# THE IMMUNOGENICITY OF HUMAN HL-A HAPLOTYPES AS MEASURED BY SKIN GRAFT SURVIVAL TIMES AND MIXED LEUKOCYTE REACTIONS*

By H. F. SEIGLER, M.D., F. E. WARD, PH.D., D. B. AMOS, M.D., M. B. PHAUP, AND D. L. STICKEL, M.D.

*(From the Departments of Surgery, Microbiology, and Immunology of the Duke University Medical Center and the Durham Veterans' Administration Hospital, Durham, North Carolina 27706)*

(Received for publication 24 September 1970)

The HL-A antigens, with the possible exception of the ABO blood group antigens, are proposed to be the most potent transplantation antigens in man (1, 2). The evidence for this is indirect and rests mainly on the differences in skin graft rejection time and the behavior of renal allografts and bone marrow allografts between family members differing at one, two, or neither HL-A haplotypes (3), on the failure of leukocytes from HL-A–identical siblings to stimulate in the mixed leukocyte reaction (MLR)[1] test (4), and accelerated skin graft rejection in subjects preimmunized against antigens including a designated HL-A specificity (5). It has been supposed that the HL-A determinant is divided into at least two subloci (6). At one point it was thought that the first antigens to be detected serologically would prove to be the antigens most responsible for graft rejection. This has not proven to be correct.

In the analogous situation in the mouse, where it was possible to test certain isolated specificities, incompatibility for what was thought to be a single specificity correlated with delayed chronic skin graft rejection, and was easily overridden by enhancing antiserum. Klein suggested that the potency of complex antigenic systems such as H2 results from cumulative effects of incompatibility for many specificities (7). In the rat, however, Silvers could not distinguish incompatibility for Ag-B against a background of incompatibility for many non–Ag-B factors (8). In the mouse, Andrus also could not distinguish the immunogenicity for alleles b, k, d, or q against a background of non-H2 antigens (9). A distinction between Ag-B and non–Ag-B could be made in the rat after mild immunosuppression (8), and in the mouse the H2 alleles seemed to be unequal in their effect in skin graft rejection when non-H2 differences were eliminated (10). In the absence of data from immunosuppressed matched human subjects, evidence must be sought from a large series of test skin grafts between care-

* Supported in part by Grant No. 2 R01 A. I. 998–02, Am 13084, and by Veterans Administration Surgical Research Funds.

[1] *Abbreviations used in this paper:* MLR, mixed leukocyte reaction; PCA, perchloric acid; SGST, skin graft survival times.

fully serotyped nonimmunosuppressed human subjects and from results of the MLR. It must be kept in mind that it has not been proven that antigens detected serologically are those detected in the MLR. It may be that the locus that controls the MLR and the one that controls HL-A are not identical, and that the MLR activator in the responding cell may be under the control of a different genetic system altogether from that which controls the response mechanism in the cell (10). The MLR test has been reported to distinguish among related cell donors differing with respect to the number of incompatible HL-A alleles (11). It has also been stated that for related individuals there is a close correlation between the MLR and skin graft survival time (12). In the selection of related donors in our own renal transplantation program, MLR and skin grafting experiments are frequently employed to augment serological data.

Our findings suggest that the MLR test frequently does not distinguish among the three classes of related donors (none, one, or two HL-A alleles different), and the correlation between skin graft survival time and MLR is low. Results indicate that attempts to quantitate the MLR should be cautiously interpreted.

## *Materials and Methods*

31 families, each including a potential renal transplant recipient, were tested with a panel of antisera capable of detecting the World Health Organization recognized antigens HL-A 1–12 as well as additional specificities not yet officially recognized. A two-stage semimicrocytotoxicity dye-exclusion test was employed (13). The inheritance of the four HL-A alleles in each family was established by haplotype analysis (14). Skin grafts were exchanged between selected family pairs by the method previously described (3). No grafts were placed on the potential renal transplant recipient and all grafts were ABO-compatible. Whenever possible skin grafts were followed until rejected.

One-way MLR were set up between selected family members. Heparinized blood specimens were spun at 500 rpm for 15 min, and the plasma was removed and spun at 800 rpm for 10 min. The supernatant plasma was drawn off and cleared of platelets by centrifugation at 4000 rpm for 15 min and saved for incorporation into the media. At this point the white cells were resuspended in their autologous tissue culture media. The stimulator cells were then incubated with 0.05 cc mitomycin C per cc of suspension and incubated for 20 min at 37°C. The cells were then washed three times and resuspended in 3.3 cc of tissue culture media prepared with autologous plasma. Duplicates of $3 \times 10^5$ and $7 \times 10^5$ per cc of mitomycin-treated cells were utilized as stimulator cells. At this point, the target lymphocytes were added using $3 \times 10^5$ cells per cc with the total volume being set at 5.5 cc so that duplicate cultures of 2.5 cc could be prepared. The reactions were incubated at 37°C in a $CO_2$ incubator for a 7 day period. After the 7 day incubation, 20 $\mu$l of tritiated thymidine was added to each tube and incubated for 5 hr at 37°C. After this, the reactions were kept at 4°C. An additional two drops of a solution containing 1 $\mu$Ci/cc concentration of normal thymidine was added to each tube. After centrifugation at 1500 rpm for 15 min, the cells were washed three times with cold saline, and the cell pellet was precipitated using 7% perchloric acid (PCA). Each precipitate was collected on a glass filter acidified with 10 cc of 2 N hydrochloric acid, and washed with 10 cc of 95% ethanol. The filters were then thoroughly dried, placed in vials with 10 cc of scintillation cocktail, and counted in a liquid scintillation counter. The degree of stimulation in each test was expressed as a ratio between the mixture and the appropriate control, called the stimulation index (15).

## RESULTS

Duplicate MLR were compared to assess the reproducibility of the method. No significant difference between the duplicate test was found (t [*t*-test] = 0.71, df [degrees of freedom] = 35, $P = 0.5$). As a further indication of the reliability of the procedure employed, it should be noted that 45 of the 46 MLR between the HL-A–identical pairs did not result in a stimulation index greater than 2.5. Stimulation indices of 1.5–2.5 must be considered borderline values in the definition of "stimulation" and "nonstimulation". A stimulation index greater than 2.5 clearly indicates stimulation.

*Comparison of MLR with HL-A Alleles and Antigens.*—Mixed leukocyte reactions between 212 family pairs were grouped on the basis of the HL-A genotypes of the paired individuals (Table I). The tests in which the stimulating and responding cells were HL-A–identical showed a mean stimulation index of 1.1 (SE = 0.68). This was significantly different ($P = 0.05$) from that of the MLR between pairs sharing only one HL-A allele ($\bar{X}$ [mean] = 5.12, SE = 0.437) or sharing neither HL-A allele ($\bar{X} = 8.92$, SE = 1.592). The mean stimulation was greater when stimulator and responder shared neither HL-A allele than when they shared one; this difference was significant at $P < 0.05$. Stimulation indices in the three groups are plotted in Fig. 1. The distribution of the degree of stimulation for the group sharing one allele clearly overlaps that for the pairs differing with respect to two alleles. It should be further noted that there is some overlap for all three groups, occurring at the stimulation indices of 1.5–2.5. This is more clearly illustrated in Table II. None of the pairs differing with respect to two alleles failed to stimulate, but three pairs showed borderline stimulation with indices between 1.5 and 2.5. Only one pair of HL-A identical siblings had a stimulation index greater than 2.5 (the value being 2.8 in this

TABLE I

*Correlation of HLA with MLR*

| | Family pairs | | | Unrelated stimulating cell |
|---|---|---|---|---|
| | HLA identicals | One allele incompatible | Both HLA alleles incompatible | |
| Mean ($\bar{X}$) | 1.10 | 5.1 | 8.83 | 17.46 |
| Standard error (SE) | 0.07 | 0.44 | 1.33 | 3.24 |
| Number (N) | 46 | 133 | 33 | 37 |
| Coefficient of variation (C) | 0.42 | 0.98 | 0.87 | 1.13 |

Stimulation indices: means, standard errors, and coefficients of variation.

case), but eight reactions between HL-A–identical siblings had borderline indices between 1.5 and 2.5. One-fourth of the reactions between related pairs sharing only one allele showed stimulation indices less than 2.5; of these one-third clearly failed to stimulate, their indices being less than 1.5 (Table III).

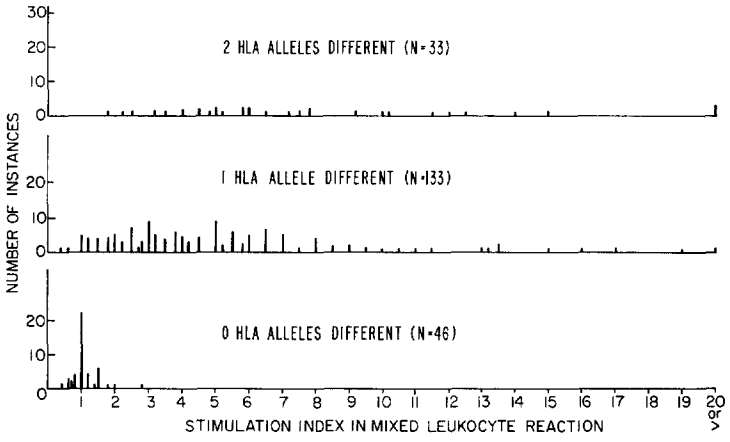


FIG. 1

TABLE II

*Correlation of HLA with MLR*

| HLA alleles not shared | Stimulation index | | | |
|---|---|---|---|---|
| | <1.5 | 1.5–2.5 | >2.5–5 | >5 |
| 0 | 37 | 8 | 1 | 0 |
| 1 | 11 | 23 | 49 | 50 |
| 2 | 0 | 3 | 9 | 21 |

$X_2^6 = 128.36$, $P < 0.005$.

Distribution of stimulation indices among HLA classes.

Thus, the degree of stimulation in each group was variable, and for those differing with respect to one allele, the range of values was considerable.

Although none of the healthy family pairs failed to respond, 11 of the 31 transplant patients failed to respond to any stimulating cell and were eliminated from all analyses. 10 of the 11 patients were on immunosuppression when the MLR were established. Considering the possibility that these patients may have been generally weak responders or stimulators and hence may have biased the data, those MLR involving patients were excluded from the analysis

TABLE III

*Nonstimulation between Pairs Incompatible for One Allele*

| Family | Genotype and HLA type of | | Stimulation index | Skin graft survival time |
|---|---|---|---|---|
| | Stimulating cells | Responding cells | | |
| 0114 | AD $\frac{2, \text{LND}}{1, 8}$ | CD $\frac{11, (\text{LND})}{1, 8}$ | 1.2 | 15 |
| 0128 | AC $\frac{1 \text{ or } 11, 8}{1 \text{ or } 11, 5}$ | BC $\frac{1 \text{ or } 11, 5}{1 \text{ or } 11, 5}$ | 1.2 | 8 |
| 0183 | CC′ $\frac{2, \text{Te10}}{1, 12}$ | AC $\frac{3, 7}{2, \text{Te10}}$ | 1.2 | 14 |
| 0113 | BC $\frac{2,}{11, 7}$ | BD $\frac{2}{2, 4c}$ | 1.2 | Not Done |
| 0104 | CD $\frac{2 \text{ or } 9}{7}$ | AD $\frac{2, 7 \text{ or } 8}{7}$ | 0.4 | Not Done |
| 0015 | AC $\frac{1, 5}{(2, 3, \text{ or } 9), 12}$ | BC $\frac{(2, 3, \text{ or } 9)}{(2, 3, \text{ or } 9), 12}$ | 1.0 | 11 |
| 0206 | BD $\frac{1}{1}$ | CD $\frac{8}{1}$ | 1.0 | >15 |
| 0206 | BC $\frac{1}{8}$ | CD $\frac{8}{1}$ | 0.6 | >15 |
| 0206 | AD $\frac{3, 7}{1}$ | AB $\frac{3, 7}{1}$ | 1.0 | >15 |
| 0206 | AD $\frac{3, 7}{1}$ | AB $\frac{3, 7}{1}$ | 1.0 | >15 |
| 0206 | BD $\frac{1}{1}$ | BC $\frac{1}{8}$ | 1.0 | Not Done |

TABLE IV

*Distribution of Stimulation Indices among HL-A Classes*

| Number of HL-A alleles not shared | Stimulation index | | | | |
|---|---|---|---|---|---|
| | <1.5 | 1.5–2.5 | >2.5–5.0 | >5 | |
| 0 | 10 | 1 | 1 | 0 | $X_6^2 = 70.95$ |
| 1 | 5 | 11 | 35 | 37 | $P < 0.005$ |
| 2 | 0 | 0 | 5 | 16 | |

MLR from patients are not included.

TABLE V

*Mixed Leukocyte Reactions Measuring the Same HL-A Incompatibility*

| Family | Stimulating cells (S) | Responding cells (R) | S:R** | No. of HL-A-incompatible alleles | Incompatible HL-A groups‡ | SGST | Stimulation index |
|---|---|---|---|---|---|---|---|
| 0099 | 03 | 02 | AD:CD | 1 | —, 4c | 22 | 2.5 |
| | 09 | 02 | AD:CD | 1 | —, 4c | 22 | 3.8 |
| | 02 | 08 | CD:BD | 1 | , 7 | 17 | 8.5 |
| | 02 | 10 | CD:BD | 1 | , 7 | 28 | 5.8 |
| | 03 | 10 | AD:BD | 1 | —, — | 14 | 4.0 |
| | 09 | 10 | AD:BD | 1 | —, — | 11 | 3.5 |
| | 09 | 08 | AD:BD | 1 | —, — | 18 | 11.0 |
| | 10 | 09 | BD:AD | 1 | —, LND | 24 | 4.5 |
| | 08 | 09 | BD:AD | 1 | —, LND | 13 | 2.2 |
| 0083 | 03 | 02 | AC:CD | 1 | —, 8 | >15 | 2.8 |
| | 04 | 04 | AC:CD | 1 | —, 8 | >15 | 3.8 |
| | 05 | 03 | BD:AC | 2 | 11, —§/, 7 | ND | 5.0 |
| | 05 | 04 | BD:AC | 2 | 11, —/, 7 | ND | 4.0 |
| 0106 | 12 | 08 | BC:BD | 1 | 9, — | ND | 16.0 |
| | 03 | 08 | BC:BD | 1 | 9, — | ND | 5.5 |
| | 12 | 07 | BC:AD | 2 | 9, /.‖, LND | ND | 26.0 |
| | 03 | 07 | BC:AD | 2 | 9, /., LND | ND | 15.0 |
| 0114 | 05 | 02 | AD:CD | 1 | 2, LND | 15 | 1.8 |
| | 03 | 02 | AD:CD | 1 | 2, LND | 15 | 1.2 |
| 0139 | 03 | 04 | AC:BD | 2 | 2, 12/2 7 | ND | 3.5 |
| | 09 | 04 | AC:BD | 2 | 2, 12/2 7 | ND | 4.5 |
| 0113 | 06 | 03 | AC:AD | 1 | —, — | ND | 2.7 |
| | 07 | 03 | AC:AD | 1 | —, — | ND | 6.5 |
| | 06 | 01 | AC:AB | 1 | (11),:¶, 7 | <15 | 5.0 |
| | 07 | 01 | AC:AB | 1 | (11), 7 | <15 | 13.2 |
| | 04 | 06 | BD:AC | 2 | 2, —/2, 4c | ND | 40.0 |
| | 04 | 07 | BD:AC | 2 | 2, —2, 4c | ND | 10.2 |

** Genotypes of stimulating and responding cells.

‡ Antigens at first and second segregant series.

§ Dash (—) indicates incompatible antigen not defined.

‖ Period (.) indicates no incompatibility at the segregant series.

¶ Parenthesis ( ) indicates uncertainty of antigen assignment.

* This symbol occurring after 4c denotes an antibody which is broader than 4c.

TABLE V—*Continued*

| Family | Stimulating cells (S) | Responding cells (R) | S:R** | No. of HL-A-incompatible alleles | Incompat ible HL-A groups‡ | SGST | Stimulation index |
|---|---|---|---|---|---|---|---|
| 0159 | 22 | 21 | BD:CD | 1 | ., — | ND | 3.5 |
| | 23 | 21 | BD:CD | 1 | ., — | ND | 3.5 |
| 0082 | 03 | 06 | BC:AC | 1 | —, — | ND | 1.8 |
| | 05 | 06 | BC:AC | 1 | —, — | ND | 3.0 |
| | 03 | 06 | BC:AB | 1 | 3, 7 | ND | 2.8 |
| | 05 | 01 | BC:AB | 1 | 3, 7 | ND | 4.2 |
| 0015 | 05 | 09 | BD:AD | 1 | (2, 3, 9), — | 11 | 2.5 |
| | 06 | 09 | BD:AD | 1 | (2, 3, 9), — | <11 | 6.5 |
| | 08 | 09 | BD:AD | 1 | (2, 3, 9), — | <11 | 5.5 |
| 0013 | 08 | 09 | AC:BC | 1 | 3, 5 | <14 | 5.2 |
| | 06 | 09 | AC:BC | 1 | 3, 5 | >14 | 6.5 |
| 0206 | 04 | 02 | AD:CD | 1 | 3, 7 | >15 | 13.0 |
| | 21 | 02 | AD:CD | 1 | 3, 7 | >15 | 8.0 |
| | 04 | 01 | AD:AB | 1 | ., — | >15 | 1.0 |
| | 21 | 01 | AD:AB | 1 | ., — | >15 | 1.0 |
| 0207 | 06 | 03 | AD:AC | 1 | 3, 7 | ND | 6.5 |
| | 06 | 05 | AD:AC | 1 | 3, 7 | <15 | 5.0 |
| | 02 | 03 | CD:AC | 1 | 3, 7 | ND | 6.0 |
| | 02 | 05 | CD:AC | 1 | 3, 7 | 13 | 5.5 |
| | 01 | 03 | AB:AC | 1 | —, (5, 7) | ND | 6.5 |
| | 01 | 05 | AB:AC | 1 | —, (5, 7) | 15 | 6.0 |
| | 05 | 06 | AC:AD | 1 | 2, — | ND | 6.5 |
| | 03 | 06 | AC:AD | 1 | 2, — | ND | 7.5 |
| | 05 | 02 | AC:CD | 1 | —, — | 16 | 3.2 |
| | 03 | 02 | AC:CD | 1 | —, — | ND | 8.0 |
| | 05 | 01 | AC:AB | 1 | 2, — | 15 | 7.0 |
| | 03 | 01 | AC:AB | 1 | 2, — | ND | 5.0 |
| 0208 | 02 | 03 | CD:AC | 1 | —, 8 | ND | 3.2 |
| | 02 | 04 | CD:AC | 1 | —, 8 | 14 | 5.0 |

TABLE V—*Concluded*

| Family | Stimulating cells (S) | Responding cells (R) | S:R** | No. of HL-A-incompatible alleles | Incompatible HL-A groups‡ | SGST | Stimulation index |
|---|---|---|---|---|---|---|---|
| 0208 | 01 | 03 | AB:AC | 1 | 11, 4c* | ND | 7.0 |
| | 01 | 04 | AB:AC | 1 | 11, 4c* | 14 | 15.0 |
| | 03 | 02 | AC:CD | 1 | 9, — | ND | 4.5 |
| | 04 | 02 | AC:CD | 1 | 9, — | ND | 5.5 |
| | 04 | 01 | AC:AB | 1 | 3, 7 | >15 | 11.5 |
| | 03 | 01 | AC:AB | 1 | 3, 7 | ND | 4.5 |
| 0209 | 02 | 03 | CD:AD | 1 | (2, 9), — | ND | 5.0 |
| | 02 | 03 | CD:AD | 1 | (2, 9), — | 13 | 2.5 |
| | 06 | 03 | AC:AD | 1 | (2, 9), — | ND | 5.5 |
| | 05 | 03 | AC:AD | 1 | (2, 9), — | ND | 6.0 |
| | 06 | 04 | AC:AD | 1 | (2, 9), — | ND | 6.0 |
| | 05 | 04 | AC:AD | 1 | (2, 9), — | ND | 10.0 |
| | 03 | 02 | AD:CD | 1 | ., 7 | ND | 8.0 |
| | 04 | 02 | AD:CD | 1 | ., 7 | ND | 7.0 |
| | 06 | 02 | AC:CD | 1 | ., 7 | ND | 2.5 |
| | 05 | 02 | AC:CD | 1 | ., 7 | ND | 4.0 |
| | 06 | 04 | AC:AD | 1 | (2, 9), — | ND | 6.0 |
| | 05 | 04 | AC:AD | 1 | (2, 9), — | ND | 10.0 |

shown in Table IV. As a result of excluding the cultures involving patients, a somewhat clearer distinction between HL-A–identical siblings and those sharing neither allele was observed. Cultures between healthy subjects incompatible for one allele still demonstrated the widespread range in the degree of stimulation. In the latter group, sample size remained relatively large.

63 pairs of reactions were studied for which stimulation by a single incompatible allele was compared with stimulation by the same incompatible allele plus another. Response to the single incompatible allele in 48 pairs was less than or equal to the response when an additional allele was incompatible. However, in 15 cases (24%) in 6 different families, a single allele stimulated more than when it was paired with an additional incompatible allele. Although polymorphism for non–HL-A antigens might account for this increased stimulation, very little evidence of stimulation by non–HL-A antigens was observed in reactions between HL-A–identical siblings. The results from these 15 cases thus are unexplained.

It was possible to measure a response to the same HL-A–incompatibilities with different family pairs in 14 families (Table V). It is readily observable that the degree of stimulation may be a function of both the stimulating and responding cell. Response to different, but HL-A–identical, siblings may be similar as shown in family 0159 or quite different, as in family 0106.

With this variability in response by an individual to the same HL-A allele, it is not surprising that the same incompatible allele stimulates different individuals to a different degree. Antigens in both segregant series were defined in

TABLE VI

*Stimulation Indices for Incompatible HL-A 3, 7 and HL-A 2, 12 Combinations*

| HL-A 3, HL-A 7 / Family | Stimulating cells (S) | Responding cells (R) | Genotypes of SR | SGST | Stimulation index |
|---|---|---|---|---|---|
| 0082 | 03 | 01 | BC:AB | ND | 2.8 |
| | 05 | 01 | BC:AB | ND | 4.2 |
| 0206 | 04 | 02 | AD:CD | 15 | 13.0 |
| | 21 | 02 | AD:CD | 15 | 8.0 |
| 0207 | 06 | 03 | AD:AC | ND | 6.5 |
| | 06 | 05 | AD:AC | 15 | 5.0 |
| | 02 | 03 | CD:AC | ND | 6.0 |
| | 02 | 05 | CD:AC | 13 | 5.5 |
| 0208 | 04 | 01 | AC:AB | 15 | 11.5 |
| | 03 | 01 | AC:AB | ND | 4.5 |
| HL-A 2, HL-A 12 | | | | | |
| 0169 | 06 | 01 | AD:AB | ND | 4.0 |
| 0139 | 07 | 04 | AD:BD | ND | 4.5 |

several families, but the degree of stimulation by the same combination of antigens was measured in different families only for HL-A 3 and 7 and for HL-A 2 and 12 (Table VI). The HL-A 3, 7 combination was associated with stimulation indices ranging from 2.8 to 13.0, while response to the HL-A 2, 12 incompatibility was essentially the same in both families in which it was measured (indices of 4.0 and 4.5). Comparisons of the mean skin graft survival times (SGST) and mean stimulation indices for the individual HL-A specificities seem to indicate no significant differences in the antigenicity or immunogenicity for the groups studied (Table VII).

*Comparison of Skin Graft Survival Time and HL-A Alleles.*—Previous publications from this group have reported the effects of HL-A relationships on SGST (16). Briefly, skin grafts exchanged between HL-A–identical siblings

were found to be distributed about a mean of 22.4 days with a range of 15–42 days, while those exchanged between siblings sharing neither HL-A allele were rejected in 6–15 days, mean 11.4. When one HL-A allele was incompatible, a wide range of survival times was observed, 8–28 days, mean 14.2 days. Although rapid rejection could not be associated with any of the individually defined HL-A antigens, a statistical association was observed between SGST and the number of incompatible HL-A specificities. Skin graft survival in these families in which MLR data is available is consistent with our earlier

TABLE VII

*Comparison of Skin Graft Survival Time (Days) and Stimulation Indices for Various Incompatible HL-A Groups: Means and Standard Errors*

| Incompatible HL-A group | | SGST | | | MLR stimulating index | | |
|---|---|---|---|---|---|---|---|
| | | $\bar{x}$ | SE | N | $\bar{x}$ | SE | N |
| First segregant series | HL-A 1 | 12.5 | 0.87 | 4 | 3.21 | 0.81 | 7 |
| | HL-A 2 | 13.2 | 0.91 | 9 | 7.68 | 1.65 | 30 |
| | HL-A 3 | 15.6 | 1.24 | 9 | 7.78 | 0.90 | 25 |
| | HL-A 9 | 18.0 | | 1 | 10.62 | 2.98 | 8 |
| | HL-A 11 | 15.5 | 1.50 | 2 | 8.00 | 2.15 | 9 |
| | Blank | 16.9 | 0.97 | 22 | 5.03 | 0.44 | 53 |
| Second segregant series | HL-A 5 | 12.8 | 0.51 | 10 | 6.25 | 1.35 | 8 |
| | HL-A 8 | 17.4 | 1.45 | 11 | 6.84 | 0.77 | 30 |
| | LND | 19.7 | 3.38 | 3 | 13.80 | 6.81 | 5 |
| | 4c | 10.0 | 0.00 | 2 | 17.50 | 6.83 | 6 |
| | 4c* | 14.0 | | 1 | 8.60 | 2.23 | 5 |
| | Te 10 | | | | 2.75 | 0.75 | 2 |
| | Blank | 15.3 | 0.72 | 26 | 5.96 | 0.68 | 87 |

findings with the single exception of a graft measuring two allelic incompatibilities which survived 16 days.

*Comparison of Mixed Leukocyte Reaction and Skin Graft Survival.*—Certain parallels may be drawn between the MLR and skin graft survival. A range of values far exceeding that of duplicate tests was observed when either the MLR or skin graft survival was measured within any of the classes defined in terms of the number of HL-A–incompatible alleles. Stimulation and SGST for the groups measuring one incompatible allele covered a wide range, but the average values fell between those for HL-A–identical and for two incompatible HL-A alleles. These observations indicate that HL-A affects both the MLR and SGST but that other factors probably influence them as well. These other factors may not be the same for MLR and for skin grafts as indicated by the low correlation between them ($r = 0.25$). As Fig. 2 and Table VIII depict, an incompatible allele for which the MLR stimulation index is high may be

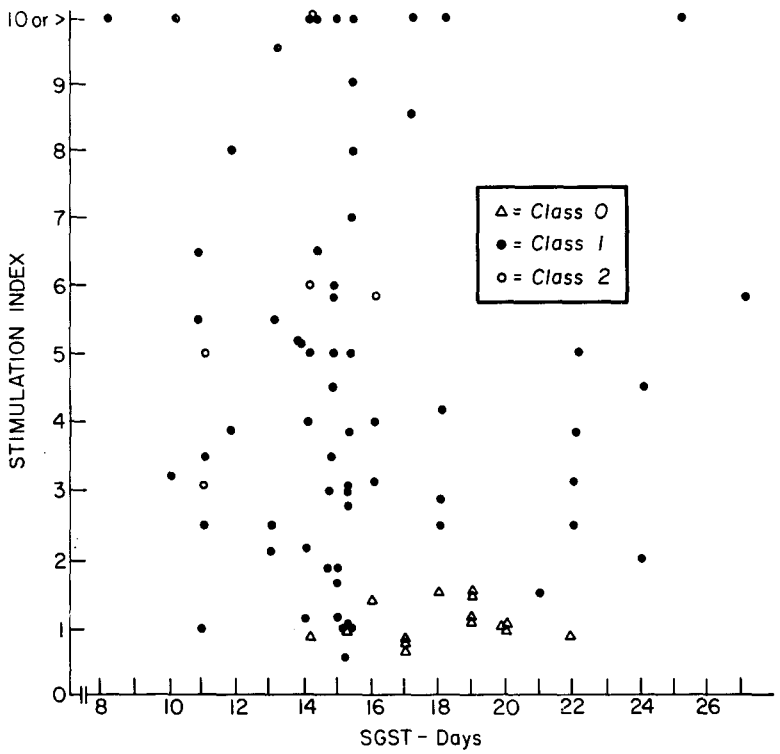


FIG. 2. Scattergram of SGST compared with stimulation indices for the three HL-A classes.

TABLE VIII

*Comparison of Skin Graft Survival Times and Stimulation Indices*

| SGST | Stimulation index | | | |
|---|---|---|---|---|
| | <1.5 | 1.5–2.5 | >2.5–5 | >5 |
| ≤15 Days | 4 | 6 | 11 | 16 |
| >15 Days | 15 | 7 | 13 | 10 |

$x_3^2 = 7.27, 0.1 > P > 0.05$

associated with a skin graft which survives as few as 8 days or as many as 28 days. Clearly, one cannot accurately predict SGST on the basis of the degree of stimulation in MLR.

## DISCUSSION

These data indicate that one cannot directly equate degrees of stimulation in the MLR with the degree of incompatibility for HL-A. One might argue

that certain HL-A alleles, when incompatible, do not provoke a response, thus accounting for the one-incompatible allele reactions with no stimulation. The probability that both incompatible alleles in the two-allele difference reactions are nonstimulating might be small enough to be undetected in this sample. One would, however, expect that a reaction measuring one incompatible allele would show less stimulation than one measuring the same allele plus another.

The immunogenicity of HL-A haplotypes has been correlated with test SGST (16). Skin graft survival time has been correlated with renal allograft survival as well as with the level of immediate and long-term renal function (17). The absence of correlation between the MLR and corresponding SGST indicates the failure of the MLR test to accurately predict the degree of compatibility between family pairs. The range of MLR stimulation index is wide and does not always reflect the degree of immunogenicity as demonstrated by SGST. The over-all association of a higher mean stimulation index with the greater number of HL-A alleles which are incompatible must be carefully examined for the numerous exceptions. Absence of different degrees of stimulation when different HL-A specificities are incompatible may reflect biochemical homogeneity underlying the separate antigenic groups.

These observations might have been predicted since the ultimate response to a foreign antigen, particularly in the intact animal, surely depends upon a number of factors, all of which are not recognizable in MLR. Ability to recognize a particular antigenic configuration may depend upon the mode of presentation and perhaps the immunological experience of the individual, particularly with respect to cross-reacting antigens. The over-all correlation of the MLR with HL-A genotyping and SGST makes it a valuable asset in donor selection, but at this point the major value of MLR is in confirming HL-A identity.

## SUMMARY

The immunogenicity of the haplotypes in 30 families was measured by survival of skin grafts between selected paired family members. These families were genotyped for HL-A using 57 selected cytotoxic alloantisera defining HL-A 1–12 as well as other nondefined specificities. Mixed leukocyte reactions were also studied in this series and the correlations between the mixed leukocyte reactions with skin graft survival times, individual HL-A specificities, and the number of incompatible HL-A alleles are reported. Comments concerning the interpretation and quantitation of the mixed leukocyte reactions are discussed.

## BIBLIOGRAPHY

1. Dausset, J., P. Ivanyi, J. Colombani, N. Feingold, and L. Legrand. 1967. The Hu-1 system. *In* Histocompatibility Testing. E. S. Curtoni, P. L. Mattiuz, and R. M. Tosi, editors. The Williams & Wilkins Company, Baltimore, Md. 189.

2. Ceppellini, R., E. S. Curtoni, P. L. Mattiuz, V. Miggiano, G. Scudeller, and A. Serra. 1967. Genetics of leukocyte antigens: a family study of segregation and linkage. *In* Histocompatiblity Testing. 149.
3. Seigler, H. F., D. B. Amos, F. E. Ward, J. G. Southworth, B. G. Hattler, and D. L. Stickel. 1970. Immunogenetics on consanguinous allografts in man. I. Histocompatiblity testing and skin allografts. *Ann. Surg.* **2**:151.
4. Bach, F. H., R. J. Albertini, H. Klostermann, and E. Day. 1967. Evaluation of compatibility at the major histocompatibility locus in man. *In* Histocompatibility Testing. 55.
5. Rapaport, F., J. Dausset, J. Converse, and H. Lawrence. 1965. Biological and ultrastructural studies of leukocyte fractions as transplantation antigens in man. *Transplantation.* **3**:490.
6. Dausset, J., R. Walford, J. Colombani, L. Legrand, N. Feingold, A. Borge, and F. Rapaport. 1969. The HL-A system sub-loci and their importance in transplantation. *Transplant. Proc.* **1**:331.
7. Klein, J. 1967. Strength of histocompatibility genes in mice. *In* Histocompatibility Testing. 21.
8. Silvers, W., D. Wilson, and J. Palm. 1967. Typing and immunosuppression in rats. *Transplantation.* **5**:1053.
9. Andrus, C. 1970. A model system for the measurement of antigenic strength. *In* Histocompatibility Testing. 417.
10. Plate, J. M., F. E. Ward, and D. B. Amos. 1970. The mixed leukocyte culture response between HL-A identical siblings. *In* Histocompatibility Testing. 531.
11. Albertini, R., and F. Bach. Quantitative assay of antigenic disparity at HL-A—the major histocompatibility locus in man. 1968. *J. Exp. Med.* **128**:639.
12. Bach, F., and W. Kisken. 1967. Predictive value of results of mixed leukocyte cultures for skin allograft survival in man. *Transplantation.* **5**:1046.
13. Amos, B., H. Bashir, W. Boyle, J. MacQueen, A. Trilikamen. 1969. A simple microcytotoxicity test. *Transplantation.* **7**:220.
14. Amos, B., F. E. Ward, C. M. Zmijewski, B. D. Hattler, H. F. Seigler. 1968. Graft donor selection based upon single locus (haplotype) analysis within families. *Transplantation.* **6**:524.
15. Bildsoe, P., S. Sorensen, O. Pettirossi, and M. Simmomsen. 1970. Heart and kidney transplantation from segregating hybrid to parental rats. *Transplant. Rev.* **3**:36.
16. Amos, B., H. Seigler, J. Southworth, and F. E. Ward. 1969. Skin graft rejection between subjects genotyped for HL-A. *Transplant. Proc.* **1**:342.
17. H. F. Seigler, D. L. Stickel, F. E. Ward, C. H. Andrus, W. J. Klein, Jr., J. C. Gunnells, D. B. Amos. 1970. Correlations of test skin grafts and renal allograft function in human subjects genotyped for HL-A. *Surgery.* **68**:86.