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# Parental HLA Compatibility, Fetal Wastage and Neural Tube Defects: Evidence for a *T/t*-Like Locus in Humans

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SUMMARY

To test the hypothesis that a locus in or near the human major histocompatibility complex (HLA) contributes to both involuntary fetal loss and neural tube defects (NTD), we evaluated sharing of antigens of the *HLA-A*, *HLA-B*, or *HLA-DR* loci of couples who had three or more first-trimester spontaneous abortions or who had a child with an NTD (myelomeningocele or anencephaly). *HLA-A* antigen sharing was increased in couples with three or more spontaneous abortions and in couples who had an anencephalic fetus, when compared with couples who had three or more pregnancies and no fetal loss. Increased sharing of antigens at the *HLA-A* and *B* loci was not seen in the entire group of couples with children with myelomeningocele, but was found in the subgroup of 36 couples whose child had a lumbar myelomeningocele. An increase in *HLA-DR* sharing was not seen in any group or subgroup when compared with the control couples. Among the aborting couples, increased sharing was not restricted to the couples who had no term pregnancies, but was also found in the couples whose fetal losses occurred after one or more normal term pregnancies. These results are consistent with the hypothesis that a locus on the *HLA-A* side of the *HLA-DR* locus contributes to some fetal loss and susceptibility to NTD. This model is proposed as an alternative to the hypothesis that the maternal immune response to paternal major histocompatibility complex (MHC) antigens is the basis for increased HLA sharing in couples with fetal wastage.

## INTRODUCTION

Several studies have demonstrated increased sharing of antigens of the human major histocompatibility complex (HLA) in couples experiencing recurrent in-

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voluntary fetal wastage, when such couples are compared with couples who have had multiple pregnancies and no fetal loss [1–5]. These studies extend earlier animal studies that demonstrated decreases in litter size in major histocompatibility complex (MHC) compatible matings [6]. Such observations have led to the formulation of an "immunological model" for the role of the MHC in fetal survival. In this model, the maternal immune response to non-MHC antigens on fetal tissue is suppressed or blocked when MHC incompatibility occurs between mother and fetus [7]. Thus, the maternal immune response to paternally derived gene products may determine pregnancy outcome [5, 8, 9]. The antigen(s) that elicit suppression or which are targets for the immune response are not well defined. The expression of classical *HLA* class I antigens of the *A*, *B*, and *C* loci on the villous trophoblast, the fetal tissue exposed to maternal circulation, is a controversial issue [10–13]. Montgomery and Lala [13] recently presented evidence of the absence of class I antigens from the syncytiotrophoblast cells of the villi and the presence of class I antigens on the cytotrophoblast cells in early gestational placentas; whether the cytotrophoblast cells are exposed to the maternal immune system is not well established [13].

A second model of possible relevance for the role of MHC-linked genes and their products to fetal outcome in humans is the murine *T/t* complex, which is linked to the mouse MHC [14, 15]. The *T/t* complex is centromeric to *H-2 K* and thus to the entire *H-2* complex. The *T/t* complex may span more than 7 cm, and the allelic relationship between mutant genes is not clear [18]. Mutant *t* alleles cause stage-specific failures of embryogenesis [16–18]. Some *t* alleles exert segregation distortion and suppression of recombination that extends into the MHC [17, 18]. Since mutant *t* alleles result in abnormal or abortive development of neuroectoderm or neural tube, it has been proposed that expression of a human analog of the murine *T/t* complex could play a role in the development of neural tube defects (NTD), as well as contributing to early fetal loss [19]. A more general formulation of this "genetic" model would propose a locus (or loci) in or near the human MHC with alleles that contribute to fetal loss or NTD and are in linkage disequilibrium with *HLA* alleles.

The "genetic" and the "immunological" models are not necessarily mutually exclusive. Evidence to support either or both models in human reproduction has been marshaled, but most of such studies have focused on either fetal loss or NTD and have not considered both types of fetal abnormalities [1–7, 19–21]. We previously reported results of a small study of *HLA-A* and *HLA-B* compatibility and fetal outcome in 77 couples. Here, we report the results of evaluation of the *HLA-A*, *HLA-B*, and *HLA-DR* compatibility of couples who have experienced multiple pregnancies with no fetal loss, couples with multiple pregnancies with recurrent fetal loss, and couples who have conceived a child with NTD.

## MATERIALS AND METHODS

### *Subjects*

Couples were referred to us by the Genetics Clinics of University Hospitals of Cleveland and of the Cleveland Metropolitan General Hospital, as well as the Myelodysplasia Clinics

of the same institutions. Over 90% of the subjects were Caucasian. The data with regard to obstetrical history presented by the physicians were reviewed with each couple. Discrepancies were clarified with the physician and, if necessary, by review of the patients' medical and hospital records. The criteria for inclusion in the study consisted of a confirmed obstetrical history that fit into one or more of five groups (table 1). Three hundred eight couples were referred for study: 50 couples were excluded because review of the obstetrical history indicated that the subjects did not meet the criteria for any group or indicated an unresolvable inconsistency or uncertainty of the facts with regard to the history. Of the 258 couples reported, approximately 50 were included in a previous report [4]. The couples from the previous study who were not included in this study were those for whom follow-up to confirm *HLA* typing and/or obstetrical history was not possible. All couples with recurrent spontaneous abortions had karyotype analysis, with G-banding. Three couples were found to have a balanced translocation in one partner and were excluded from analysis.

*Laboratory Methods*

HLA typing was performed on peripheral blood lymphocytes by standard dye-exclusion microcytotoxicity [22]. Typing for the alleles of the *HLA-A* and *B* loci was performed on T lymphocytes, and *HLA-DR* typing was performed on B lymphocytes separated on nylon wool columns as described by Lowry et al. [23]. Sera from multiparous women used for the typing were obtained from the NIAID Serum Bank, from local screening, and by private exchange. The cell panel used to define the specificity of these sera was updated in the Ninth International Histocompatibility Workshop, and all antigen assignments on cells from study subjects were consistent with these assignments. The antigens detected with the local sera panel included: A1, A2, A3, A9, A10, A11, Aw23, Aw24, A25, A26, A28, A29, Aw30, Aw31, Aw32, Aw33, Aw34, Aw36, B5, B7, B8, B12, B13, B14, B15, Bw16, B17, B18, Bw21, Bw22, B27, Bw35, B37, Bw38, Bw39, B40, Bw41, Bw42, Bw44, Bw45, Bw47, Bw48, Bw49, Bw50, Bw51, Bw52, Bw53, Bw54, Bw55, Bw56, Bw57, Bw58, Bw59, Bw60, Bw61, Bw62, Bw63, DR1, DR2, DR3, DR4, DR5, DRw6, and DR7.

Chromosomal analysis was performed on peripheral blood lymphocytes. Lymphocytes were cultured for 70 hrs using 200–400 μl of blood in 5 ml of RPMI 1640 with 16% fetal bovine serum, 7.6 mg/ml penicillin, 6.2 mg/ml streptomycin, and 25 μl/ml M-phytohemagglutinin (Gibco, Grand Island, N.Y.). Cells were harvested after 30-min exposure to 0.06 μg/ml Colcemid, exposure to hypotonic (0.075 M) KCl, and fixed in 3:1 methanol:acetic acid. G-banding was performed by the method of Seabright [24]. All preparations were

TABLE 1

CRITERIA FOR STUDY GROUPS

| GROUP | NO. COUPLES TYPED FOR *HLA-A,B* | NO. COUPLES TYPED FOR *HLA-DR* | CRITERIA* |
|---|---|---|---|
| 1 | 49 | 35 | Three or more pregnancies, all live births, no congenital abnormalities |
| 2 | 45 | 33 | Three or more pregnancies, one or two first-trimester spontaneous abortions |
| 3 | 54 | 40 | Three or more pregnancies, three or more first-trimester spontaneous abortions, normal chromosomes in both partners |
| 4 | 69 | 37 | Child with myelomeningocele |
| 5 | 38 | 28 | An infant with anencephaly |

* In all cases, the criteria relate to a single mating couple.

TABLE 2

ANTIGEN DETECTION IN EACH GROUP

| | CLASS I LOCI | | CLASS II LOCI |
|---|---|---|---|
| | *HLA-A* | *HLA-B* | *HLA-DR* |
| 1 | 180/196 (91.8%) | 176/196 (89.8%) | 115/140 (82.1%) |
| 2 | 165/180 (91.6%) | 168/180 (93.3%) | 114/132 (86.4%) |
| 3 | 196/216 (90.7%) | 202/216 (93.5%) | 146/160 (91.3%)* |
| 4 | 247/276 (98.5%) | 259/276 (98.3%) | 130/148 (87.8%) |
| 5 | 137/152 (90.1%) | 147/152 (96.7%)† | 106/112 (94.6%)‡ |

NOTE: Antigens detected/alleles tested (%).
* $\chi^2 = 4.70$, $P = .030$.
† $\chi^2 = 5.15$, $P = .023$.
‡ $\chi^2 = 7.89$, $P = .005$.

evaluated for resolution by the number of bands of chromosome 10 [25]. All preparations met or exceeded the Paris Conference standard of 400 bands per haploid set [26].

*Analysis*

Antigen sharing between mates was scored on the basis of the highest level of distinction of variants or "splits" afforded by the reagents used for typing. For example, if different splits of HLA-B40, Bw60, or Bw61 were detected in the husband and wife in a mating, this was not scored as a compatibility. If the same variant were detected in both husband and wife or if B40 could not be resolved in both subjects, then a compatibility was scored. If the B40 could be resolved in one subject but not in the mate, then compatibility was not scored. The total number of alleles at each locus shared by mates within each group was divided by the number of alleles to be accounted for by the number of couples within the group, each couple contributing four alleles at each locus. Comparisons between and among groups were made using a contingency chi-square test with Yates' correction.

## RESULTS

HLA-antigen ascertainment was calculated for each group to determine if the incidence of blank alleles or of homozygosity differed among the groups (table 2). Antigen detection was greater than 89% in all groups at the *HLA-A* and *HLA-B* loci, and greater than 82% at the *HLA-DR* locus. Small, but statistically significant, increases in antigen detection were noted for the *HLA-B* locus for group 5 (96.7%, $P = .023$) and for the *HLA-DR* locus for group 3 (91.3%, $P = .030$) and group 5 (94.6%, $P = .005$), when compared with the control group, group 1. Without typing of family members to allow determination of genotype, it is impossible to determine if these small increases in antigen detection at the *B* locus in group 5 and at the *DR* locus in groups 3 and 5 represent decreases in true blanks or in homozygotes. Family studies of Caucasian subjects have suggested that with an extensive serum panel as was used in this study, genetic homozygosity is a more frequent cause of less than full-house typings than the presence of blank or unknown alleles [4].

When antigen sharing was evaluated in the study groups, significant deviations from control values were found (table 3). Increased sharing of *HLA-A*-locus antigens was found in groups 3 and 5, as compared with group 1. In group 3, 68

TABLE 3

HLA ALLELES SHARED WITHIN COUPLES

| GROUP | GROUPS SHARED ALLELES/TOTAL POSSIBLE ALLELES | | |
|---|---|---|---|
| | *A* locus | *B* locus | *DR* locus |
| 1 | 38/196 (19%) | 20/196 (10%) | 24/140 (17%) |
| 2 | 46/180 (26%) | 16/180 (9%) | 14/132 (11%) |
| 3 | 68/216 (31%)* | 34/216 (16%) | 40/160 (25%) |
| 4 | 66/276 (24%) | 38/276 (14%) | 16/148 (11%) |
| 5 | 44/152 (29%)† | 26/152 (17%) | 20/112 (18%) |
| Analysis of subgroups | | | |
| 3A: | | | |
| Aborters with no term pregnancies | 18/64 (28%) | 14/64 (22%)‡ | 10/48 (21%) |
| 3B: | | | |
| Aborters with one or more term pregnancies | 42/144 (29%)§ | 12/144 (8%) | 26/92 (28%) |
| 4 (myelomeningocele): | | | |
| Level of lesion: | | | |
| Thoracic | 10/44 (23%) | 6/44 (14%) | 0/28 (0%)** |
| Lumbar | 36/120 (30%)‖ | 24/120 (20%)# | 18/64 (28%) |
| Sacral | 8/32 (22%) | 0/36 (0%) | 0/24 (0%) |
| Sex: | | | |
| Female | 24/108 (22%) | 14/108 (13%) | 6/76 (8%) |
| Male | 36/136 (26%) | 20/136 (15%) | 18/68 (26%) |
| 5 (anencephaly): | | | |
| Sex: | | | |
| Female | 18/68 (26%) | 14/68 (21%) | 12/52 (23%) |
| Male | 22/68 (32%)†† | 6/68 (9%) | 12/60 (20%) |

* $\chi^2 = 7.25$, $P = .007$.
† $\chi^2 = 3.83$, $P = .050$.
‡ $\chi^2 = 4.80$, $P = .028$.
§ $\chi^2 = 3.88$, $P = .049$.
‖ $\chi^2 = 4.10$, $P = .043$.
# $\chi^2 = 5.17$, $P = .023$.
** $\chi^2 = 4.29$, $P = .038$.
†† $\chi^2 = 4.12$, $P = .042$.

of 216 (31%) possible alleles were shared by husband and wife, compared to 38 of 196 (19%) possible alleles in group 1 ($P = .007$). *HLA-A*-locus sharing was elevated to 44 of 152 (29%) possible alleles in group 5 ($P = .050$). No significant increase in antigen sharing was noted at the *HLA-B* locus when the experimental groups were compared with controls. Group 2, which consists of couples with three or more pregnancies and one or two fetal losses, is a heterogeneous group, and no difference between group 2 and the control group was found for any locus. Of note is the absence of an increase in sharing at the *DR* locus in any study group. If groups 3, 4, and 5 are taken together and compared with the controls, then a significant increase in sharing of antigens at the *HLA-A* locus ($P = .029$), and not at the *HLA-B* or *HLA-DR* loci, is noted. Similar results would be obtained for all of these comparisons had we calculated the proportion of shared alleles in relation to detected alleles rather than in relation to the total number of possible alleles.

Based on the data with regard to the sequence of pregnancies in 52 of 54 couples in group 3, this group could be divided into those couples in which all pregnancies end in first-trimester abortions (subgroup 3A) and those couples in which at least one pregnancy was carried to term (subgroup 3B). All couples in subgroup 3B had had one or more of their fetal losses occur after the birth of a live, healthy infant. Thus, in no case in this subgroup did all three or more fetal losses occur prior to a live birth. In subgroup 3A, with no live births and three or more spontaneous abortions, *HLA-B*-locus sharing was elevated to 14 of 64 (22%) possible alleles, compared with 20 of 196 (10%) possible alleles in the control group ($P = .028$). *HLA-A*-locus sharing was not elevated in subgroup 3A. Of 144 possible alleles at the *A* locus in subgroup 3B, 42 (29%) were shared by husband and wife, compared to 38 of 196 (19%) in the control group ($P = .049$).

Published reports have indicated an excess of females affected with NTD, varying with the incidence of NTD [26–29]. The excess of females was more profound for anencephaly than for myelomeninogocele [28]. Our study group was not collected with the aim of defining the incidence rates in the two sexes. Group 4 (myelomeningocele) and group 5 (anencephaly) were analyzed with regard to sex, however, in order to evaluate for possible variation in potential contribution of HLA in the different sexes. The sex of the affected child in group 4 was known for 61 couples, and the male:female ratio was 34:27 (1.3). No significant increase in *HLA-A*- or *HLA-B*-locus sharing was noted in either subgroup—the parents of a female affected or the parents of a male affected (table 3)—when compared with each other or with group 1. An increase in *HLA-DR* sharing was noted in the parents of male children in group 4, when they were compared with the parents of an affected female ($P = .006$). This increase in sharing was not significant when compared, however, with the controls ($P = .95$). The sex of the fetus with anencephaly was known for 34 couples in group 5; the male:female ratio was 1.0. As noted above, group 5 demonstrated a shift to increased *HLA-A*-locus sharing (table 3). The 17 couples who had had a male anencephalic had an increase in sharing at the *HLA-A* locus, so that of 68 posssible alleles at this locus, 22 (32%) were shared between husband and wife ($P = .042$). The 17 couples who had had a female anencephalic did not demonstrate an increase in *HLA-A*-antigen sharing when compared with group 1.

Level of the lesion was known for 50 of the 69 couples in group 4. Thirty of these 50 couples had had a child with lumbar myelomeningocele. *HLA-A*- and *HLA-B*-antigen sharing was increased to 36 (30%) and 24 (20%) of 120 possible alleles, respectively, in this subgroup ($P = .023$ and $P = .043$) when compared with the control group. In the small group of couples with a child with thoracic myelomeningocele, *HLA-DR* sharing was significantly decreased to 0 of 28 (0%) possible alleles ($P = .038$).

## DISCUSSION

Our data support the previously reported increase in *HLA* compatibility in couples experiencing involuntary fetal wastage [1–5]. We have, however, extended these studies to evaluate with substantial numbers three *HLA* loci (*HLA-A*, *HLA-B*, and *HLA-DR*). As summarized by Gill, there is little published information

with regard to sharing of alleles at the *HLA-D/DR* locus (loci) in fetal wastage [7]. In our study, increased *HLA* sharing between couples with involuntary fetal loss occurs primarily for the *HLA-A* locus.

We have compared couples with three or more pregnancies and no fetal loss, not only to couples with recurrent fetal wastage, but also to couples experiencing the conception of a fetus with NTD. The reason for the latter comparison was the fact that the murine *T/t* complex linked to the mouse MHC has alleles that cause stage-specific failures in embryogenesis [14, 16, 18]. The increased sharing in our data is strikingly limited to the *HLA-A* locus and to a lesser degree to the *HLA-B* locus in groups 3, 4, and 5, and groups 3–5 combined. There was no increase in sharing at the *HLA-DR* locus. Thus, our data can be explained by a model in which a locus (loci) on the *HLA-A* side of *HLA-DR* has alleles that result in abnormal fetal development, leading either to fetal loss or NTD. This model would require that alleles at the putative locus (loci) are subject to the linkage disequilibrium observed for the *HLA* complex.

The immunological model for the basis of increased *HLA* compatibility in fetal loss has received much attention and is the basis for immunological intervention in affected matings. At least two groups have initiated programs of leukocyte immunization for women experiencing recurrent fetal loss with no gynecological or chromosomal abnormalities, when *HLA* sharing between husband and wife is extensive [5, 9]. Our observation of increased *HLA-A* sharing in couples who have experienced recurrent fetal wastage *after* one or more normal pregnancies, as well as the recently reported data from Ober et al. with regard to *HLA* compatibility and reproductive performance among Hutterites, suggests that *HLA* sharing per se may not be ineluctably or solely responsible for fetal loss [27]. Further, the increased compatibility in couples who suffer recurrent fetal wastage after the birth of one or more children suggests that maternal immunological naïvete with regard to paternal antigens is not necessary in order to observe fetal loss in *HLA*-compatible couples. Our results, thus, are not consistent with the immunological model as the entire explanation for the increase in parental *HLA* compatibility in fetal loss, since such a model would not predict an increase at only one locus and would predict that the impact of *HLA* sharing would be greatest in those couples whose fetal loss occurs prior to live births. We have observed a significant increase in *HLA-B* sharing in couples with three or more first-trimester spontaneous abortions and no live births, and a significant increase in *HLA-A*-antigen sharing in couples who have had abortions after the birth of a live child. This distinction has not been evaluated in the previous studies. Three of 10 aborting couples studied by Beer et al. had had a first pregnancy result in a live birth, but these couples are not considered separately [5]. Our observation may reflect the impact of two different mechanisms giving rise to increased HLA class I compatibility in couples experiencing recurrent fetal loss with the determining factors at or near different *HLA* loci.

The significance of the elevated sharing limited to lumbar myelomeningocele and anencephaly, particularly with regard to male anencephalics, is unknown. Leck suggested that the rate of NTD varies more over time and ethnicity in females than in males [28]. The balance of genetic and environmental factors

contributing to NTD may vary with the level of the lesion, so that the impact of *HLA* is more clearly seen with lumbar NTD. Holmes et al. suggested that in consideration of the recurrence risks of NTD one must consider the etiologic heterogeneity of NTD, but did not suggest heterogeneity with regard to meningomyeloceles [30]. The genetics of NTD are complex, with no clear evidence of a single gene etiology. The model most consistent with the extensive epidemiologic data is one in which one or more genetic factors interact with one or more environmental factors to cause NTD [28]. The environmental factor(s) are unknown, although, at present, there is much interest and speculation about a role for vitamin supplementation in decreasing the risk of NTD [31]. Several studies failed to demonstrate any evidence of classic linkage between a susceptibility locus for NTD and *HLA* [19, 20]. Nonetheless, analysis of recurrence risks in comparison with occurrence risks, of ethnic diversity in the risk of NTD, and maintenance of relatively high or low risks despite emigration suggest a genetic contribution to the development of NTD [28]. Whether one of the genetic factors is linked to the *HLA* complex has not been rigorously excluded, although the data of Bobrow et al., demonstrating *HLA*-identical sib-pairs divergent for NTD, clearly exclude a single gene model [19]. Our observation of increased *HLA-A* compatibility in couples who have had a child with anencephaly or a lumbar myelomeningocele, as well as in couples experiencing involuntary fetal loss, are consistent with a model in which alleles at a locus near the *HLA-A* locus contribute to the risk of NTD or fetal loss but do not always lead to these outcomes. Such a model, one of incomplete penetrance, would assume interaction with other genetic factors and/or with environmental factors to be necessary to result in NTD or fetal loss.

Finally, the locus we have proposed might be expected to lead to segregation distortion of the *HLA* complex. Segregation distortion of *HLA* haplotypes has been difficult to test with conflicting reports on the literature. Segregation distortion was not observed in two studies, while Albert et al. reported an excess of the high-frequency haplotype *A2-B12* in 535 families [32–34]. It may be necessary to evaluate haplotypes more extensively defined, since Awdeh et al. reported transmission bias of an *HLA* haplotype when the complement and glyoxylase alleles were included [35]. Epistatic interaction between *HLA* and other loci may require that evidence of transmission bias be sought in families in which segregation of other loci can also be analyzed.

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