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# Genetic control of major histocompatibility complex-linked immune responses to synthetic polypeptides in man: Poly(L-phenylalanine, L-glutamic acid)-poly(DL-alanine)--poly(L-lysine) and L-glutamic acid, L-alanine, L-tyrosine (60:30:10)

**(human heteropolymer stimulation/*Ir* gene complementation)**

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ABSTRACT **Vigorous lymphoproliferative responses to synthetic polypeptides poly(L-phenylalanine, L-glutamic acid)-poly(DL-alanine)--poly(L-lysine) [(Phe,G)-A--L], and L-glutamic acid, L-alanine, L-tyrosine (60:30:10) (GAT) were observed in cells from 92 unrelated subjects. Thirty-three percent responded to (Phe,G)-A--L and 77% to GAT. No HLA association was observed with responses to these two antigens. Family studies indicated that two complementary immune response (*Ir*) genes are required for response to each antigen. Eleven matings were informative for linkage analysis between *HLA* and these *Ir* genes. Families in which the complementary genes are in coupling gave maximal lod scores (log of the odds) of 4.50 for (Phe,G)-A--L and 7.57 for GAT for $\theta = 0$. In a *HLA-B/D* recombinant family, the *Ir-PheGAL* genes are mapped towards the *HLA-D* region. The localization of *Ir-GAT* genes close to *HLA-B* was provided by a *HLA-A/B* recombinant.**

Major histocompatibility complex (MHC)-linked immune response (*Ir*) genes encoding recognition of various synthetic polypeptide antigens and native proteins have been well-documented in laboratory animal species (1, 2). In 1981 we reported similar *Ir* genes in man (3). We demonstrated that human peripheral blood lymphocytes proliferated vigorously to *in vitro* challenge with the synthetic polypeptides poly(L-histidine, L-glutamic acid)-poly(DL-alanine)--poly(L-lysine) [(H,G)-A--L] and poly(L-tyrosine, L-glutamic acid)-poly(DL-alanine)--poly-(L-lysine) [(T,G)-A--L], without prior *in vivo* immunization, indicating that the responses are secondary rather than primary. Family studies showed that, as in experimental animals, responsiveness is controlled by two MHC-linked complementary loci. Except for the matings in which the complementary genes are in repulsion, inheritance follows a Mendelian dominant mode. The study reported here extends and substantiates these findings by using two other synthetic antigens, poly(L-phenylalanine, L-glutamic acid)-poly(DL-alanine)--poly-(L-lysine) [(Phe,G)-A--L] and L-glutamic acid, L-alanine, L-tyrosine (GAT). As with (T,G)-A--L and (H,G)-A--L, lymphoproliferative responses to these two antigens are controlled by *HLA*-linked complementary genes that we have designated as *Ir-PheGAL-1*, *Ir-PheGAL-2* and *Ir-GAT-1*, *Ir-GAT-2*. One family with an intra-*HLA* recombination between the *HLA-B* and *D* loci was informative for gene localization for (Phe,G)-A--L and one with a recombinant between the *HLA-A* and *B* loci was informative for GAT.

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## MATERIALS AND METHODS

**Subjects.** Cell donors were 92 healthy, unrelated volunteers selected to include all known *HLA-A*, *-B*, *-C* and *DR* specificities and members of 35 nuclear families with 3 or more children. Eight of the families had an offspring who carried an intra-*HLA* recombinant haplotype.

**Antigens.** Synthetic polymers (Phe,G)-A--L (lot MP1, $M_r$ 260,000) and GAT (lot GAT 12, $M_r$ 46,200) were obtained from Miles–Yeda (Elkhart, IN). Both antigens were readily soluble in RPMI 1640 medium. Before use, the pH of each antigen solution was adjusted to 7.2 and the solution was filter-sterilized (Millipore, 0.45 μm). As described previously (3), each antigen preparation was tested for contamination with mitogenic material by coculturing with human cord blood lymphocytes and with spleen cells from unimmunized female mice of nonresponder and high-responder strains.

***In Vitro* Lymphocyte Stimulation.** Human peripheral blood lymphocytes were isolated by the standard Ficoll-Hypaque gradient technique. Varying concentrations of each antigen were cultured in triplicate with $8 \times 10^4$ peripheral blood lymphocytes in 0.2 ml of RPMI 1640 medium with 10% (vol/vol) human group AB serum, in round-bottom microtiter plates (Linbro). On day 7, 1 μCi of [$^3$H]thymidine (specific activity, 6.7 Ci/mmol; 1 Ci = 37 GBq) was added to each well. The cultures were harvested 16–18 hr after labeling and samples were assayed for radioactivity in a Beckman model LS 7000 liquid scintillation counter. Results were expressed as the stimulation index (SI): SI = mean cpm of culture with antigen/mean cpm of culture without antigen.

Each subject was tested at least twice with seven serial dilutions of each antigen. As previously reported, the antigen concentration eliciting maximal response varied from individual to individual (3). For the majority of subjects tested, the optimal antigen concentration was 80 μg per well for both (Phe,G)-A--L and GAT. The mean maximal SI obtained for each antigen was used to classify the subjects into responder and nonresponder groups. Subjects with SI >3 were considered as responders, in accordance with accepted procedures (4–6). There was a small number of subjects [12 for (Phe,G)-A--L and 9 for GAT] whose results were repeatedly borderline—i.e., between 2.5 and 3.0. Cells of these subjects were classified as nonresponders on the basis of negative results of secondary stimulation. The secondary cultures were carried out as follows: After primary antigen stimula-

Abbreviations: (Phe,G)-A--L, poly(L-phenylalanine, L-glutamic acid)-poly(DL-alanine)--poly(L-lysine); GAT, L-glutamic acid, L-alanine, L-tyrosine (60:30:10); (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); SI stimulation index; MHC, major histocompatibility complex.

Table 1. Distribution of (Phe,G)-A--L phenotypes in 35 Caucasian families

| Mating ($n$ = 35)* | | | Offspring ($n$ = 175) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Responder | | Nonresponder | | | |
| Type | Observed | Expected | Observed | Expected | Observed | Expected | $\chi^2$ | $P$ |
| Responder × responder | 6 | 4 | 17 | 28 | 18 | 7 | 21.29 | 0.000003 |
| Responder × nonresponder | 9 | 15 | 18 | 21 | 20 | 17 | 0.88 | >0.25 |
| Nonresponder × nonresponder | 20 | 16 | 0 | 0 | 102 | 102 | | |
| Total | 35 | 35 | 35 | 49 | 140 | 126 | | |

*$\chi^2$ = 5.12; $0.05 < P < 0.10$.

tion for 10–12 days, the cultured cells were centrifuged, washed, resuspended in fresh medium, and cultured with antigen at various concentrations for an additional 3 days. No significant proliferative response was observed in any of the borderline cultures as compared to cultures from responder individuals.

## RESULTS

**Responses by Unrelated Volunteers.** Thirty-three percent of the subjects (30/92) responded to (Phe,G)-A--L with SIs ranging from 3 to 22.4 (mean SI, 6.8) as compared to 77% for GAT (71/92) with SIs from 3 to 27.6 (mean SI, 7.4). The SIs for (Phe,G)-A--L nonresponders were from 0.2 to 2.8 with a mean of 1.1 and those for GAT nonresponders were from 0.5 to 2.8 with a mean of 1.8. As was demonstrated for (T,G)-A--L and (H,G)-A--L (3), replicate experiments carried out on different days with different blood samples were highly correlated. For the 92-cell panel the correlation coefficient was 0.93 for (Phe,G)-A--L and 0.89 for GAT. Also as previously shown (3), individuals showed selective responsiveness—i.e., some subjects responded to only one antigen, whereas others responded to both. When results were analyzed according to age, sex, and *HLA* specificities, no statistically significant association was observed.

**Family Studies.** *(Phe,G)-A--L.* The distribution of response phenotypes by mating type and among the 175 children of the 35 families is shown in Table 1. In the parental sample of 70 there were 49 nonresponders. Based on the phenotype frequencies of the unrelated sample, 47 would be expected. The $\chi^2$ test for goodness-of-fit showed that the deviation from expectation was no greater than would occur by chance.

It was observed that, in this sample, matings between (Phe,G)-A--L nonresponders produced only nonresponder children and matings in which one or both parents were responders produced at least 1 responder child. Therefore, $\chi^2$ analysis was carried out to test whether the distribution of phenotypes among the offspring conformed to autosomal dominant inheritance. As shown in Table 1, there was a highly significant excess of nonresponder offspring from responder × responder matings. Only 7 were expected but 18 were observed.

Eleven informative matings indicated genetic linkage between *HLA* and responsiveness to (Phe,G)-A--L. In 4 of the matings responsiveness segregated as a Mendelian dominant trait linked to *HLA* (Fig. 1*a*). In the other 7 families, *HLA*

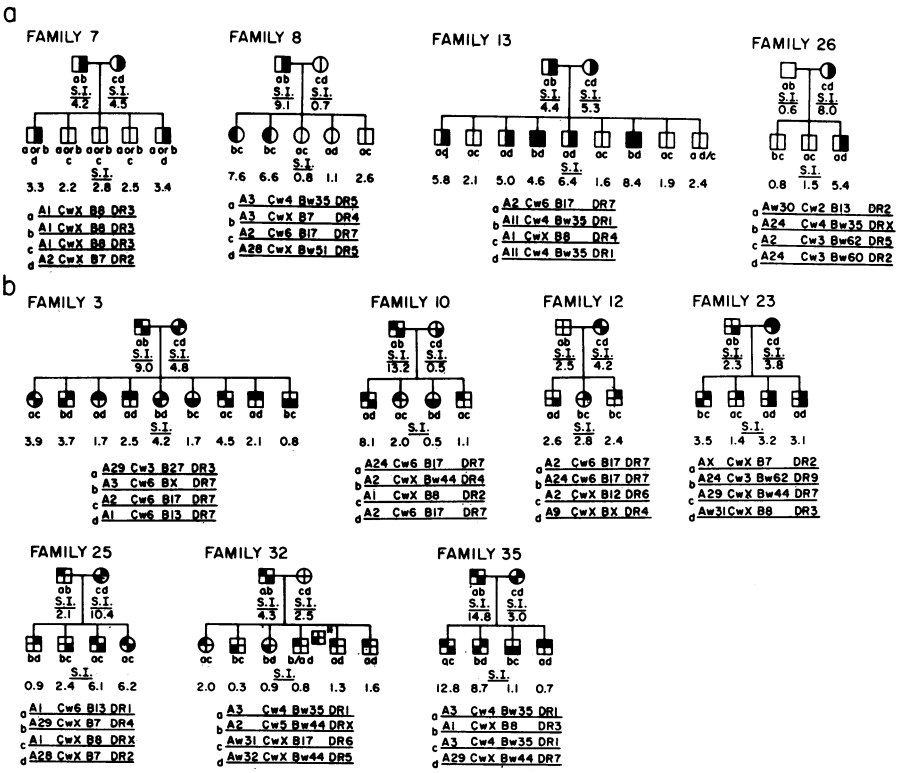


FIG. 1. Pedigrees informative for genetic linkage between *HLA* and *in vitro* lymphoproliferative response to (Phe,G)-A--L. (*a*) Families showing responsiveness segregated as an *HLA*-linked Mendelian dominant trait. ■, Responder allele; □, nonresponder allele. (*b*) Families indicating complementary *HLA*-linked genes are required for response ⊞, *Ir-PheGAL-1*; ⊟, *Ir-PheGAL-2*.

Table 2. Lod scores for linkage between *HLA* and *Ir-PheGAL*

| Family | Recombination fraction ($\theta$) 0.00 | 0.05 | 0.10 | 0.20 | 0.30 | 0.40 |
|---|---|---|---|---|---|---|
| | Complementing loci in coupling | | | | | |
| 7 | 0.87 | 0.76 | 0.67 | 0.47 | 0.27 | 0.08 |
| 8 | 1.20 | 1.09 | 0.98 | 0.72 | 0.44 | 0.15 |
| 13 | 1.83 | 1.63 | 1.42 | 0.99 | 0.55 | 0.16 |
| 26 | 0.60 | 0.54 | 0.47 | 0.32 | 0.17 | 0.05 |
| Total | 4.50 | 4.02 | 3.54 | 2.50 | 1.43 | 0.44 |
| | Complementing loci in repulsion | | | | | |
| 3 | −29.09 | −2.51 | −1.52 | −0.70 | −0.30 | −0.08 |
| 10 | −7.10 | −0.46 | −2.23 | −0.06 | −0.01 | −0.00 |
| 12 | −7.40 | −0.72 | −0.44 | −0.19 | −0.08 | −0.02 |
| 23 | −7.10 | −0.46 | −0.23 | −0.06 | −0.01 | −0.00 |
| 25 | −7.10 | −0.46 | −0.23 | −0.06 | −0.01 | −0.00 |
| 32 | −14.80 | −1.44 | −0.89 | −0.39 | −0.15 | −0.04 |
| 35 | −14.85 | −1.49 | −0.93 | −0.42 | −0.17 | −0.04 |
| Total | −87.44 | −7.54 | −4.24 | −1.88 | −0.73 | −0.18 |

linkage was conditional on the assumption that the two complementing *Ir* genes were in repulsion in one or both parents and the coupling phase of each *Ir* gene with the appropriate *HLA* haplotype could be presumed from the phenotype of the offspring (Fig. 1*b*).

The LIPED computer program (7) was used to test for linkage with *HLA*. Table 2 (top) gives the lod scores (log of the odds) (sexes combined) for the 4 families in which the complementary genes appeared to be in coupling. The maximal lod scores of 4.50 were obtained at a recombination fraction ($\theta$) of 0. This means that the odds favoring linkage with no recombination are >10,000:1. As expected, there was no evidence for linkage in those families with the complementary genes in repulsion (Table 2, bottom).

Although 8 intra-*HLA* recombinant families were studied, only 1 provided information for localization of the (Phe,G)-A--L *Ir* genes. Family 13 (Fig. 1*a*) is an intercross mating between (Phe,G)-A--L responders. The children of genotypes *ad* and *bd* are responders, whereas the 3 *ac* offspring are nonresponders; thus, the responder genes were assigned to haplotypes *b* and *d*. The youngest child was a maternal recombinant who inherited *HLA-A11*, *-Cw4*, *-Bw35* of the *d* haplotype with the *DR4* of the *c* haplotype. Inasmuch as he was a nonresponder—i.e., he had the phenotype of his *ac* sibs rather than the *ad* sibs—the *Ir-PheGAL* genes can be assigned to a region proximal to *HLA-B*, toward the *HLA-D* region.

*GAT.* The same 35 families were tested for responsiveness to GAT. Fifteen of the 70 parents were nonresponders, a frequency of 21% as compared to 23% in the unrelated series, an insignificant difference. Again, the responder–nonresponder frequencies of the unrelated series were used to calculate goodness-of-fit of the mating types and no significant deviation was observed (Table 3). As with (Phe,G)-A--L, nonresponder × nonresponder matings produced only nonresponder offspring, indicating that responsiveness may be a simple dominant trait. Indeed, unlike (Phe,G)-A--L, the phenotypes of offspring from the other two mating types did not deviate significantly from expectation (Table 3). Nevertheless, under the assumption that the *Ir* genes are localized within the MHC, pedigree analysis required the assignment of complementing loci in the repulsion phase in 3 of the 11 informative matings. The 8 matings in coupling phase are shown in Fig. 2*a*, whereas those in repulsion phase are shown in Fig. 2*b*. Analysis of linkage between *HLA* and GAT *Ir* genes in coupling gave maximal lod scores of 7.57 for $\theta = 0$ (Table 4, top). Those presumed to be in repulsion, as expected, gave negative lod scores (Table 4, bottom).

Information on the localization of *Ir-GAT* genes was provided by the *HLA-A/B* recombinant in family 24 (Fig. 2*b*). Family 24 is a mating between a responder father and a nonresponder mother. The *bc* child is a nonresponder, whereas the *ac* and *bd* offspring are responders. The second child who is a maternal recombinant between *HLA-A* and *HLA-B* loci (*bc/d*) has the new maternal haplotype *A2*, *Bw44*, *DR4*. She is a responder like her *bd* sib. To explain this response pattern, we assume that the father has both *Ir-GAT-1* and *Ir-GAT-2* on his *a* haplotype but only the *GAT-1* responder allele on his *b* haplotype. His spouse has only the *GAT-2* responder allele on her *d* haplotype but both nonresponder alleles on her *c* haplotype. As a result, the recombinant and her *bd* sib are responders since they are *Ir-GAT-1+/Ir-GAT-2+* heterozygotes. The response pattern of the recombinant child suggested that the *Ir-GAT* genes are proximal to *HLA-A*. Unfortunately, family 13 with the *HLA-B/D* recombination was uninformative because all members were GAT responders.

## DISCUSSION

In 1981, using an *in vitro* lymphoproliferative assay, we demonstrated that human lymphocytes responded to synthetic polypeptides (T,G)-A--L and (H,G)-A--L without any deliberate immunization (3). Our family studies (3) supported *HLA* linkage if one assumed gene complementation, which we believed to be a tenable hypothesis inasmuch as it had been well-documented in the mouse. A *HLA-B/D* recombinant localized *Ir-TGAL-1*, *Ir-TGAL-2*, *Ir-HGAL-1*, and *Ir-HGAL-2* distal to *HLA-D* (3). In a later study, a family with a *HLA-A/B* recombinant gave further evidence that the *Ir-TGAL* and *Ir-HGAL* genes are distal to *HLA-B*, toward the *HLA-A* region (unpublished data).

In the present study, we have observed vigorous primary responses to synthetic antigens (Phe,G)-A--L, and GAT. Of the unrelated sample of 92, 33% were responders to (Phe,G)-A--L. This frequency was not significantly different from that of the parents in 35 matings. The combined sample of 162 had a responder frequency of 31%. The GAT responder frequency of the sample of 92 was 77%, whereas that of the random plus parental sample was 78%. Inasmuch as all mat-

Table 3. Distribution of GAT phenotypes in 35 Caucasian families

| Mating ($n$ = 35)* | | | Offspring ($n$ = 175) Responder | | Nonresponder | | | |
|---|---|---|---|---|---|---|---|---|
| Type | Observed | Expected | Observed | Expected | Observed | Expected | $\chi^2$ | $P$ |
| Responder × responder | 23 | 21 | 99 | 98 | 12 | 13 | 0.10 | >0.75 |
| Responder × nonresponder | 9 | 12 | 23 | 28 | 19 | 14 | 1.54 | >0.10 |
| Nonresponder × nonresponder | 3 | 2 | 0 | 0 | 22 | 22 | | |
| Total | 35 | 35 | 122 | 126 | 53 | 49 | | |

*$\chi^2 = 1.89$; $0.25 < P < 0.5$.

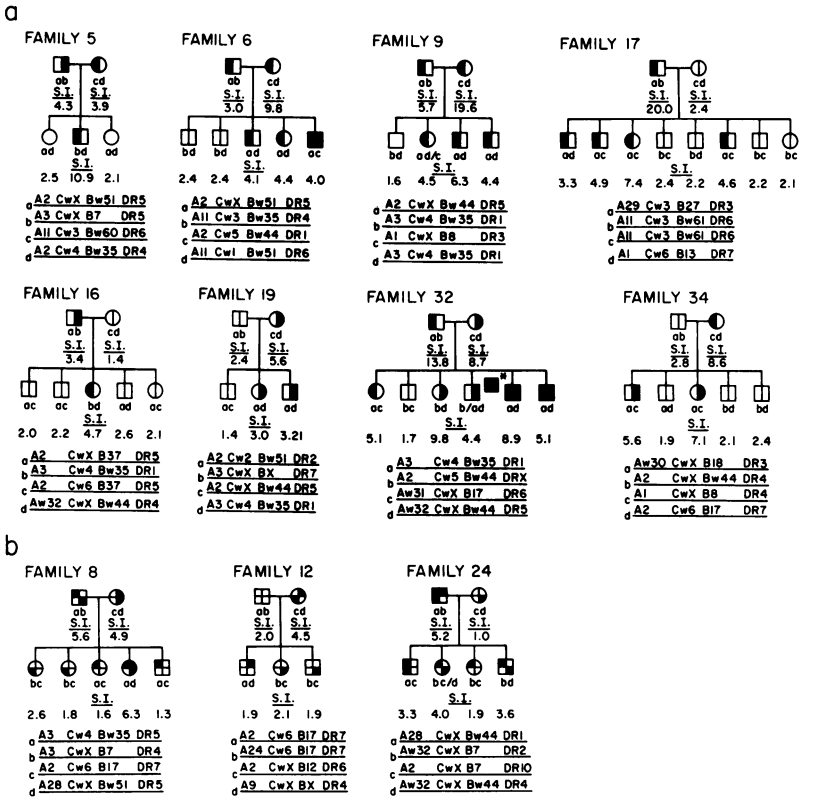


FIG. 2. Pedigrees informative for genetic linkage between *HLA* and *in vitro* lymphoproliferative response to GAT. (*a*) Families showing responsiveness segregated as an *HLA*-linked Mendelian dominant trait. ■, Responder allele; □, nonresponder allele. (*b*) Families indicating complementary *HLA*-linked genes are required for response ⊞, *Ir-GAT-1*; ⊞, *Ir-GAT-2*.

ings between nonresponders produced only nonresponder offspring, responses to both antigens were tested for fit to a Mendelian dominant mode of inheritance. In (Phe,G)-A--L responder × responder matings there was a significant excess of nonresponder children ($\chi^2 = 21.29$, $P = 0.000003$) (Table 1). In contrast, the inheritance of GAT responses conformed to dominant inheritance for all mating types, although there was a slight excess of nonresponder offspring from responder × nonresponder matings (Table 3). We believe that the finding of an excess of (Phe,G)-A--L nonresponder offspring from responder × responder matings supports the hypothesis of complementary genes rather than inadequate *in vivo* exposure to antigen because (*i*) all subjects were >10 years of age; (*ii*) 15 of the 19 families informative for either or both antigens were Old Order Amish, who are noted for a particularly uniform lifestyle; and (*iii*) all members of non-Amish families resided in the same household. Furthermore, in the (T,G)-A--L, (H,G)-A--L study, we have nonresponders who produced responder children. The exposure of the parents should be equal to, if not greater than, that of their responder children.

Table 4. Lod scores for linkage between *HLA* and *Ir-GAT*

| | Recombination fraction ($\theta$) | | | | | |
|---|---|---|---|---|---|---|
| Family | 0.00 | 0.05 | 0.10 | 0.20 | 0.30 | 0.40 |
| | Complementing loci in coupling | | | | | |
| 5 | 0.73 | 0.62 | 0.51 | 0.31 | 0.14 | 0.03 |
| 6 | 0.98 | 0.85 | 0.71 | 0.45 | 0.22 | 0.06 |
| 9 | 0.25 | 0.19 | 0.14 | 0.06 | 0.02 | 0.00 |
| 16 | 1.20 | 1.09 | 0.98 | 0.72 | 0.44 | 0.15 |
| 17 | 2.11 | 1.93 | 1.74 | 1.33 | 0.87 | 0.35 |
| 19 | 0.60 | 0.54 | 0.47 | 0.32 | 0.17 | 0.05 |
| 32 | 0.50 | 0.42 | 0.34 | 0.20 | 0.09 | 0.02 |
| 34 | 1.20 | 1.09 | 0.98 | 0.72 | 0.44 | 0.15 |
| Total | 7.57 | 6.73 | 5.87 | 4.11 | 2.39 | 0.81 |
| | Complementing loci in repulsion | | | | | |
| 8 | −13.77 | −0.51 | −0.06 | +0.21 | +0.20 | +0.08 |
| 12 | −7.40 | −0.72 | −0.44 | −0.19 | −0.08 | −0.02 |
| 24 | −14.80 | −1.44 | −0.89 | −0.39 | −0.15 | −0.04 |
| Total | −35.97 | −2.67 | −1.39 | −0.37 | −0.03 | −0.02 |

Of the 4 (Phe,G)-A--L intercross matings (Fig. 1), the parents in 2 are responders by virtue of complementarity. Six of their 13 children are responders, not significantly different from the expected 1:1 ratio. Of the 14 offspring of the 2 matings in which the complementary genes appeared to be in coupling (Fig. 1*a*), 7 are responders, which is not significantly different from the expected 3:1 ratio. The data from this small group of families suggest that the responder alleles of the complementary genes occur in coupling or repulsion with approximately equal frequencies. In contrast, since the population frequency of GAT responsiveness is 77%, it is to be expected that a greater proportion of haplotypes would carry the complementary alleles in coupling. Indeed, of the 30 parental haplotypes whose GAT coupling phase could be assigned, 21 (70%) appeared to be in coupling (Fig. 2*a*). It is, thus, not surprising that GAT responsiveness appeared to be inherited as a simple dominant trait.

There were 25 families with 2 *HLA* identical sibs, 9 families with 3 *HLA* identicals, and 1 with 4, a total of 35 opportunities for discordance of responsiveness. In all instances, *HLA* identical sibs were invariably concordant for response phenotype. If responsiveness required the interaction of non-*HLA* genes, some *HLA* identical sibs should have been discordant. An alternative to intra-*HLA* complementary loci consistent with these data is that there is an *Ir* locus and an *Is* locus controlling responsiveness. Even in the presence of appropriate *Ir* alleles the $Is^+$ allele produced suppressor cells

that prevent response. (In principle, this does not differ from two complementing loci.)

Eleven matings were informative for analysis of linkage between *HLA* and each antigen (Figs. 1 and 2). Of the 24 uninformative families for (Phe,G)-A--L, 20 had only nonresponder offspring and 3 had only responder offspring. The fourth was uninformative because both parents were *HLA* homozygous. The uninformative GAT families included 3 in which all children were nonresponders, 19 in which all children were responders, and 2 with *HLA* homozygous parents. The sum of the lod scores for the 4 matings in which *Ir-PheGAL-1* and *IrPheGAL-2* were in coupling phase was 4.50 for $\theta = 0$. The similar sum for the 8 GAT matings in coupling was 7.57 for $\theta = 0$. As expected, the lod scores were negative for the repulsion phase, regardless of recombination fraction.

In an informative *HLA-A/C* recombinant family, Taussig (8), using a mouse helper factor to (Phe,G)-A--L, mapped acceptor gene(s) for (Phe,G)-A--L to the *B-D* end of the recombination site. Similarly, in our *HLA-B/D* recombinant family (Fig. 1*a*), genes regulating *in vitro* responsiveness to (Phe,G)-A--L are mapped toward the *HLA-D* region. Information on the localization of *Ir-GAT* genes was provided by a *HLA-A/B* recombinant (Fig. 2*b*). These genes mapped with the *B-D* segment of the MHC. Informative *B/D* recombinants were not available to determine whether the *Ir-GAT* genes are nearer to *HLA-D* than to *B*.

Inasmuch as the *Ir-TGAL* and *Ir-HGAL* genes mapped toward the *HLA-A* region, whereas *Ir-PheGAL* and *Ir-GAT* are proximal to *HLA-B*, we conclude that there are at least two *I* regions controlling immune responsiveness to various antigens in man. This is in contrast to the mouse, in which *Ir* genes appear to be clustered in one region of the MHC. This disparity with mouse is not surprising, however, because, in addition to *HLA-D*, genes controlling secondary mixed lymphocyte reaction have been assigned to the *HLA-A* region (9, *) and to *SB*, a region at least two centimorgans proximal to *HLA-D* (10). Furthermore, in an *HLA-A/C* recombinant family, Taussig (8) localized the genes for mouse (T,G)-A--L helper factor toward *HLA-A*.

In summary, these data confirm our previous report of *HLA*-linked *Ir* genes in man homologous to those observed in experimental animals. Responsiveness to (Phe,G)-A--L and GAT is controlled by complementary genes that map proximal to *HLA-A*, toward the *HLA-B-D* region, whereas those controlling (T,G)-A--L and (H,G)-A--L responses map distal to *HLA-B*, toward *HLA-A*.

*Quintieri, F. B., Sheehy, M. J. & Yunis, E. J., Eighth American Association for Clinical Histocompatibility Testing Annual Meeting, Apr. 25, 1982, San Francisco, abstr. A-12.

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1. McDevitt, H. O. & Benacerraf, B. (1969) *Adv. Immunol.* **11,** 31–74.
2. Benacerraf, B. & Katz, D. H. (1975) *Adv. Cancer Res.* **21,** 121–173.
3. Hsu, S. H., Chan, M. M. & Bias, W. B. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 440–444.
4. Kaplan, J. G. & Bona, C. (1974) *Exp. Cell Res.* **88,** 388–394.
5. Gunther, E. & Rude, E. (1976) *Immunogenetics* **3,** 261–269.
6. Merryman, C. F., Maurer, P. H., Lai, C. H. & Zeiger, A. R. (1979) *Immunogenetics* **9,** 183–192.
7. Ott, J. (1974) *Am. J. Hum. Genet.* **26,** 588–597.
8. Taussig, M. J. (1978) in *Ir Genes and Ia Antigens*, ed. McDevitt, H. O. (Academic, New York), pp. 493–501.
9. Eijsvoogel, V. P., van Rood, J. J., du Toit, E. D. & Schelekens, P. T. A. (1972) *Eur. J. Immunol.* **2,** 413–418.
10. Shaw, S., Pollack, M. S., Payne, S. M. & Johnson, A. H. (1980) *Hum. Immunol.* **1,** 177–185.