. Individuals whose cells gave SI above 3.0 were classified as responders. Responders fell into two discrete categories: those with mean SI between 3.6 and 4.2 with none lower than 3.0, and those with mean SI above 6.0. We tentatively categorized the former group as intermediate responders and the latter as high responders. Although the number of subjects with each phenotype was too small to analyze statistically, the classifications were confirmed by replicate testing of those subjects from whom a second blood sample could be obtained. The correlation coefficients of SI between duplicate tests with the antigen concentration eliciting maximal response were 0.94 and 0.97 for (H, G)-A--L and (T, G)-A--L, respectively (Table 1).

Tables 2 and 3 show typical dose–response patterns for the three response phenotypes and illustrate that an individual can be a high or an intermediate responder to both antigens or a high responder to one antigen but an intermediate responder or a nonresponder to the other. No secondary restimulation to (T, G)-A--L was observed in the seven (T, G)-A--L nonresponders. Restimulation data of nonresponders to (H, G)-A--L are still incomplete. There was no association between response to a given antigen and a particular *HLA-A, B, C,* or *D (DR)* allele in the unrelated series.

Genetic Control of Immune Response to Synthetic Antigens in Families. Ten nuclear families with three or more offspring were included in the study. Four matings were between nonresponders to both antigens, who produced only nonresponder children, suggesting a dominant mode of inheritance of responsiveness. This mode of inheritance was supported by another four matings in which at least one parent was a responder to either antigen or to both antigens and produced at least one responder child (Figs. 1, 2, and 5).

Anomalous responses in offspring were obtained, however, from two matings between nonresponders who produced at least one responder child (Figs. 3 and 4). These anomalous responses could be due to incomplete penetrance of the trait or lack of antigen exposure in the parents. The latter explanation is rejected because all members of both families had been living in

Table 3. Proliferative response of human lymphocytes stimulated with (T, G)-A--L

(T, G)-A--L, μg/well	High responders: Subject no.			Intermediate responders: Subject no.			Nonresponders: Subject no.		
	45	21	22	31	9	2	15	6	28
0	700	420	3,300	2,600	4,300	3,700	1400	1700	830
6	410 (0.6)	910 (2.2)	*31,000 (9.4)*	4,000 (1.5)	6,100 (1.4)	8,500 (2.3)	*1600 (1.1)*	2100 (1.2)	1300 (1.5)
12	720 (1.0)	2400 (5.8)	23,000 (6.9)	4,700 (1.8)	9,900 (2.3)	13,000 (3.6)	1100 (0.8)	2000 (1.1)	540 (0.7)
24	2,700 (3.8)	*3200 (7.7)*	12,000 (3.5)	11,000 (4.4)	16,000 (3.8)	*15,000 (4.1)*	770 (0.5)	*4200 (2.4)*	590 (0.7)
48	*16,000 (22.2)*	1000 (2.5)	5,000 (1.5)	*12,000 (4.5)*	17,000 (3.9)	12,000 (3.3)	990 (0.7)	2900 (1.6)	540 (0.6)
96	6,300 (8.9)	1100 (2.7)	4,100 (1.3)	8,600 (3.3)	*19,000 (4.3)*	8,700 (2.3)	760 (0.5)	590 (0.3)	*600 (0.7)*

Values are given as cpm; SI are in parentheses; peak individual responses are italicized.

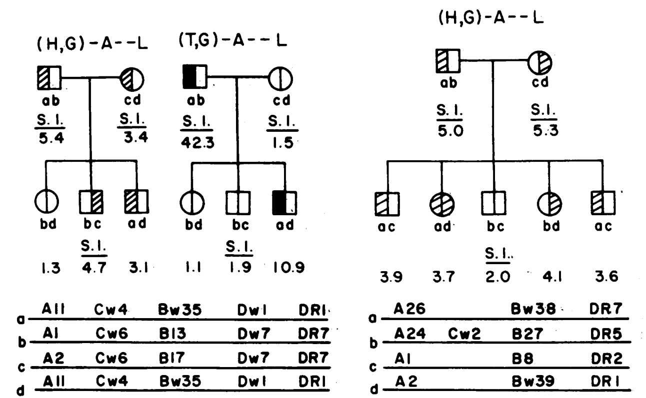

FIG. 1. Pedigrees showing inheritance of *in vitro* lymphoproliferative responses to (T, G)-A--L and (H, G)-A--L as Mendelian dominant traits linked to *HLA*. (*Left*) Family 4222; (*Right*) family 4389. Responses to these two antigens appear to be under separate gene control. ■, High-responder allele; ▨, intermediate responder allele; □, nonresponder allele.

their respective households at the time of our study; thus, the parents would have experienced exposure to antigen at least as great as their children. One mechanism for incomplete penetrance is the absence of an interacting or modifying gene. Inasmuch as studies in experimental animals have shown that response to the synthetic polymers requires interaction of two MHC-linked genes (5), we favor this explanation for the unexpected results.

Genetic linkage between *HLA* and immune response to either (T, G)-A--L or (H, G)-A--L was indicated by six of the families. Family 4222 (Fig. 1 *Left*) is a mating between a (T, G)-A--L high-responder father and a nonresponder mother, but is an intercross mating for intermediate response to (H, G)-A--L. In this family, only the child who inherited the paternal *a* haplotype is a (T, G)-A--L responder, indicating that the response phenotype is a dominant trait. This family further showed that responses to (T, G)-A--L and (H, G)-A--L are not controlled by the same *Ir* gene. The child with *HLA* genotype *bd* is a nonresponder to both antigens. The child with *bc* genotype is nonresponsive to (T, G)-A--L but gives an intermediate response to (H, G)-A--L, whereas the child with *ad* genotype responds to both antigens. Thus, pedigree analysis indicated that response alleles for both (T, G)-A--L and (H, G)-A--L are coupled to the paternal *a HLA* haplotype, and the mother carries an (H, G)-A--L responder gene on her *c* haplotype coupled with a nonresponder allele to (T, G)-A--L.

The segregation pattern of (T, G)-A--L and (H, G)-A--L in family 4389 (Fig. 1 *Right*) also illustrated the distinct genetic control of immune response to these antigens. This family is an intercross mating for both (T, G)-A--L and (H, G)-A--L, and all five children, representing all four possible haplotype combinations, are (T, G)-A--L responders, with S.I. ranging from 5.0 to 19.6. However, as shown, the child with *bc* genotype is a nonresponder to (H, G)-A--L, indicating coupling of the (H, G)-A--L nonresponse allele to the *b* and *c* haplotypes and separate gene control of the two antigens. In this family, one or both parents must be homozygous for (T, G)-A--L responsiveness.

Family 1140 (Fig. 2) suggested that high and intermediate responsiveness may be controlled by allelic genes. This intercross between two high responders to both (T, G)-A--L and (H, G)-A--L produced three high-responder offspring of *HLA* genotypes *ac* and *bc*. A fourth child, with *bd* genotype, was an intermediate responder to both antigens. Our interpretation is that genes for high response are on the *a* and *c* haplotypes and alleles for intermediate response are carried on *b* and *d* or, alternatively, one parent has a nonresponder allele and the *bd* child is a heterozygous (H, G)-A--L intermediate responder/nonresponder.

Evidence for gene complementation in response to (T, G)-A--L and (H, G)-A--L was provided by two families. Fig. 3 (family 347) depicts a mating between two nonresponders. If responsiveness is controlled by a single gene, this mating should produce only nonresponder children. Indeed, two offspring with the *ac* genotype are nonresponders; however, the *bd* child is a high responder to both antigens. This observation is consistent with the finding in the mouse that specific immune response may require the interaction of alleles at two loci mapping within

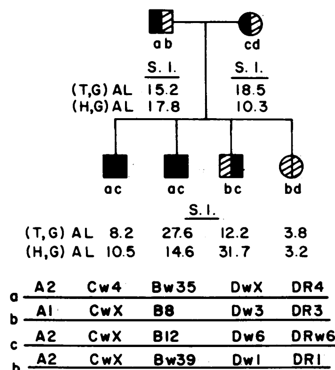

FIG. 2. Family 1140, illustrating that the level of responsiveness may be controlled by allelic genes. ■, High-responder allele; ▨, intermediate-responder allele.

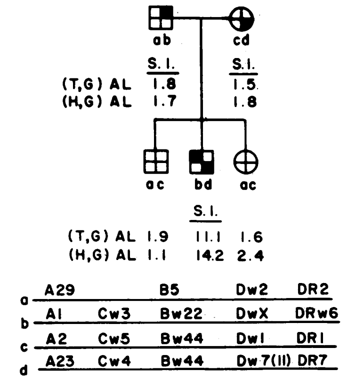

FIG. 3. Family 347, a mating between nonresponders who produced an offspring responsive to both (T, G)-A--L and (H, G)-A--L, indicating that complementing *HLA*-linked genes are required for the *in vitro* lymphoproliferative response. ⊞, *Ir-TGAL-1, Ir-HGAL-1*; ⊕, *Ir-TGAL-2, Ir-HGAL-2*.

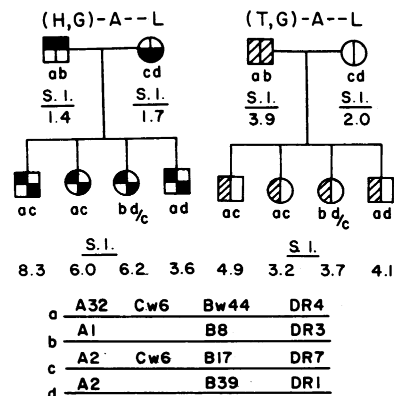

FIG. 4. A second family (family 4427) showing gene complementation with differing patterns of coupling of the relevant alleles for *Ir-TGAL-1* and *Ir-TGAL-2* and *Ir-HGAL-1* and *Ir-HGAL-2*. ⊞, *Ir-HGAL-1*; ⊕, *Ir-HGAL-2*.

the MHC (5–8). We have designated one set of complementing loci as *Ir-TGAL-1* and *Ir-TGAL-2* and the other set as *Ir-HGAL-1* and *Ir-HGAL-2*. In family 347, *Ir-TGAL-1* and *Ir-HGAL-1* are coupled on the paternal *b* haplotype, whereas *Ir-TGAL-2* and *Ir-HGAL-2* are coupled on the maternal *d* haplotype.

To be consistent with the hypothesis of complementing loci, the families depicted in Figs. 1 and 2 must have the responder alleles in coupling for both (T, G)-A--L and (H, G)-A--L.

Family 4427 (Fig. 4) is a mating between two nonresponders to (H, G)-A--L. As in family 347 (Fig. 3), this mating produced responder children. As illustrated, we made the assumption that the father is homozygous for the *HGAL-1+* allele and is a homozygous nonresponder for the complementing *HGAL-2* allele (i.e., *HGAL-2−*). Conversely, his spouse is a *HGAL-2+* homozygote but has the nonresponder allele of *Ir-HGAL-1*. Given this mating type, as expected, all children are responders to (H, G)-A--L because they are *HGAL-1+/HGAL-2+* heterozygotes, in repulsion. For (T, G)-A--L, the father is an intermediate responder and the mother is a nonresponder. All children are intermediate responders. Our assumption is that the father is homozygous for both *TGAL-1* and *TGAL-2* responder alleles. The third offspring in the family is a maternal intra-*HLA* recombinant, but provided no gene mapping information, given the parental genotypes.

Family 4000 (Fig. 5) is an intercross mating between (T, G)-A--L and (H, G)-A--L responder parents. The *ad* child is a nonresponder whereas three offspring of *ac* and one of *bd* genotype are all responders. The *bd* child is a high responder, suggesting further genetic complexity, perhaps analogous to the observations of Melchers and Rajewsky (8) in the mouse. Pedigree analysis in this family is consistent with the coupling of the (T, G)-A--L nonresponse alleles to the *a* and *d* haplotypes whereas the low-response alleles are located on the *b* and *c* haplotypes. The youngest child in this family is a maternal recombinant between the *HLA-B* and *HLA-D* loci (*ad/c*). He inherited the *HLA-A11*, *Cw4*, and *Bw35* specificities of the *d* haplotype with the *DR4* specificity of the *c* haplotype. He is a (T, G)-A--L nonresponder, as is the *ad* sibling. Because the response pattern in this family localized the (T, G)-A--L nonresponse genes to the *d* haplotype, this recombinant offspring provided evidence that the *Ir-TGAL* genes may map closer to *HLA-B* than to *D*. As for (H, G)-A--L, all three *ac* children are (H, G)-A--L nonresponders, thus localizing the response alleles of *Ir-HGAL* to the *b* and *d* haplotypes. This assignment is confirmed by the findings that the *ad* and *bd* children are (H, G)-A--L intermediate responders. The *ad/c* recombinant child is also an intermediate (H, G)-A--L responder, showing, as with (T, G)-A--L, that the *Ir-HGAL* genes map closer to the *HLA-A–B* interval, separate from the *D* region.

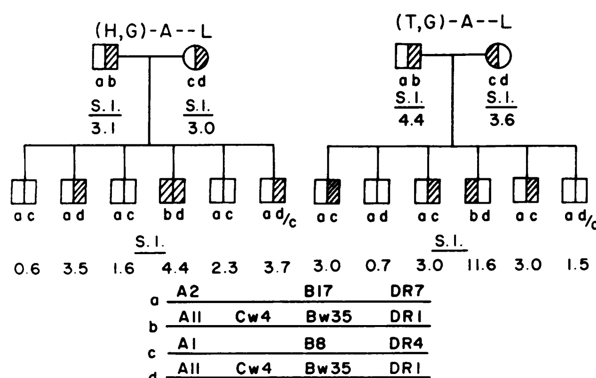

FIG. 5. Family 4000, with a *HLA-B/D* recombinant informative for gene mapping. The inheritance pattern indicates that the *Ir* loci for both (T, G)-A--L and (H, G)-A--L map in the *HLA-A–B* interval, separate from *HLA-D*. ▨, Intermediate-responder allele; □, nonresponder allele.

These six nuclear families do not provide enough data for formal linkage analysis. However, data from these preliminary studies are consistent with linkage of *HLA* and immune response to (T, G)-A--L and (H, G)-A--L and with the requirement of complementation of alleles at two MHC-linked loci as observed in experimental animals (5–8).

DISCUSSION

Hensen and Elferink (9) were unable to obtain responses to (T, G)-A--L with human lymphocytes in primary culture, but did observe proliferation after *in vitro* sensitization with this antigen. The maximal amount of [^{3}H]thymidine uptake they observed after secondary challenge with (T, G)-A--L was only about 4-fold higher than the negative control culture. This small increment in secondary proliferation response was comparable to that of our intermediate responders in primary culture. Inasmuch as they did not present experimental procedures and data for their primary cultures, the apparent discrepancy between their results and ours cannot be explained. The (T, G)-A--L used in both studies is from the same source; in our study, 54% of subjects responded to (T, G)-A--L. This finding is similar to that of Young and Engleman (10), who also observed vigorous primary response in 54% of normal subjects to this polymer. We further observed that 65% of the volunteers were responsive to (H, G)-A--L. Our observed variation of *in vitro* responses of human lymphocytes to (T, G)-A--L and (H, G)-A--L is similar to the early classical *in vivo* responses in laboratory animals that led to the postulation of immune response (*Ir*) genes.

Although we observed concordant inheritance of *HLA* and responsiveness, association between response to (T, G)-A--L or (H, G)-A--L and particular *HLA-A, B, C,* and *D* alleles was not apparent in this study. This may be due to the small sample size of our unrelated series or to the lack of linkage disequilibrium between different immune response genes and particular *HLA* alleles. Because responsiveness is a frequent phenomenon and our unrelated panel was selected to include all *HLA-A, B, C,* and *D* alleles, the latter possibility is more likely.

In our experiments we ruled out the possibility that the lymphoproliferative responses to these synthetic antigens were attributable to contamination of the synthetic antigen preparations with other mitogenic material. Prior to testing in human lymphocytes, the copolymers were cultured with spleen cells of unimmunized mice of both responder and nonresponder strains. There were no proliferative responses.

Our experiments indicate that the *in vitro* response of human lymphocytes is a secondary, rather than a primary, response. This conclusion results from the failure of cord blood lymphocytes from 12 newborns to respond to any of the synthetic antigens. Our assumption is that each of the synthetic polypeptides shares one or more epitopes with natural environmental antigenic substances. Support for the hypothesis that human lymphocytes possess receptors for the synthetic polymers comes from the observations of Taussig (11). He showed that human lymphocytes were able to absorb mouse T-cell helper factor specific for (T, G)-A--L and (Phe, G)-A--L. In an attempt to explain this finding, he proposed that human lymphocytes possess acceptors for these antigens. His family studies showed that the acceptor phenomenon was inherited as a dominant trait linked to *HLA*.

In our studies, the dominant inheritance of response to (T, G)-A--L and (H, G)-A--L was supported by the segregation of both responder and nonresponder offspring from intercrosses between responder parents and between matings of responders and nonresponders. Furthermore, the responder and nonre-

sponder alleles segregated with appropriate *HLA* haplotypes. It is clear from our studies in unrelated subjects and in families that a high responder to one antigen may be an intermediate responder or a nonresponder to the other antigen. This indicated that different genes control immune response to each synthetic antigen. In our families, *HLA* identical siblings were invariably concordant for response phenotype. This suggested that there are multiple alleles of *Ir-TGAL* and *Ir-HGAL* that control the levels of responsiveness, with a hierarchy of dominance.

Ir gene complementation in antibody response to (T, G)-A--L, (H, G)-A--L, Glu-Lys-Leu, Glu-Lys-Phe, and lactate dehydrogenase B has been reported for both mice and rats (6, 8, 12). MHC-linked complementation was also observed in lymphocyte proliferative response to (T, G)-A--L and Glu-Lys-Phe in the mouse (13) and to (H, G)-A--L in the rat (12). In both species, gene complementation in cellular responses was demonstrated in F_1 hybrids from crosses made between nonresponder congenic strains or between low responder and nonresponder strains. Most of the F_1 progeny were high responders, but intermediate responders were also obtained.

Data from the families depicted in Figs. 3 and 4 were consistent with complementation between at least two *HLA*-linked genes controlling responsiveness to (T, G)-A--L and (H, G)-A--L. In Fig. 5, a mating between nonresponders, the responder alleles of loci designated *Ir-TGAL-1* and *Ir-HGAL-1* are syntenic on the paternal *b* haplotype whereas the maternal *Ir-TGAL-2* and *Ir-HGAL-2* alleles are coupled on the *d* haplotype. The parents each have nonresponder alleles at the complementing loci. This gene arrangement permits responsiveness to both antigens only in the *bd* genotype.

In Fig. 6, the parents were (H, G)-A--L nonresponders but all children were responders. This pedigree is consistent with the hypothesis that the father is homozygous for one (H, G)-A--L responder locus and the mother is homozygous for the other. They can produce only responder children heterozygous for both *Ir-HGAL-1* and *Ir-HGAL-2*.

Ir-1, the first immune response locus, was recognized because of the differential response of certain mouse strains to (T, G)-A--L and (H, G)-A--L (14, 15). Intra-*H2* recombinations demonstrated that *Ir-1* belonged to a region mapping between, but separate from, *H2-K* and *H2-D* that contained multiple discrete immune response loci. The *HLA-D* region of man is considered to be the homologue of the *I* region of mouse inasmuch as both control allogeneic lymphoproliferative responses and contain genes encoding membrane antigens with structural homologies that are expressed preferentially on B lymphocytes.

Chromosomal rearrangement during phylogeny that could be explained by a simple transposition (Fig. 6) has resulted in the localization of the mouse *I* region between *H2-K* and *H2-D*, whereas *HLA-D* lies outside the homologous *HLA-A* and *HLA-B* regions. Our data and Taussig's (11) suggest that such rearrangement may have resulted in the localization of genes with similar functions in nonhomologous regions. In an informative *HLA-A/C* recombinant family, Taussig mapped the acceptor gene(s) for (T, G)-A--L close to the *A* locus and the acceptor gene(s) for (Phe, G)-A--L to the *C* side of the recombination site. Similarly, in our intra-*HLA* recombinant family (Fig. 5), genes regulating *in vitro* responsiveness to (T, G)-A--L and (H, G)-A--L map in the *HLA-A-B* interval rather than in *HLA-D*. This recombinant family also indicated that the complementing loci for each antigen map in the same interval.

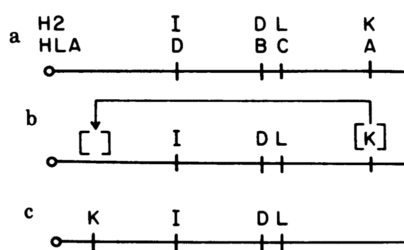

FIG. 6. Postulated gene rearrangement during phylogeny. In this scheme *H2-K* is the homologue of *HLA-A* and was transposed to a position left of *I*. This assumes that the human MHC sequence is the prototype, an assumption based on similar gene arrangements found in rhesus monkey, rat, guinea pig, and dog. (*a*) Prototype MHC; (*b*) transposition in *H2*; (*c*) modern mouse *H2*.

These data of *in vitro* lymphoproliferative response to synthetic polypeptides in cells from 50 unrelated subjects possessing all known *HLA-A, B, C,* and *D* specificities and from inheritance patterns in 10 families provide evidence that (*i*) there are *HLA*-linked genes homologous to those observed in experimental animals that control immune response to these antigens; (*ii*) the *in vitro* responses are not primary responses but, rather, represent recall responses to epitopes on "natural" antigens shared by the copolymers; (*iii*) the human *Ir* genes are polymorphic, with different alleles controlling the level of responsiveness; and (*iv*) the response to a single synthetic antigen requires complementation of at least two *HLA*-linked genes. The data also suggest that different combinations of alleles at the complementing loci may determine particular levels of responsiveness. Extension of these studies to include other structurally defined antigens should provide the data required to elucidate gene organization and function within the human MHC.

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