Objective: To examine the contribution of genetic and environmental factors to disease occurrence in 26 families with two or more members affected with systemic lupus erythematosus (SLE).

Methods: Genetic and environmental factors were examined by HLA-A, B, C/DR typing and by determining the presence of lymphocytotoxic antibodies (LCA) in patients and their consanguineous and non-consanguineous relatives.

Results: No association between SLE and HLA-A, B, C antigens was found. There was, however, a significant association with HLA-DR2 in white subjects with SLE. The most striking finding was that HLA sharing was increased among the affected members, suggesting genetic similarities. Seven of 14 sib pairs (50%) who had concordant SLE were HLA identical as opposed to an expected 25%. Another striking finding was that 15/18 (83%) patients with SLE and 11/22 (50%) consanguineous relatives had LCA, while 1/9 (11%) spouses, and 2/42 (5%) healthy controls had these antibodies.

Conclusion: Genetic factors have a role in the development and expression of SLE. Environmental factors may trigger the disease in genetically susceptible hosts.

Despite the extensive studies, the cause of systemic lupus erythematosus (SLE) is unknown. Accumulating reported evidence suggests that both environmental and genetic factors play a part.

The genetic predisposition was supported by the occurrence of SLE in more than 10% of a member of a family as well as in identical twins. Approximately 10% of lupus patients have a first or second degree relative with SLE or closely related disease. The sib recurrence ratio (λs) of SLE, a more recently defined measure of familial aggregation, has been estimated to be approximately 25. Concordance for SLE varies widely but is consistently higher in monozygotic (25–70%) than in dizygotic twins (2–9%). Further evidence for the influence of genetics is the association between SLE and genetically determined HLA antigens.

The detection of antinuclear antibodies and lymphocytotoxic antibodies (LCA) in relatives living in the same house as patients with SLE, but not in relatives living away, was hailed by the supporters of the environmental theory.

Familial SLE provides a unique opportunity to examine the relative contributions of several genetic markers to intrafamilial disease expression. This study began in 1978 and continued until 2000 to examine the contributions of genetic and environmental factors to disease occurrence in 26 families with two or more members affected with SLE.

PATIENTS AND METHODS

Patient group

Twenty six families having two or more members with SLE were selected from the lupus clinic at the University of Colorado Medical Centre in Denver between 1978 and 1989 and the Tulane University Medical Centre in New Orleans between 1990 and 2000. A total of 150 subjects, 61 with SLE and 89 healthy first degree relatives, were included in the study. Of the 61 patients, 44 were female and 17 were male with ages ranging from 15 to 62 (median 28). The age at onset was recorded as the time of recognition of multisystem disease fulfilling the American Rheumatism Association (ARA) criteria in order to diminish the possibility of inaccurate diagnosis. All the living patients, except two who were diagnosed as having probable SLE, fulfilled the ARA criteria for definite SLE. Seven patients with SLE were dead. There were four pairs of twins, one dizygotic and three monozygotic, in four different families.

Although HLA typing was performed on 26 families, testing for LCA was carried out in nine families because they were available and willing to be studied.

Control group

Forty two healthy volunteers, without a personal or family history of connective tissue disease and who were not taking any drugs, served as controls for LCA studies. They were matched with SLE families for age, sex, and race.

HLA-A, B, C/DR typing

HLA-A, B, and C antigens were determined using the standard NIH microlymphocytotoxic technique. HLA-DR antigens were determined on B lymphocytes isolated on a nylon wool column.

LCA testing

Serum samples from patients and their family members and control families were absorbed with pooled platelets to eliminate antibodies to HLA as described previously. They were then tested against a cell panel composed of cells obtained from 60 different people of known HLA-A, B, and C phenotypes using the microcytotoxicity technique previously described. Briefly, 1 μl cells (200) was incubated with 1 μl serum at 5°C for one hour. After addition of pooled rabbit complement (Pel-Freeze Biological, Rogers, AK) incubation was continued for two more hours at room temperature. Before stopping the reaction, 3 μl of eosin Y (5% in H₂O) was added, followed by 5 μl of formalin (37%) to fix the cells. The plates were examined with an inverted phase contrast microscope with 10-fold objective. Each assay was run in the presence of two negative and two positive controls.

Abbreviations: LCA, lymphocytotoxic antibodies; SLE, systemic lupus erythematosus
Statistical methods
The association of HLA alleles with SLE was analysed by the $\chi^2$ test with Yates’s correction. The level of significance was selected as 0.05. Ninety five per cent confidence intervals are given for differences in proportion. The binominal exact test was used to compare the observed proportion with the expected proportion for both, single and non-shared haplotypes.

RESULTS
Segregation analysis
Ten of our families were Caucasian, nine were African American, and seven were Mexican American. Although more white families than African American and Mexican American were studied, the number of Mexican American patients was the highest of the three groups because they tend to have larger families. No obvious mode of inheritance is discernible in these families. All patterns of father/daughter/son, grandmother/mother/grandchildren, brother/brother, sister/sister, etc is possible. In our series, however, sister/sister was the most common (31%) (table 1) and women predominated (72%). When the sister/sister pattern was seen as mother/maternal aunt/son or daughter or it was not included as sibling pairs to avoid counting twice. But they were included as sibling pairs in the haplotype analysis. Children were born to both healthy parents or one healthy and one affected parent.

HLA-A, B, C/DR and haplotype analysis
Class I antigen typing of the patients with SLE and their relatives showed no significant differences in the frequency of any HLA-A, B, C antigens. There was, however, increased frequency of HLA-DR2 in white Americans ($p=0.003$) but not in African and Mexican Americans.

HLA haplotype analysis was carried out in 14 sibling pairs concordant for SLE. Monozygotic twins were not included as they were expected to be HLA identical. Seven pairs (50%) were identical for both haplotypes, five pairs (36%) for one haplotype, and two pairs (14%) were non-identical, yet the expected distribution is 25%, 50%, and 25%. The results were statistically significant ($p=0.05$).

Lymphocytotoxic antibodies
Figure 1 shows the prevalence of LCA in patients with SLE, their consanguineous and non-consanguineous relatives, and control families. As seen, 15/18 (83%) patients and 11/22 (50%) consanguineous relatives were LCA positive, whereas only 1/9 (11%) non-consanguineous relatives (spouses) and 2/42 (5%) members of the control group were positive. When the frequency and titre of antibodies were compared in patients and relatives, it was easily seen that they were higher and stronger in patients with SLE.

DISCUSSION
In SLE, support for the existence of genetic predisposition is derived from several lines of evidence. The first is based on the prevalence of SLE in families with multiple cases. Several studies indicate a significant increase in the prevalence of SLE among relatives of patients with the disease compared with controls.1–4 Secondly, there is also greater concordance for SLE in monozygotic twins than in dizygotic twins.5–7 Our studies enhance the evidence for a genetic connection in SLE as we present here 26 families in which at least two members are

Table 1  Siblings and parent offspring with SLE

<table>
<thead>
<tr>
<th>Number in group</th>
<th>Percentage in group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sister/sister</td>
<td>8</td>
</tr>
<tr>
<td>Sisisters</td>
<td>3</td>
</tr>
<tr>
<td>Sister/brother</td>
<td>1</td>
</tr>
<tr>
<td>Brother/brother</td>
<td>1</td>
</tr>
<tr>
<td>Mother/maternal aunt/1 daughter</td>
<td>1</td>
</tr>
<tr>
<td>Mother/maternal aunt/daughter</td>
<td>1</td>
</tr>
<tr>
<td>Mother/maternal aunt/son</td>
<td>1</td>
</tr>
<tr>
<td>Grandmother/mother/daughter</td>
<td>2</td>
</tr>
<tr>
<td>Father/son/daughter</td>
<td>1</td>
</tr>
<tr>
<td>Mother/son</td>
<td>1</td>
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<tr>
<td>Mother/daughter</td>
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</tbody>
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*Percentages were calculated in 26 families.

Figure 1  The percentage frequency against the cell panel is shown by bars—that is, 50% reactivity means that the serum had LCA against half of the cell panel.
affected with the disease. It is particularly interesting to see the affected members in three generations, grandmother/mother/grandchild (table 1). Also in our series, marked concordance was found between the identical twins. All three monozygotic twins were affected with the disease.

Further evidence is derived from the association between SLE and the HLA system. Firstly, in 1972, McDevitt and Bodmer reported an association between SLE and HLA-B8.13 This was followed by other reports stating an association between SLE and HLA-A1, B8, B13, and B17.14–17 There were also reports of associations between SLE and HLA-DR2 and DR3.18–20 These findings were not, however, confirmed unanimously. In our study there was no association between HLA class I alleles and SLE. There was, however, significant association between HLA-DR2 and SLE in white patients (p=0.003), but not in African American and Mexican American patients. (Some studies have identified a higher frequency of HLA-DR3 in African Americans,21,22 but we found no such association.)

Contrary to recent reports, our studies show a striking increase in HLA sharing among affected family members. Three monozygotic twins were not included in this analysis because they were expected to be HLA identical. Seven of 14 sib pairs shared two haplotypes, which differed significantly from the expected pattern. There was not, however, any specific haplotypes common to all 14 sib pairs. Haplotype sharing is particularly important, because specific HLA alleles may only be markers for other as yet undefined but closely linked genes within the HLA region. Thus the association of certain HLA haplotypes with disease in multiple affected members of a family may provide more inclusive genetic information than the single alleles. In one of our families both the mother and son had SLE and were HLA identical. This is a rather rare situation and, presumably, would be due to shared HLA antigens between the parents.

To assess the role of environmental factors, we examined the presence of LCA in patients with SLE and their relatives. Figure 1 shows that 15/18 (83%) of the patients and 11/22 (50%) of their consanguineous relatives were LCA positive while only 1/9 (11%) of non-consanguineous relatives (spouses) and 2/42 (5%) in the control group were positive. These findings are highly significant and show that the occurrence of LCA is due to close kinship not close contact. There was no association between any specific HLA allele and LCA. LCA in patients with SLE has been reported previously by us and by others.23–28 It has also been reported in other autoimmune disease29 and in some viral infections such as infectious mononucleosis, measles, rubella,30 and AIDS.31–33 In these studies it was concluded that there was an association between LCA and viral infections. Even so, our study suggests that they occur in genetically predisposed hosts.

There was no association between the level of LCA and the clinical activity of the disease.

In summary, our studies emphasise previous studies indicating that genetic factors do have a role in the development and expression of SLE. Environmental factors may, perhaps, trigger the disease in genetically susceptible subjects.

ACKNOWLEDGMENTS

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