# Familial Rheumatoid Arthritis

## LINKAGE OF HLA TO DISEASE SUSCEPTIBILITY LOCUS IN FOUR FAMILIES WHERE PROBAND PRESENTED WITH JUVENILE RHEUMATOID ARTHRITIS

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ABSTRACT The occurrence of a chronic seronegative polyarthritis has been studied in four families in which the proband presented with some form of juvenile rheumatoid arthritis. In these families, histocompatibility testing suggested that susceptibility to arthritis was controlled by a dominant allele with variable penetrance and expressivity at the rheumatoid-like arthritis, first locus (*RLA-1*). The combined lod scores for the four families (2.70) indicated that the odds in favor of genetic linkage between the major histocompatibility complex and the postulated disease susceptibility gene, *RLA-1*, were 500:1. In one family, a recombinant event permitted localization of *RLA-1* centromeric to *HLA-D*. Of major interest was the fact that there was significant pleomorphism in the clinical manifestations of arthritis in affected individuals. In some, symptoms first occurred in childhood and in others, in adult life. Even among those with childhood-onset arthritis, different types of juvenile rheumatoid arthritis were observed within the same family.

## INTRODUCTION

Studies of populations with rheumatic diseases suggest that certain HLA antigens occur more or less frequently in diseased individuals than expected by chance alone (1–13). Data associating HLA-B27 with Reiter's Syndrome (2) and ankylosing spondylitis (AS)[1] (3, 10) are most persuasive. Recent studies from three laboratories (5, 9, 13) suggest that certain HLA-D antigens, most notably Dw4, are found more often than expected in patients with adult-onset, sero-positive rheumatoid arthritis (RA), especially in those with relatively severe disease (5, 13). Abnormal frequencies of certain HLA-D antigens have also been observed in patients with juvenile rheumatoid arthritis (JRA) (7, 9).

Studies in widely different geographic areas have also developed evidence both for and against an association between HLA-B27 and JRA (1, 8, 9). A crucial issue in these population studies was the inclusion (or systematic exclusion) of patients with early onset of AS (11, 14).

These investigations have established that there is a relationship between certain HLA markers and the subsequent occurrence of rheumatic disease, but it is the nature of population surveys that they cannot ascertain the genetic basis for these relationships. On the other hand, by examining the relationship between the occurrence of rheumatic complaints and the inheritance of the major histocompatibility complex (MHC) in families, one may test for genetic linkage between the MHC and a postulated gene regulating susceptibility to disease. This study presents investigations in four families, each of which has at least

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[1] *Abbreviations used in this paper*: ANA, antinuclear antibody; AS, ankylosing spondylitis; CIC, circulating immune complexes; GLO, Glyoxalase I; JRA, juvenile RA; MHC, major histocompatibility complex; RA, rheumatoid arthritis; *RLA-1*, rheumatoid-like arthritis, first locus.

one child with JRA. The data suggest that there is a high likelihood that a major gene, rheumatoid-like arthritis, first locus (*RLA-1*), controlling the susceptibility to arthritis in these families, is linked to HLA and resides centromeric to HLA-D.

## METHODS

Families participating in this study had one or more children under treatment at the Rheumatic Disease Clinic at the Texas Children's Hospital. In addition to the proband, one or more additional family members had symptoms of arthritis. Among families selected for study, all clinical presentations of JRA were represented. Diagnostic classification followed criteria established by the JRA Subcommittee of the American Rheumatism Association (15), and those established earlier by Ropes et al. (16) for adult-onset RA.

Family members were examined by Dr. Brewer. Sacroiliac and spine films were taken to evaluate patients for AS; such x rays were obtained in asymptomatic family members as well, when the pattern of inheritance suggested the possibility of AS. In addition to the physical examination and x rays, serum was obtained from family members for measurement of rheumatoid factor (latex-fixation test), antinuclear antibody (ANA), and circulating immune complexes (CIC). Serum was stored in small aliquots at −70°C until assayed. ANA was measured by indirect immunofluorescence using frozen sections of mouse liver as substrate (14). ANA titers >1:32 were considered abnormal. CIC were measured by the Clq binding test (15). 95% of the sera from 179 healthy blood donors had Clq binding activities <6.2%. Abnormal values for ANA, CIC, and rheumatoid factor are shown where applicable in tables that depict results from each family.

HLA serotyping was performed by the standard National Institutes of Health microcytoxicity test using antisera recognizing 11 HLA-A antigens and 15 HLA-B antigens (17). In repeat typing studies of family 1, we used sera recognizing 17 HLA-A, 22 HLA-B, and 4 HLA-Cw antigens. One-way mixed leukocyte culture (MLC) tests were performed (18), using cultures containing $2.5 \times 10^5$ responder cells and equal numbers of mitomycin-treated donor cells per well in 20% pooled AB,Rh(D) positive plasma or plasma from the responder cell donor. The results of the MLC tests were expressed as counts per minute less background incorporated by responder cells. Stimulation indices were calculated as the ratio of counts of [$^3$H]thymidine incorporated by cells of the responder, stimulated by mitomycin-treated donor cells, to counts of [$^3$H]-thymidine incorporated by cells from the same recipient stimulated by mitomycin-treated autologous cells. Stimulation index = counts per minute (A × Bm)/(A × Am). Individuals were considered haploidentical if the stimulation index was ≤12.76 (±9.48 SD) and probably identical at HLA-D if the stimulation index was ≤1.18 (±0.53 SD). This data was obtained from MLC tests performed in 100 haploidentical and 106 identical pairs, respectively. Relationship at *HLA* in these family members was originally demonstrated serologically. HLA-DR typing was carried out using peripheral blood B cells isolated as described by Ting et al. (19), and sera capable of recognizing the HLA-DRw1 to -DRw7. Glyoxalase I [EC:4.4.1.5] (GLO) was phenotyped with erythrocyte lysates as previously described (20).

Genetic linkage analysis of the pedigrees (Figs. 1–4) was accomplished with the aid of a computer program, LIPED, which allows one to test linkage where one of the genes is <100% penetrant (21, 22). The assumptions in this analysis were: susceptibility to chronic peripheral arthritis is a dominant monogenic trait; patients with only AS were not considered as afflicted with peripheral arthritis; variation in phenotype (age of onset, severity, symptoms, and signs of disease, etc.) is an environmental effect uncorrelated among individuals in the same family or at least not associated with HLA; there is no phenotypic association of HLA-A and B with RA in the population studied. Contribution of the susceptibility gene from mates outside the line of descent was allowed by setting the frequency of the gene in the population at 1%. This was considered to be an upper limit for the frequency of the proposed gene in the population (23). Differences resulting from sex and possible mutation of genes were not considered in this analysis. We considered that family members <30 yr of age at the time of this study were still at risk to acquire the disease at a later date. For this reason, unaffected individuals under the age of 30 were considered to have an indeterminant or unknown genotype with respect to the disease.

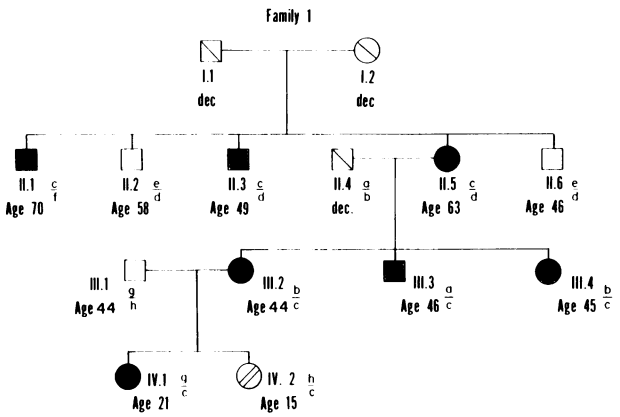


FIGURE 1 Chromosome 6 haplotypes, family 1. GLO typing indicated that III.1, III.2, IV.1, and IV.2 were heterozygous for *GLO*. (GLO 2-1). Thus, a GLO type could not be assigned to a specific haplotype in these individuals. Symbols used in pedigrees: □○, Normal male and female, respectively (with respect to peripheral rheumatoid arthritis). ■●, Diseased male and female, respectively. ⊠⊘, Untested male and female, respectively. ▨⊘, Status with respect to peripheral rheumatoid arthritis indeterminant or unknown (see text). a, *Bw35,A10* or *B blank,A10*; b, *B13,Aw30*; c, *DRw7,Bw35, Cw4,A11*; d, *Bw22,A11*; e, *B8,A1*; f, *B12,A1*; g, *DRw7,Bw35 Cw4,Aw24*; h, *DRw5,B40,Cw3,Aw24*; dec, deceased.

*HLA-A* and *B* loci were treated as a single segregating unit (haplotype) because HLA genotypes were ascertained in all but three individuals (see below) and recombinants were not observed between the *A* and *B* loci (22, 24). Probabilities for individuals, I.1, I.2, and II. 6 in family 2 being homozygous or heterozygous were calculated from population data derived from a United States Caucasian population (24). These probabilities were used to weight the likelihoods of all the permutations incurred because of indeterminant genotypes.

The scheme for HLA linkage analysis (21) was modified for individuals I.1 and I.2 in family 2 who could be either homozygous or heterozygous at the HLA locus. The HLA genotype of I.1 in family 2 could be homozygous *B7,A2* or any heterozygous genotype with *B7,A2* as one of the haplotypes. Similarly, II.6 in family 2 could be homozygous *B40,A2* or *B40, blank* or any other heterozygous genotype with *B40,A2* or *B40, blank* as one of the haplotypes. If $u$ or $v$ are two of the four HLA alleles in a simple scheme (21) with corresponding gene frequencies $P_u$ and $P_v$, whose values can be

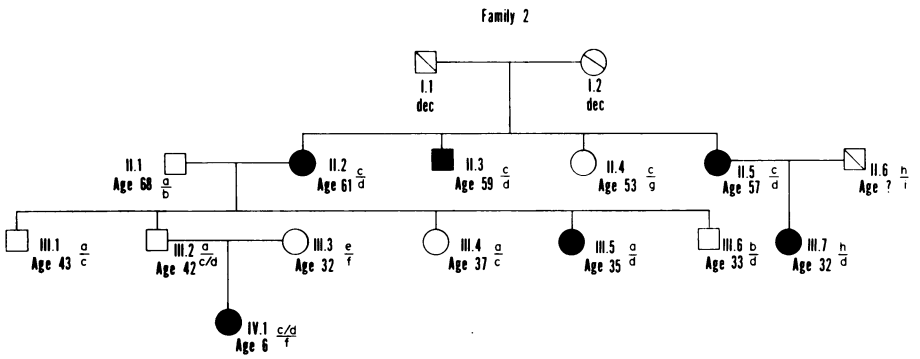


FIGURE 2 Chromosome 6 haplotypes, family 2. a, *GLO²,DRw5,B12,Aw31*; b, *GLO²,DRw5, B12,A2*; c, *GLO¹,DRw2,B7,A2*; d, *GLO²,DRw1,Bw35,A2*; c/d, *GLO²,DRw2,B7,A2*; e, *GLO²,DR blank,B18,A3*; f, *GLO²,DR blank,B blank,A29* or *GLO²,DR blank,B18,A29*; g, *B18,Aw30*; h, *(B40,A2)* or *(B40,A blank)*; dec, deceased; i, *(B?,A?)*; *individual was untyped; second haplotype could not be assigned through testing of progeny. See Fig. 1 for legend to symbols.

chosen freely, then an individual is homozygous *uu* and heterozygous *uv* with prior probabilities $P_u 2$ and $2P_uP_v$. The ratio of the probabilities for homozygosity and heterozygosity is then given by $P(uu):P(uv) = P_u:2P_v$, i.e., as an extension of the simple scheme of HLA linkage analysis (21), the proper prior probability of homozygosity, as determined from the table of haplotype frequencies (24), can be obtained simply by the appropriate ratio of $P_u$ to $P_v$. For the purposes of the computer analysis, an artificial phenotype is then given to such an individual so that it can only have the genotypes *uu* and *uv*. The relative probability of these alternatives was taken from a table of population haplotype frequencies (24). This table was adapted to the specificities typed for in Houston, i.e., some specificities in the table had to be merged with the blank alleles.

## RESULTS

*Family 1 (Fig. 1).* There were seven people with some manifestations of arthritis in this family (Table I). The proband (IV.1) had pauciarticular JRA. Her mother, III.2, has had severe polyarticular JRA, still active at this time, and the mother's siblings, III.3 and III.4, have had objective and subjective evidence, respectively, of arthritis. Moreover, III.2 and III.4 had abnormal levels of antinuclear antibody. All family members with arthritic manifestations inherited the c haplotype (*Drw7,Bw35,Cw4,A11*) from the maternal grandmother, I.2, who had a history consistent with JRA in childhood. Sibling IV.2, who is haploidentical with the proband as indicated by serotyping and the MLC tests (Table II), also inherited the c haplotype but not the disease. Because IV.2 could develop arthritis later on, we classified her phenotype with respect to arthritis as "unknown" in the pedigree analysis.

*Family 2 (Fig. 2).* In this family, all members with a history or objective evidence of arthritis except the proband inherited the d haplotype (*GLO²,DRw1, Bw35,A2*). MLC and DR testing (Table III) indicated that the reference patient's father (III.2) was HLA-D identical to his brother and sister, III.1 and III.4, both of whom had inherited the c haplotype (*GLO¹, DRw2,B7,A2*) from their afflicted mother, II.2. GLO typing, however, showed that III.2 carried *GLO²*,

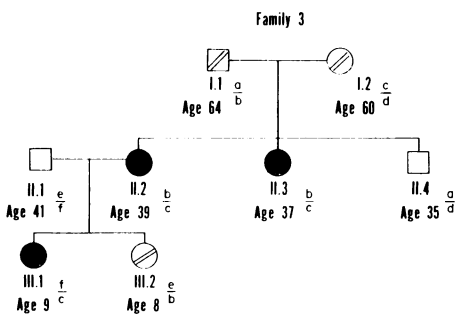


FIGURE 3 Chromosome 6 haplotypes, family 3. a, *B12,A11*; b, *B blank, A11*; c, *B7,A3*; d, *Bw35,A3*; e, *B12,A2*; f, *B15,A9*. See Fig. 1 for legend to symbols.

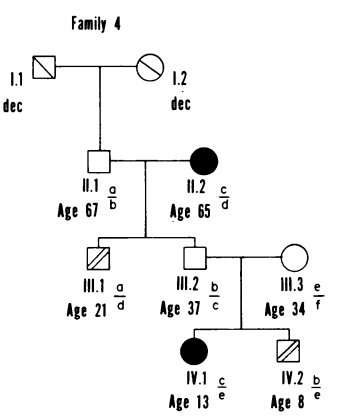


FIGURE 4 Chromosome 6 haplotypes, family 4. a, *B8,A2*; b, *B27,A3*; c, *B40,A2*; d, *B12,A1*; e, *B7,A11*; f, *B27,A3*; dec, deceased. See Fig. 1 for legend to symbols.

## TABLE I
*Clinical Features of Disease in Family 1*

| Identity | Diagnosis | Age at onset | Duration of disease | Course of involvement | Joints involved | MS Swelling POM LOM | X-ray findings | ANA RF | Source of information |
|---|---|---|---|---|---|---|---|---|---|
| | | | *yr* | | | | | | |
| Family 1 | | | | | | | | | |
| II.1 Maternal great-uncle | Probable RA (16) | 50 | 12 still active | Continuous | (L) Shoulder cervical, and thoracic spine | LOM and POM (L) shoulder and cervical spine | Neg-(L) shoulder | ANA and RF Neg. | Investigator E.B. |
| II.3 Maternal great-uncle | Probable RA (16) | 44 | 5 yr still active | Continuous in shoulders, flare in hips every 2–3 mo | Hips, shoulders | POM and LOM shoulders and hips | Neg-Hips | ANA and RF Neg. | Investigator E.B. |
| II.5 Maternal grandmother | Unclassified probable arthritis as child | 7–8 | Unknown | Unknown | Hips | POM-Hips, had to be carried | Not done | Not done | By history |
| | | 62 | 1 yr still active | Continuous | Shoulders with bursitis | POM and LOM shoulders | Not done | | Attending physician |
| III.2 Mother | JRA polyarticular (15) | 7 | 37 yr still active | Continuous | TMJ, shoulders, elbows, wrists, MCP, PIP, DIP, hips, knees, MTP, toes, cervical, thoracic, and lumbar spine SI joint | POM-7 swelling-6 joints LOM-59 joints MS | Cervical spine and hands, marked osteoporosis, bony ankylosis, dislocations, fusion | ANA->1:40 RF-Neg. | Investigator E.B. |
| III.3 Maternal uncle | Definite RA (16) | 38 | 8 yr still active | Continuous with flare every 7–10 d | TMJ, elbows, MCP, PIP, (R) hip, tarsus | POM-28 joints, swelling—8 joints | Hands, possible early erosion | ANA-Neg. RF-Neg. | Investigator E.B. |
| III.4 Maternal aunt | Possible RA (16) | 33 | 11 yr still active | Continuous with increased pain on prolonged use | Hands, cervical and lumbar spine | POM-4 joints MS | Neg. | ANA->1:40 RF-Neg. | Investigator E.B. |
| IV.1 Reference patient | Pauciarticular JRA onset, pauciarticular course (15) | 14 | 4 yr remission | Continuous for 1 yr then occasional flares | MTP, cervical and thoracic spine | POM-3 joints, swelling-1 joint | Cervical spine erosion in $C_3$–$C_6$ | ANA-Neg. RF-Neg. | Investigator E.B. |

Abbreviations used in this table: DIP, distalinterphalangeal; L, left; LOM, loss of motion; MCP, metacarpophalangeal; MS, morning stiffness; PIP, proximalinterphalangeal; POM, pain on motion; R, right; SI, sacroiliac; TMJ, temporomandibular joint.

TABLE II
*MLC Testing in Family 1*

| Responder cell (haplotype) | Stimulator cell (mitomycin-treated) | | | | | | |
|---|---|---|---|---|---|---|---|
| | IV.1m* | III.1m | III.2m | IV.2m | Xm | Ym | PHA‡ |
| IV.1§ | 2,714‖ | 49,288 | 35,618 | 38,937 | 62,826 | 110,677 | 64,398 |
| (g/c) | 1.0 | 18.2 | 13.1 | 14.9 | 23.2 | 15.0 | 23.7 |
| III.1¶ | 53,215 | 1,013 | 46,734 | 33,206 | 9,946 | 9,595 | 83,017 |
| (g/h) | 52.5 | 1.0 | 46.1 | 32.6 | 9.8 | 9.5 | 82.0 |
| III.2** | 35,113 | 55,837 | 1,409 | 15,353 | 23,257 | 17,112 | 59,706 |
| (b/c) | 24.9 | 39.6 | 1.0 | 10.9 | 16.5 | 12.1 | 47.4 |
| IV.2‡‡ | 40,787 | 39,340 | 25,060 | 576 | 32,412 | 28,703 | 66,830 |
| (h/c) | 70.8 | 68.3 | 43.5 | 1.0 | 56.3 | 49.8 | 463.3 |
| X§§ | 42,437 | 30,536 | 28,878 | 32,353 | 523 | 12,150 | 69,897 |
| | 81.1 | 58.4 | 55.2 | 61.9 | 1.0 | 23.2 | 133.7 |
| Y§§ | 45,089 | ND‖‖ | 29,317 | 24,619 | 14,327 | 775 | 294,661 |
| | 58.2 | | 37.8 | 31.8 | 18.5 | 1.0 | 380.2 |
| IV.1¶¶ | 2,452 | 26,771 | 24,619 | 52,924 | 74,234 | 59,709 | 249,756 |
| | 1 | 10.9 | 10.0 | 21.6 | 30.3 | 24.4 | 101.9 |

* Mitomycin-treated.
‡ Phytohemagglutinin.
§ Proband.
‖ Counts per minute of [$^3$H]thymidine incorporation above, stimulation index below.
¶ Proband's father.
** Proband's mother.
‡‡ Proband's sibling.
§§ Unrelated lymphocyte donors.
‖‖ Test was not done.
¶¶ Cultures done in plasma autologous to responder cell.

TABLE III
*MLC Testing in Family 2*

| Responder cell (haplotype) | Stimulator cell (Mitomycin-treated) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | II.2m | III.1m | III.2m | III.4m | III.5m | III.6m | Xm | Zm | PHA | SK-SD |
| II.2 | 775 | 13,391 | 19,956 | 16,748 | 16,469 | 12,582 | 28,397 | 22,584 | 261,411 | 36,927 |
| (c/d) | 1.0 | 17.3 | 25.8 | 21.6 | 21.3 | 16.2 | 36.6 | 29.1 | 337.0 | 47.7 |
| III.1 | 18,766 | 1,769 | 1,821 | 1,534 | 14,176 | 19,190 | 25,439 | 11,621 | 224,825 | 24,170 |
| (a/c) | 10.6 | 1.0 | 1.0 | 0.9 | 8.0 | 10.9 | 14.4 | 6.6 | 127.0 | 13.7 |
| III.2 | 22,108 | 2,670 | 2,183 | 3,178 | 18,099 | 30,220 | 22,681 | 12,511 | 356,300 | 30,327 |
| (a/cd) | 10.2 | 1.2 | 1.0 | 1.5 | 8.3 | 13.8 | 10.4 | 5.7 | 164.0 | 13.9 |
| III.4 | 9,692 | 1,421 | 2,020 | 2,149 | 11,225 | 15,307 | 14,440 | 8,971 | 168,515 | 21,868 |
| (a/c) | 4.5 | 0.7 | 0.9 | 1.0 | 5.2 | 7.1 | 6.7 | 4.2 | 78.4 | 10.2 |
| III.5 | 11,199 | 13,068 | 13,568 | 12,583 | 1,282 | 7,257 | 14,049 | 8,258 | 203,932 | 33,446 |
| (a/d) | 8.7 | 10.2 | 10.6 | 9.8 | 1.0 | 5.7 | 11.0 | 6.4 | 159.0 | 26.1 |
| III.6 | 10,202 | 12,924 | 19,733 | 19,357 | 6,732 | 997 | 12,570 | 10,822 | 174,789 | 48,208 |
| (b/d) | 10.2 | 13.0 | 19.8 | 19.4 | 6.8 | 1.0 | 12.6 | 10.9 | 175.0 | 48.3 |
| X | 28,329 | 19,765 | 20,570 | 19,572 | 19,551 | 11,655 | 1,794 | 11,059 | 323,718 | 24,382 |
| | 15.8 | 11.0 | 11.5 | 10.9 | 10.9 | 6.5 | 1.0 | 6.2 | 180.0 | 13.6 |
| Z | 24,482 | 12,810 | 19,838 | 18,360 | 17,608 | 28,114 | 14,652 | 1,357 | 263,598 | 15,008 |
| | 18.0 | 10.2 | 14.6 | 13.5 | 13.0 | 20.7 | 10.8 | 1.0 | 194.0 | 11.1 |

X, Z, unrelated cell donor. Other legends as in Table II.

usually coupled with the d haplotype (*GLO*$^2$,*Drw1*, *Bw35*,*A2*) in this family. Thus, the proband's father was the product of a crossover centromeric to *HLA-D* between the maternal c and d haplotypes. The reference patient inherited c/d (*GLO*$^2$,*DRw2*,*B7*,*A2*) from her father indicating that she also inherited sixth chromosome genes centromeric to *HLA-D* on the d haplotype. Not considering those who were c/d recombinants, five of the six carrying the d haplotype had some manifestation of peripheral arthritis (Table IV). In the paternal grandmother (II.2) and paternal aunt (III.5) the disease was classified, respectively, as pauciarticular JRA and systemic onset JRA. In other family members, II.5 and III.7, the manifestations of disease were largely subjective and did not conform to standard classification criteria.

*Family 3 (Fig. 3).* This family exhibited a complex relationship between HLA and disease. Members of this family had two commonly associated manifestations of rheumatic disease in children; arthritis and iritis, in various combinations. Arthritis was inherited with the c haplotype (*B7*,*A3*) derived from the grandmother, I.2. She had no history of arthritis, but she did have ANA and was the only subject in any of these four families to have a positive C1q binding test for circulating immune complexes. The grandfather (I.1) had episodes of joint pain and swelling beginning at age 12 that generally lasted <3 d, without any history of ocular abnormality. For the purpose of the linkage analysis, both I.1 and I.2 were classified as having an indeterminant genotype with respect to arthritis. In generation II, iritis occurred together with arthritis in the reference patient's mother, II.2, and her MLC identical sibling, II.3 (see Table V). II.3 had objective evidence of arthritis whereas the manifestations of disease in II.2 were chiefly subjective (Table VI). In generation III, III.1 had typical pauciarticular onset JRA with iritis. Her MHC nonidentical sibling, III.2, has had iritis but has not had arthritis up to now. For the purpose of linkage analysis, she was also classified as having an indeterminant genotype for arthritis. It is interesting that results of the MLC test suggest that III.2 and her grandfather, I.1, have very similar HLA-D antigens (see Table V).

In testing linkage between HLA and a proposed gene for iritis in this family, we obtained a negative lod score at all penetrance values. Thus the calculations favored the interpretation that iritis occurred independent of the inheritance of MHC. In contrast, the analysis of linkage of HLA to the disease susceptibility gene in this family produced a lod score of 0.461, assuming 80% penetrance, and a 5% recombination frequency.

*Family 4 (Fig. 4).* In this family, there were four members with arthritis (Table VII). The proband (IV.1) had polyarticular JRA. Her father (III.2) had AS and her grandmother (II.2) had severe, erosive adult-onset RA. Her great-grandfather (I.1), whom we could not study, had a history consistent with AS. For the purpose of linkage analysis in this family, we considered peripheral polyarthritis as impenetrant in the proband's father (III.2), who had only AS.

*Linkage analysis.* Because there were no previous estimates for the penetrance of a susceptibility gene for arthritis, penetrance was established jointly with the recombination fraction by first calculating the likelihood of the pedigrees for various penetrance values and recombination frequencies and then selecting that penetrance value and recombination frequency, giving the maximum likelihood (not lod score) for the combined pedigrees (see Table VIII). The maximum-likelihood estimates (Table VIII) were 80% for penetrance and 5% for the recombination frequency. This table also shows that the estimate of penetrance is the same whether the recombination fraction is arbitrarily set at 50% or is obtained simultaneously with the estimate of the recombination fraction. The value, $f = 0.80$, for the penetrance is also compatible with the estimated gene frequency, $P = 0.01$, and the published value, $r = 1.5$ to 2.5%, for the incidences of RA in the population (23), because for a dominant mode of inheritance, these three values have an approximate relationship given by $r = 2fP$.

The estimate of the penetrance was obtained without correcting for the fact that our protocol required more than one affected member for the family to be included in this study. An ascertainment correction would decrease the estimate of the penetrance somewhat, but probably not to a value <70%. For these four families, lower penetrance values are associated with somewhat smaller lod scores (Table VIII) so that the reported lod scores uncorrected for ascertainment may be a little too high. Lod scores were calculated using 80% penetrance (22) as shown in Table IX. The cumulative lod score for the four families of 2.7 at a recombination frequency of 5% means that the odds for a close linkage of HLA with the postulated disease susceptibility gene, *RLA-1*, are 500:1.

## DISCUSSION

Because many patients in these families complained of rheumatic disease that did not meet the criteria for classical or definite RA, it was often difficult to determine whether we should classify a family member as affected. At the outset, we chose to call affected all family members having symptoms of persistent arthritis, including many who did not have classical or definite RA. We chose to include people with less well defined disease because even among family members who fit stringent diagnostic criteria there was considerable heterogeneity. For example, in family 1,

## TABLE IV
*Clinical Features of Disease in Family 2*

| Identity | Diagnosis | Age at onset | Duration of disease | Course of involvement | Joints involved | MS swelling POM LOM | X-ray findings | ANA RF | Source of information |
|---|---|---|---|---|---|---|---|---|---|
| | | *yr* | | | | | | | |
| Family 2 | | | | | | | | | |
| II.2 Paternal grandmother | Pauciarticular onset, JRA poly-articular course (15) | 6 | 55 still active | Continuous with flares | (L) Elbow PIP, DIP, knees, MTP, hips, thoracic spine | LOM-18 joints POM-25 joints swelling-18 joints | Fingers, erosive changes of PIP and DIP | ANA-Neg. RF-Neg. | Investigator E.B. |
| II.3 Paternal great uncle | Probable RA (16) | 38 | 17 remis-sion now | Continuous swelling with several severe flares per year | (R) Elbow, shoulders, hands | MS and swelling POM (R) elbow, and hands, POM-shoulders | Neg. | ANA-Neg. RF-Neg. | Investigator E.B. |
| II.5 Paternal great-aunt | Unclassified probable arthritis as a child | 9 | Several | Continuous to teenage | Hips, ankles, hands, shoulders | POM-hips, ankles, hands, shoulders | Neg. | ANA-Neg. RF-Neg. | Investigator E.B. |
| III.5 Paternal aunt | Systemic JRA with rash (15) | 15 | 21 still active | Continuous with intermittent flares | Knees, cervical spine, PIP, DIP | MS, POM-knees LOM-knees, cervical spine, swelling-PIP, DIP | Neg. | ANA-Neg. RF-Neg. | Investigator E.B. |
| III.7 Paternal 2nd cousin | Possible RA (16) | Teen-age | 16 still active | Has occasional flare with complete remission | SI joint, lumbar spine, legs | POM and LOM of spine, MS | Neg. | ANA-Neg. RF-Neg. | Investigator E.B. |
| IV.1 Reference patient | Systemic JRA with rash (15) | 5 | 4 still active | Continuous with flares | Elbows, knees, (L) wrist 2nd MCP | POM-elbows, knees, swelling-2nd MCP, Knees, 2nd PIP (R) | Neg. | ANA-Neg. RF-Neg. | Investigator E.B. |

For abbreviations see Table I.

TABLE V
*MLC Testing in Family 3*

| Responder cell (haplotype) | Stimulator cell (mitomycin-treated) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | I.1m | II.4m | II.3m | II.2m | III.2m | III.1m | Xm* | Ym* | PHA |
| I.1 | 1,317 | 16,582 | 17,731 | 16,169 | 2,516 | 25,858 | 21,015 | 32,069 | 314,181 |
| (a/b) | 1.0 | 10.3 | 13.5 | 12.3 | 1.9 | 19.6 | 16.0 | 24.4 | 238.6 |
| II.4 | 9,618 | 1,527 | 12,753 | 13,922 | 4,655 | 12,212 | 10,114 | 23,813 | 300,982 |
| (a/d) | 6.3 | 1.0 | 8.4 | 9.1 | 3.1 | 8.0 | 6.6 | 15.6 | 1197.1 |
| II.3 | 10,698 | 12,168 | 1,423 | 3,098 | 4,101 | 12,434 | 13,354 | 10,653 | 309,406 |
| (b/c) | 7.5 | 8.6 | 1.0 | 2.2 | 2.9 | 8.1 | 9.4 | 7.5 | 217.4 |
| II.2 | 6,787 | 10,911 | 1,074 | 590 | 3,327 | 9,200 | 10,233 | 16,598 | 349,087 |
| (b/c) | 11.5 | 18.5 | 1.8 | 1.0 | 5.6 | 15.6 | 17.3 | 28.1 | 591.7 |
| III.2 | 1,111 | 8,674 | 6,361 | 5,127 | 518 | 12,307 | 8,208 | 15,540 | 457,022 |
| (e/b) | 2.1 | 16.8 | 12.3 | 9.9 | 1.0 | 23.2 | 15.9 | 30.0 | 882.3 |
| III.1 | 14,971 | 8,454 | 6,635 | 10,542 | 9,180 | 1,117 | 9,856 | 15,617 | 193,637 |
| (f/c) | 13.4 | 7.6 | 5.9 | 9.4 | 8.2 | 1.0 | 8.8 | 14.0 | 173.4 |
| X* | 17,859 | 32,822 | 26,319 | 24,562 | 21,916 | 22,914 | 1,133 | 11,268 | 326,038 |
| | 15.8 | 29.0 | 23.2 | 21.7 | 19.3 | 29.9 | 1.0 | 10.0 | 287.7 |
| Y* | 35,334 | 41,047 | 31,255 | 10,464 | 25,413 | 13,018 | 12,208 | 1,361 | 221,825 |
| | 26.9 | 31.2 | 23.8 | 8.0 | 19.3 | 9.9 | 9.3 | 1.0 | 168.6 |

* X, Y, unrelated individuals. Other legends as in Table II.

the reference patient had pauciarticular onset JRA and her mother had polyarticular JRA. In family 2, the proband and her paternal aunt had systemic JRA with rash whereas her paternal grandmother had pauciarticular onset JRA with a polyarticular course. In family 4, the proband had polyarticular onset JRA whereas her paternal grandmother had classical RA, adult-onset type.

TABLE VI
*Clinical Features of Disease in Family 3*

| Identity | Diagnosis | Age at onset | Duration of disease | Course of involvement | Joints involved | MS swelling POM LOM | X ray findings | ANA RF | Source of information |
|---|---|---|---|---|---|---|---|---|---|
| | | *yr* | | | | | | | |
| Family 3 | | | | | | | | | |
| I.2* Maternal grandmother | No complaints | — | — | — | — | — | — | ANA->1:40 RF-Neg. Clq binding activity 12.1% | Investigator E.B. |
| II.2 Mother | Unclassified arthralgia with iritis | 24 | 15 | Remission with yearly flares | Elbows, wrists | POM-elbows and wrists | Neg. | ANA-Neg. RF-Neg. | Investigator E.B. |
| II.3 Maternal aunt | Probable pauciarticular onset with iritis (15) | 15 | 22 | Remission with flares few times per year | Hips, wrists, lumbar spine | POM and swelling of tarsal area, and wrists, POM-hips, lumbar spine | Neg. | ANA-Neg. RF-Neg. | Investigator E.B. |
| III.1 Reference patient | Pauciarticular onset JRA with iritis (15) | 8 | 8 still flares | Continuous with flares | Knees, ankles, elbows, shoulders, hips and wrists | POM-elbows and wrists | Neg. | ANA/Neg. RF-Neg. | Investigator E.B. |
| III.2 Sister | Iritis no arthritis | — | — | — | — | — | — | ANA-Neg. RF-Neg. | Investigator E.B. |

* Classified as phenotype indeterminant for purpose of linkage analysis, see Results.
For abbreviations see Table I.

## TABLE VII
*Clinical Features of Disease in Family 4*

| Identity | Diagnosis | Age at onset | Duration of disease | Course of involvement | Joints involved | MS swelling POM LOM | X ray findings | ANA RF | Source of information |
|---|---|---|---|---|---|---|---|---|---|
| | | | *yr* | | | | | | |
| Family 4 | | | | | | | | | |
| I.1 Paternal great-grandfather | Possible AS | Unknown | Unknown, deceased at age 70 | Continuous from onset | Severe erosive destructive disease | LOM-severe of entire spine | Unknown | Unknown | By history |
| II.2 Paternal grandmother | Classical RA (16) | 48 | 15 still active | Continuous from onset | Toes, ankles, MCP, PIP, DIP, hips, TMJ, cervical spine | MS POM-fingers, hips, ankles, cervical spine, LOM-fingers, hips, cervical spine | Severe erosive destructive disease of fingers, and 3 cervical vertebrae | Not done | Attending physician and investigator E.B. |
| III.2 Father | AS | 17 | 20 still active | Continuous from onset | Lumbar, thoracic and cervical spine, knees, MCP, MTP | Swelling-knees, MTP, POM-Toes and entire spine LOM-entire spine | Sclerosis of SI joint, degenerative changes of cervical spine | ANA-Neg. RF-Neg. | Investigator E.B. |
| IV.1 Reference patient | Polyarticular JRA (15) | 12 | 2.5 in remission | Continuous with flares then remission | (L) Hip, (R) shoulder, (R) elbow, lumbar spine, knees, ankles | POM-knees, (R) shoulder, (R) elbow, lumbar spine swelling-(L) ankle | Neg. | ANA-Neg. RF-Neg. | Investigator E.B. |

For abbreviations see Table I.

TABLE VIII

*Total $Log_{10}$ Likelihood (L, Adjusted to Obtain Positive Values) and Lod Scores (z) for the Four Families*

| Recombination fraction | Penetrance | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 95% | 90% | 85% | 80% | 75% | 70% | 50% | 25% |
| 0.50 | | | | | | | | |
| *L* | 7.069 | 7.920 | 8.282 | 8.420 | 8.418 | 8.310 | 7.049 | 0.280 |
| *z* | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.40 | | | | | | | | |
| *L* | 7.516 | 8.359 | 8.712 | 8.841 | 8.830 | 8.714 | 7.419 | 0.646 |
| *z* | 0.446 | 0.439 | 0.430 | 0.421 | 0.412 | 0.403 | 0.370 | 0.365 |
| 0.30 | | | | | | | | |
| *L* | 8.281 | 9.120 | 9.460 | 9.574 | 9.547 | 9.414 | 8.050 | 1.285 |
| *z* | 1.212 | 1.200 | 1.178 | 1.154 | 1.129 | 1.103 | 1.001 | 1.005 |
| 0.20 | | | | | | | | |
| *L* | 9.050 | 9.906 | 10.248 | 10.356 | 10.321 | 10.177 | 8.766 | 2.004 |
| *z* | 1.981 | 1.986 | 1.965 | 1.936 | 1.903 | 1.867 | 1.716 | 1.723 |
| 0.15 | | | | | | | | |
| *L* | 9.362 | 10.236 | 10.586 | 10.698 | 10.662 | 10.518 | 9.098 | 2.335 |
| *z* | 2.293 | 2.316 | 2.303 | 2.277 | 2.244 | 2.207 | 2.048 | 2.054 |
| 0.10 | | | | | | | | |
| *L* | 9.584 | 10.487 | 10.852 | 10.972 | 10.940 | 10.798 | 9.385 | 2.624 |
| *z* | 2.514 | 2.567 | 2.570 | 2.551 | 2.522 | 2.487 | 2.335 | 2.344 |
| 0.05 | | | | | | | | |
| *L* | 9.635 | 10.593 | 10.984 | 11.119* | 11.097 | 10.962 | 9.590 | 2.848 |
| *z* | 2.566 | 2.673 | 2.702 | 2.699* | 2.680 | 2.652 | 2.540 | 2.567 |
| 0.00 | | | | | | | | |
| *L* | 9.034 | 10.124 | 10.572 | 10.745 | 10.760 | 10.667 | 9.573 | 2.949 |
| *z* | 1.964 | 2.203 | 2.289 | 2.325 | 2.342 | 2.356 | 2.524 | 2.668 |

* Position of maximum likelihood.

It may be worth pointing out that HLA typing and MLC testing results were assembled independently from the clinical evaluations. Data on the HLA phenotype and a decision concerning the presence or absence of rheumatic disease were entered into the computer. The program, LIPED, then systematically calculated the likelihood of the phenotypes occurring as they did because of genetic linkage.

In assigning the phenotypes for disease, we permitted ourselves three choices: affected, not affected, and indeterminant or "unknown." We did not classify people who had osteoarthritis, nor those who had definite AS as affected. We excluded people with AS because AS has very different clinical manifestations and course and is unquestionably associated with HLA-B27, whereas RA in adults and juveniles is not associated with any particular HLA-A or B specificity. The indeterminate or unknown classification was reserved for people who did not have peripheral arthritis but might later get the disease,

TABLE IX

*Individual and Cumulative Lod Scores for Genetic Linkage between HLA-A,B and the Disease Susceptibility Gene (Penetrance, 80%)*

| Family | Recombination fraction | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0.00 | 0.05 | 0.10 | 0.15 | 0.20 | 0.30 | 0.40 |
| 1 | 1.893 | 1.702 | 1.503 | 1.293 | 1.073 | 0.611 | 0.167 |
| 2 | −0.385 | 0.263 | 0.402 | 0.428 | 0.399 | 0.262 | 0.108 |
| 3 | 0.523 | 0.461 | 0.397 | 0.331 | 0.265 | 0.138 | 0.039 |
| 4 | 0.295 | 0.273 | 0.250 | 0.226 | 0.200 | 0.143 | 0.077 |
| Total | 2.325 | 2.699* | 2.551 | 2.277 | 1.936 | 1.154 | 0.421 |

* Position of maximum likelihood.

either because they were still young or because they had clinical manifestations often associated with RA. For example, in family 3, the grandfather (I.1) of the proband had had joint pains for short periods (<3 d) as a child. Because the pains were not persistent, he was given an indeterminant classification. Similarly, his wife (I.2) had no joint complaints but she was the only person in the whole study who had an abnormal C1q binding test consistent with the presence of circulating immune complexes. Her ANA was also abnormally high (>1:40). Thus it was possible that she had a subclinical collagen-vascular disease. She was given an indeterminant classification as well. Analysis of the pedigree in family 3 indicated that the haplotype bearing the susceptibility allele for arthritis was inherited from this lady (I.2). Considering her as indeterminant for RA in the linkage analysis produced a lod score of 0.461 in this family. Had she been classified as affected, the lod score for linkage between HLA and the occurrence of arthritis would increase to 0.750. This would increase the combined lod score to 2.99 with an increase in the odds in favor of linkage to 974:1, for all four families.

To include people with relatively mild or atypical disease in such a study may seem heretical in view of the great efforts that have been made to classify patients with adult- (16) or childhood-onset RA (15) on the basis of objective clinical criteria. It may seem particularly unreasonable to suggest that there can be a common genetic basis for such diverse disease manifestations. However, considering the difficulties involved in classifying rheumatic disease, it may be even more arbitrary to exclude individuals from analysis in family studies because their clinical presentation does not fit accepted patterns for presentation of rheumatoid arthritis. This may be particularly important in children with rheumatic diseases in whom symptoms and signs of disease may change substantially as the illness progresses. Indeed, in a recent 5-yr study of 186 cases in both the United States and Russia, 8 and 10%, respectively, of the cases with pauci- and polyarticular-onset JRA and 64% of those with systemic-onset disease transformed into a different type of JRA sometime during the 5-yr follow-up period. (Data from the ongoing United States-Russia collaborative study group for JRA.) Thus, clinical features that permit initial classification of JRA are hardly a permanent feature of the disease.

Similar variability in clinical manifestations is seen in other inherited diseases, notably sickle cell anemia (25) and the bleeding disorder, Von Willebrand's disease (26). Von Willebrand's disease is a classical example of disease with an autosomal dominant pattern of inheritance that shows as great a clinical variability as is seen in these family members with rheumatic complaints. Bleeding can be spontaneous and severe or the clotting abnormality may be evident only upon laboratory testing. Factor VIII levels may vary from 0 to 40% of normal in afflicted people (26).

Although the genetic lesion in sickle-cell disease is restricted to a single amino-acid substitution, there is great variability among individuals in the degree of organ involvement and the severity of symptoms. Recently, it has been recognized that the disease can be attenuated by persistence of fetal hemoglobin in the erythrocyte (25, 27), an event presumably under separate genetic control. The clinical manifestations of $\beta$ thalassemia may also be favorably influenced by the persistence of fetal hemoglobin (27). Thus, in other well-characterized hereditary diseases, considerable variation in signs, symptoms, and clinical course can occur that apparently reflects the influence of environmental or genetic factors independent of the genes causing the disease in the first place.

Our data suggest that the situation may be similar in certain types of rheumatoid arthritis and support the hypothesis that a single major gene, *RLA-1*, linked to the MHC may be responsible for occurrence of disease.

Of course the clinical manifestations may also be influenced by environmental or genetic influences independent of MHC. For example, pauciarticular arthritis and iritis frequently occur together in female children with JRA. However, they were not separate manifestations of the same genetic influences in family 3. The linkage analysis in this family supported the hypothesis that HLA was linked to the disease susceptibility locus, *RLA-1*, for arthritis, whereas iritis occurred independent of the inheritance of HLA. Genetic factors that could modify the signs and symptoms of the disease include: (*a*) linked or nonlinked minor modifying genes, (*b*) a *RLA-1* locus which is in fact a cluster of very closely linked genes exerting additive effects on the phenotype, or (*c*) a *RLA-1* locus that may have multiple alleles for susceptibility. Each allele may have a different influence on the disease (28).

The idea that multiple interacting genetic influences are responsible for the clinical manifestations of arthritic disease has been raised previously by Kidd et al. (29) in discussing the genetic basis for AS. The assumed impenetrance of AS (30) may not actually be impenetrance in the true sense, but an effect of independent genes, more than one of which are required for the acquisition of the disease (29).

However in our study, the pattern of inheritance in families 2 and 4 suggest a monogenic, rather than a multigenic mode of inheritance for RA. This point is supported strongly in the immediate family of individual III.2 of family 2. This healthy individual appears to represent classical impenetrance of a domi-

nant gene. He and individual III.2 in family 4 appear to be unaffected carriers of *RLA-1*. Individual III.6 in family 2, who also inherited the HLA haplotype linked to *RLA-1*, also appears to have been healthy up to age 33. Note that the estimated high penetrance of *RLA-1* in this study (80%) has not been corrected for biases introduced by the selection of families having two or more afflicted members.

Placement of the *RLA-1* gene in relation to *HLA-D* and *HLA-DR* was possible in family 2 because of an informative recombinant located centromeric to *HLA-DR*. The father (III.2) of the reference patient was not only impenetrant for *RLA-1*; he also demonstrated a crossover between *GLO* and *HLA-D* in the maternal c and d haplotypes. Because the d haplotype was found in other affected family members, and III.2 inherited $GLO^2$ from the d haplotype along with the *HLA-D* antigens of the c haplotype, (Table III), we postulated that *RLA-1* was centromeric to *HLA-D* on the sixth chromosome.

Although it might appear unusual that we should have an HLA recombinant in one of only four families, Raum et al. (31) have previously pointed out that there is an unexpectedly high frequency of recombination events in families of patients with JRA or systemic lupus erythematosus.

Stastny and his collaborators (4–6) have observed a strong association between the occurrence of seropositive, definite or classical adult-onset RA and inheritance of HLA-Dw4 or DRw4. Surprisingly, Dw4 occurs with a significantly reduced frequency in populations with JRA in which the frequency of HLA-Dw7 and -Dw8 is significantly increased (7). Stastny and Fink did not observe abnormal frequencies of HLA-A or -B antigens in JRA, but the incidence of positive reactions with a newly discovered typing cell, TMo, were also increased, especially in patients with persistent pauciarticular disease (7). HLA-D typing studies in 46 children with JRA in which two typing cells specific for Dwl and two specific for Dw3 were used, have suggested that HLA-Dw3 may also occur more frequently than expected in patients with JRA (9). Thus, genes conferring a susceptibility for both adult-onset RA and juvenile RA may be associated with certain HLA-D antigens. However, the fact that different HLA-D antigens occur with increased frequency in populations with JRA and the adult-onset disease, raises the possibility that there may be different susceptibility genes for these nosologic entities. This consideration is strengthened by Stastny and Fink's (7) observation that the lowest frequency of HLA-Dw4 occurs in children who had persistent pauciarticular-onset disease, the same group that had the highest frequency of HLA-Dw7, -Dw8 and TMo.

Although some dissent to this concept has been registered recently (32, 33), there is evidence that HLA-Dw4 is principally associated with a subset of patients with adult-onset RA. The frequency of HLA-Dw4 is especially increased in Caucasians who have severe, seropositive Ra which is relatively refractory to treatment (13, 34, 35). For example, DRw4 was found in 60% of patients who required gold, penicillamine, or steroids to control disease activity whereas only 20% of those adequately treated with aspirin had DRw4 (35). Considering the strength of these associations, it is not surprising in kindreds with multiple cases of severe seropositive adult-onset RA that all affected individuals had HLA-DRw4 (35, 36).

Clinically, the arthritis in the adult family members reported in the present study was very different from that reported in HLA-Dw4-associated RA. A few of our patients had abnormal titers of ANA but none were seropositive and in a number of cases there was no evidence of progressive, deforming, or erosive arthritis. Neither HLA-DRw4 nor -DRw3 were found in the two families in the present study who were tested for HLA-DR. Thus, on clinical and serologic grounds, the arthritis in these families is different from that associated with HLA-Dw4 or -DRw4 in adults.

Further investigation will be necessary to determine whether *RLA-1* confers susceptibility to a different type of arthritis than has been associated with -Dw7, -Dw8, and TMo in children. However, at present, we cannot exclude the possibility that *RLA-1* is only one of a number of genes conferring susceptibility to the clinical variants of rheumatoid arthritis.

In two of the four families in the present study, disease occurred in people who had HLA-Bw35. Recent reports associated *Bw35* with necrotizing cutaneous venulitis (37), nonstreptococcal glomerulonephritis (38) and, in one family with inheritance of many of the clinical features of systemic lupus erythematosus (39). As Cleland et al. (39) observed, the relationship of these diseases to *HLA-Bw35* is not clear. However, it could indicate an inherited predisposition to the formation of soluble circulating antigen-antibody complexes, a recognized feature of all of these diseases including JRA (14). This observation does not invalidate the fourth assumption under which this analysis was performed. However, it raises the possibility that genes in linkage disequilibrium with *Bw35* can modify the expression of various collagen-vascular diseases.

These family studies provide important new information concerning genetic mechanisms that predispose to rheumatoid arthritis. They may ultimately provide a basis for explaining some of the abnormal immunological mechanisms associated with RA.

The interest in studying diseases associated with or linked to the MHC arises from data that indicate that genes regulating immune responses are closely linked to those that regulate expression of MHC

antigens (40). Conceivably, MHC linked genes such as *RLA-1* described here, may have alleles that result in abnormal immune responses. This could happen in the negative sense in which an individual has a lacunar immune-response defect so that he cannot mobilize a sufficiently effective immune response to eradicate a specific infectious agent. It could also happen in the positive sense in which some usually innocuous stimulus triggers an excessive or inappropriate immune response, for example, to autologous tissue or plasma protein antigens.

There are a large number of inappropriate immune responses seen in both children and adults with RA (14). These include antiglobulins of all immunoglobulin classes, and antigenic specificities (41–44), as well as antibodies to nuclear antigens (14), and circulating immune complexes (45, 46). Lymphocyte cytotoxic responses to synovial cells have also been described, but their presence has not been demonstrated by all investigators (47) and the role of antibody-dependent cell-mediated cytotoxicity to synovial cells remains a point of controversy (48). Recently, antibodies reactive with a subset of thymic-dependent (T) cells have been detected in sera of children with active JRA (49, 50). These antibodies appear to be similar to some of the anti-lymphocytic antibodies found in patients with systemic lupus erythematosus in that they are selectively targeted against suppressor T cells (51).

It may be valuable in future studies to determine whether family members who are relatively unaffected by rheumatic disease but have the same MHC haplotype as the reference patient can make the same spectrum of abnormal immune responses.

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