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Does HLA-Dependent Chimerism Underlie the Pathogenesis of Juvenile Dermatomyositis?1

Ann M. Reed,2,* Kelly McNallan,* Peter Wettstein,† Richard Vehe,‡ and Carole Ober§

Juvenile dermatomyositis (JDM) is a multisystem autoimmune disease that at times resembles chronic graft-vs-host disease. This led us to suggest that nonself cells may play a role in the disease process. In this study we examined the relationship between HLA genotype and the presence of maternally derived chimeric cells in JDM patients and healthy controls, and assessed immunologic activity in the chimeric cells. We identified chimeric cells more often in children with JDM (60 of 72) than in their unaffected siblings (11 of 48) or in healthy controls (5 of 29). The presence of chimerism in the JDM patients, their healthy siblings, and unaffected control children was associated with a HLA-DQA1*0501 allele in the mother (p = 0.011). Further, we show that maternally transferred chimeric T cells are responsive to the host’s (JDM child’s) lymphocytes (33.75 ± 8.4 IFN-γ-producing cells from JDM cells vs 5.0 ± 1.25 from maternal cells), and that this is a memory response. These combined data indicate that chimeric cells play a direct role in the JDM disease process and that the mother’s HLA genotype facilitates the transfer and/or persistence of maternal cells in the fetal circulation. The Journal of Immunology, 2004, 172: 5041–5046.

Reciprocal trafficking of cells between the fetal-maternal circulation is a normal event during pregnancy. The presence of fetal cells in the circulation of healthy parous women and those with autoimmune diseases has been described (1–5). These nonself, or chimeric, cells can persist for many years after pregnancy (1, 2) and may contribute to the peak onset of autoimmune diseases in women during and after their childbearing years (6). We and others have described the presence of maternal cells in a childhood-onset autoimmune disease, juvenile dermatomyositis (JDM)3 (7, 8), suggesting that the passage and persistence of maternal cells in the child’s circulation may also contribute to the pathogenesis of some autoimmune diseases.

JDM is a multisystem autoimmune disease characterized by vascular inflammation that primarily affects muscle and skin. At times, a similar disease process is seen in chronic graft-vs-host disease (cGVHD). In particular, cGVHD often includes symptoms of inflammatory myositis (polymyositis and dermatomyositis), systemic lupus erythematosus, scleroderma, primary biliary cirrhosis, and Sjogren’s syndrome. Muscle biopsies in these patients show lymphocytic and phagocytic infiltrates in muscle fiber, similar to findings in JDM. This similarity led us to speculate that the underlying pathophysiology in JDM may be similar to that in cGVHD, in which the persistent maternal (chimeric) cells serve as the “graft” (9).

The most important determinant of cGVHD after bone marrow transplantation is HLA differences between the donor and recipient. Most often, HLA-incompatible donor cells stimulate persistent T cell activation against host cells. However, cGVHD can also occur in cases of HLA similarities, presumably due to differences in minor histocompatibility Ags (10, 11). Recently, Lambert and colleagues (12) demonstrated that the presence of fetal cells in the circulation of both healthy women and women with scleroderma was significantly associated with the HLA-DQA1*0501 allele of their children (donor’s genotype) and more modestly associated with their own HLA-DQA1*0501 (host’s genotype). The HLA-DQA1*0501 allele is a significant risk factor for JDM (13–15), and chimeric maternally derived cells have been identified in the circulation of these patients (15). However, the relationship between HLA genotype and the presence of chimeric cells in children as well as the immune reactivity of chimeric cells have not been investigated.

In the present study we sought to replicate our earlier finding of increased microchimerism in JDM patients in a larger sample. In addition, we asked two new questions. 1) Is the presence of chimeric cells in the child related to the HLA genotype of the mother (donor’s genotype) and/or the child (host’s genotype)? 2) Do chimeric cells in the child have immunologic activity? We demonstrate, in a larger cohort of patients, that the presence of maternally derived chimeric cells are present more often in children with JDM than in their healthy siblings and in unrelated healthy offspring, and show that the presence of chimerism is most significantly related to the HLA genotype in the mothers of both JDM and healthy children. Further, the maternally derived chimeric cells are activated when exposed to the JDM child’s lymphocytes, indicating that they are immunologically active and suggesting a direct role for the chimeric cells in the disease process.

Materials and Methods

Subjects

JDM patients. We studied 38 female and 34 male unrelated JDM patients (mean age, 10.6 years; range, 1–16 years) and their mothers. The diagnosis of JDM was based on the criteria of Bohan and Peters. Five to 40 cc of blood was obtained for DNA extraction and PBMC separation. JDM patients had varying disease states from newly diagnosed to 3 years postdiagnosis. The ethnic composition of these patients was 74% Caucasian, 13%...
African American, and 10% Hispanic. All results were consistent when analyses were performed in the Caucasian subsets of patients and families. We studied muscle biopsy samples, obtained for diagnostic reasons, from 20 male JDM and 10 control subjects who were found not to have an inflammatory myopathy. All patients were recruited from pediatric rheumatology clinics at the University of North Carolina (Chapel Hill, NC), Duke University Medical Center (Durham, NC), Pediatric Rheumatology (Roanoke, VA), and University of Minnesota (Minneapolis MN). Consent was obtained for these studies at each participating institution by the investigators. **JDM families.** We selected families of the children with JDM described above who also had at least one healthy sibling and a mother who were willing to provide informed consent. Five to 40 cc of blood was collected for DNA extraction and PBMC isolation. The 33 families who met these criteria included 48 healthy siblings (mean age, 10.2 years; range, 4–18 years). The ethnic composition of these families was 83% Caucasian, 10% African American, and 7% Hispanic. **Healthy controls.** We obtained PBMCs from 29 healthy male subjects (mean age, 20 years; range, 6–37 years) whose 16 mothers are participants in ongoing studies of HLA fertility in one of our laboratories (16). These subjects were unselected with respect to disease status. In particular, there was no autoimmune disease present in these families. All subjects were Caucasian and homogeneous with respect to genetic background. **Genotyping** Genomic DNA was extracted from whole blood mononuclear cells using commercially available kits (Qiagen, Valencia, CA; Puregene, London, U.K.) and stored at −4°C. The JDM patients, siblings, and parents were genotyped for HLA-DRB1 and HLA-DQA1 alleles using Biostest HLA typing kits and for HLA-DQA1 alleles using sequence specific oligonucleotide probes, as previously described (9, 17). The methods for HLA genotyping in the 29 healthy controls and their 16 mothers has been described previously (18). The sensitivity of HLA typing even in the presence of chimeric cells at ratios of 1:100, 1:1,000, and 1:10,000 demonstrated no difficulty in establishing an individual’s HLA genotype. This was determined by comparing typing samples with cells of a different genotype at ratios of 1:10 through 1:10,000. In our study population ratios of chimeric cells were 1:100 to 1:1,000. **Detection of microchimerism** We detected microchimerism using two approaches: PCR amplification of a maternally noninherited HLA allele and detection of female cells in male subjects using fluorescent in situ hybridization (FISH), as described previously (7, 19). In the PCR studies, a sample was considered chimeric if a noninherited maternal HLA allele was amplified. In the FISH studies, a male subject was considered chimeric if 0.1% female cells (XX) were detected, which is above published background rates. The PCR studies were performed without knowledge of the results of the FISH studies and vice versa. Both studies were performed without knowledge of affection status, and the FISH studies were performed without knowledge of the HLA types of the sons or their mothers. **PCR amplification for chimeric cells** All subjects were tested for the presence of chimeric cells using PCR amplification of the noninherited maternal HLA-DQA1 allele. We used HLA-DQA1 polymorphisms for microchimerism studies because of our laboratory’s experience with this gene, the moderate degree of polymorphism of this gene (compared with other HLA genes), the robustness of the PCR assay, and the high degree of reproducibility of HLA-DQA1 typing (>99% in our laboratory). HLA-DQA1 alleles could not be determined for three of 72 JDM patients, two of 48 siblings, and 11 of 29 healthy control subjects because of similarity with parental alleles. In these cases we used nested PCR to identify the nontransmitted maternal HLA-A or HLA-DR allele. PCR amplification for detection of maternal noninherited HLA-DQA1 alleles includes 1) a positive control for the allele being tested (maternal DNA); 2) a negative control with the same HLA genotype as the subject’s, but with a different maternal genotype; and 3) a negative control reaction that did not include DNA (blank). An allele-specific nested PCR was performed, as previously described, as a variation of the method of Olerup et al. (7, 19). Amplification of noninherited maternal HLA-DQA1 alleles could be obtained from cell ratios ranging from 1:50,000 to 1:100,000 based on amplification of the different primers used. The PCR products for each experiment were visualized on agarose gels for the presence or absence of amplified product. The presence of an amplified product corresponding to the maternal noninherited allele was considered evidence of chimerism, which was noted as present or absent based on these experiments. **FISH** To visualize and quantify maternally derived chimeric cells, we performed FISH on PBMCs from 34 male JDM patients, 39 male healthy siblings, and 29 healthy control males. A male sample was considered chimeric if 0.1% female cells (XX) were detected. For these studies, PBMCs were isolated by Ficoll-Hypaque centrifugation and washed with PBS. Recovered cells were suspended in 50 μl of fresh methanol-acetic acid solution (diluted 3:1) for fixation. Ten microliters of cell suspension was added to a clean glass slide, approximating 5000 cells/cm². The CEP X/orange)/YsaFlI (green) (Vysis, Downers Grove, IL) was added. Cells were stained with 4,6-diamidino-2-phenylindole dihydrochloride and examined using a fluorescence microscope (Leitz, Wetzlar, Germany). Alignment of the three-color images was performed with the fourth image captured through a triple bandpass filter (FITC/Texas Red/4,6-diamidino-2-phenylindole dihydrochloride) as a way to assure individual cells have informative fluorescent signals. Two thousand cells were analyzed per sample to establish rates of chimerism and to assure adequate numbers of cells to assess the presence of chimerism. Signals were visualized with a Leitz fluorescence microscope, and 2000 nuclei were examined per slide. All techniques are routine within this laboratory. All samples were studied in random order and blind to disease status (7). We also performed FISH on 10-μm-thick muscle biopsy samples from 20 random JDM patients and 10 unrelated control males who underwent muscle biopsy for diagnostic purposes, but were not diagnosed with JDM. Muscle biopsy samples were fixed with the procedure for FISH and visualization as stated above. **Phenotyping and reactivity of chimeric cells** We isolated PBMCs from 40 ml of whole blood from four male JDM patients and two healthy male sons with documented microchimerism and from their mothers to determine the phenotype of the chimeric cell and if the cells are responsive to the host’s lymphocytes. Chimeric cells in the PBMCs from JDM patients and controls were purified using Abs specific for the maternal noninherited HLA-A allodextrin. Cells isolated were examined as to there origin (XX, XY) and were found to be 89–92% female. FACs analysis was performed and demonstrated that 85–90% of the isolated cell population was positive for the HLA-A maternal Ag. Cell phenotyping was performed by FACS analysis with Abs specific for CD4 (Th; L12; PharMingen, San Diego, CA), CD8 (T suppressor cells; RPA74), CD19 (B cells; HIB19), CD14 (monocytes/macrophages; M699), CD56 (NK cells; B159), and maternal HLA-A alloantigen (One A. Canoga Park, CA). Because only 2 × 10⁶ cells were available for analysis, we used a combination of Abs with different fluorochromes to conserve Neutrophils were obtained from the red cell pellet and assessed for maternal cell chimerism using FISH for XX and XY cells in the sons. ELISPOT (Cellular Technology, Cleveland, OH) was used to detect a T cell-secreting cytokine that is active in this inflammatory reaction (IFN-γ) (20) to determine whether the chimeric cells were responsive to the host’s lymphocytes. MACS anti-HLA-DR microbeads (Miltenyi Biotech, Cologne, Germany) were used to isolate the maternally derived chimeric cells using maternal anti-HLA-A Abs in a positive selection column. Cell purity was confirmed using FISH and was found to be 95% maternal XX. The ELISPOT was performed as a mixed lymphocyte culture (MLC) using as responders 1) purified chimeric PBMCs from JDM subjects, 2) unsorted maternal PBMCs (alloreactive control), and 3) unsorted JDM patients PBMCs (negative control). Stimulator cells (10⁵) from maternal PBMCs and patient PBMCs were irradiated and added to 5 × 10⁶ responders/well using the procedure described previously (20). The frequencies of spots were estimated using Cellular Technology (Cleveland, OH) software in our laboratory. All analyses were performed in triplicate. To assess whether the reactivity of chimeric cells was a naive or a memory response, we performed MLCs using PBMCs from the JDM patients and controls described above and measured IFN-γ production by competitive PCR. Incubated cells were harvested at 18 and at 24 h after restimulation. This allowed us to distinguish a naive response, which requires restimulation, vs a memory response, which in our experience peaked at 18 h after the primary stimulations for IFN-γ production. A competitive PCR was performed using a titration of a known competitive RNA transcript to the mRNAs, at each time point, as previously described (21). **Statistical methods** To determine whether HLA genotype influences the presence of chimeric cells, we stratified the mothers, JDM patients, healthy siblings, and controls by the presence or the absence of microchimerism in themselves (patients,
siblings, and controls) or in their children (mothers). Two mothers of sibling controls and one mother of a population control had both a chimeric and a nonchimeric child in this study. These mothers were considered in the chimeric child groups for these analyses. We compared the frequencies of each HLA allele between the chimeric and nonchimeric individuals within each group using Fisher’s exact test. The Bonferroni correction for multiple testing is conservative in this context, because this correction assumes that the tests are independent. However, the alleles at each HLA locus as well as the alleles present at different HLA loci are not independent of each other. Nonetheless, we followed published protocols (12) and corrected each p value for the number of families of alleles or alleles present in each sample: eight DRB1 families, eight DQA1 alleles in the JDM families and six in the controls, and 18 DQB1 alleles in the JDM families and eight in the controls. We considered results to be significant at a corrected value of $p < 0.05$. All other comparisons between JDM and control subjects were made using Fisher’s exact test.

Results

Studies in JDM patients

A noninherited maternal HLA allele was detected in 60 of 72 (83%) JDM patients. The HLA-DQB1*03 allele was more common in the patients with chimerism than in those without chimerism, although this difference was not significant after adjusting for multiple comparisons (Table I, Children). In their mothers, the DRB1*03, DQA1*0501, and DQB1*03 alleles differed between the groups, and differences in the DQA1*0501 allele frequencies approached significance after adjustment for multiple comparisons (Table I, Mothers).

Studies in JDM families

Twenty-eight of 33 (85%) JDM patients in the family studies were chimeric for maternal cells compared with 11 of 48 (25%) of their healthy siblings (JDM vs siblings, $p < 10^{-5}$). Among the siblings, the frequencies of DRB1*03, DRB1*11, and DQA1*0501 differed between children with and without chimerism, but these differences were not significant after adjusting for multiple comparisons (Table II, Children). Further, among the three chimeric children who did not inherit a DQA1*0501 allele, the noninherited maternal allele was DQA1*0501 in all cases (and DRB1*03 and DQB1*02 in two of these cases). Thus, all healthy siblings with chimeric cells either inherited a DQA1*0501 allele ($n = 8$) or had DQA1*0501 chimeric cells ($n = 3$). The frequency of the DQA1*0501 allele was also increased in the mothers of the siblings with chimerism, but this difference was not significant after adjusting for multiple comparisons (Table II, Mothers).

The mean age was 11.8 years (range, 6–18 years) among the siblings with chimerism and 9.8 years (range, 4–18 years) among the siblings without chimerism ($p = 0.8$), suggesting that the increased prevalence of chimerism in the patients was not due to differences in the age structure between the patients and siblings.

Studies in healthy controls

Five of 29 (17%) healthy control males were chimeric for maternal cells by PCR amplification of the nontransmitted maternal allele and FISH (described in the FISH studies below; Table III). The mean age was 23 years (range, 6–37 years) in the chimeric sons and 19 years (range, 6–36 years) in the nonchimeric sons. This difference was not statistically significant ($p = 0.4$), again indicating that the presence of chimeric cells is not strongly correlated with age.

FISH studies

We used FISH to visualize and quantify female (XX) cells in a subset of 30 male JDM patients and 39 of their healthy brothers. XX (maternal) cells were detected in the PBMC in 22 (73%) JDM patients and in 12 (31%) male siblings ($p = 0.008$). The frequency of XX (chimeric) cells in the peripheral blood was not significantly different among chimeric JDM children (range, 0.2–2%) and chimeric sibling controls (range, 0.5–1%). Maternal cells were also present in the affected muscle tissue in 16 of 20 (80%) JDM children compared with 2 of 10 (20%) unrelated male control muscle tissues ($p = 0.001$). Among the 16 JDM children with chimeric cells in their muscle tissue, 15 also demonstrated microchimerism in their PBMCs by both the PCR and FISH studies (data not shown).

Because the results observed in the healthy siblings of JDM patients could be influenced by the presence of a disease haplotype in these families, we also performed FISH on the PBMCs from 29 healthy male controls without a history of autoimmune disease in their families. XX cells were detected in five (17%) male controls, which was not significantly different from the frequency of chimerism among the healthy siblings. As in the healthy siblings, the DRB1*03 and DQA1*0501 alleles were more common in sons with chimerism than in sons without chimerism (40 vs 0% for both alleles; uncorrected $p = 0.024$). Further, all four mothers of these five chimeric sons (including one set of brothers) had a DRB1*03-DQA1*0501-DQB1*02 haplotype compared with none of 12 mothers with nonchimeric sons ($p = 0.00055$). The individual frequencies of these three alleles also differed significantly between mothers of chimeric and nonchimeric sons, and these differences remained significant after adjusting for multiple comparisons (Table III, Mothers).

Phenotyping of chimeric cells

All cell types were identified in the chimeric cell population in the following proportions: 38–45% CD4+, 12–18% CD8+, 22–25% CD19+, 2–4% CD14+, and 5–12% CD56+. On the average, 0.2–1% of cells were of maternal origin. Chimeric cells were also identified in the neutrophil population in the JDM and control groups. Because of the normally short half-life of neutrophils, this finding indicates the presence of maternally derived chimeric stem cells giving rise to neutrophils.

Chimeric cell activity

We examined chimeric cell activity in the JDM patients by testing for the cells’ ability to up-regulate and secrete IFN-γ, as a marker of T cell activation, when stimulated with the JDM patients’ HLA alloantigens. Four chimeric JDM children who weighed enough to

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**Table I. The presence of HLA alleles in the JDM patients and their mothers by chimerism status (determined by PCR amplification) of the child**

<table>
<thead>
<tr>
<th>HLA Allele</th>
<th>Number (%) Negative for Chimeric Cells ($n = 12$)</th>
<th>Number (%) Positive for Chimeric Cells ($n = 60$)</th>
<th>Uncorrected $p$ Value</th>
<th>Corrected $p$ Value</th>
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<td>Children</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DQB1*03</td>
<td>2 (17)</td>
<td>32 (53)</td>
<td>0.027</td>
<td>0.46</td>
</tr>
<tr>
<td>Mothers</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>DRB1*03</td>
<td>1 (8)</td>
<td>26 (43)</td>
<td>0.025</td>
<td>0.16</td>
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<tr>
<td>DQA1*0501</td>
<td>5 (40)</td>
<td>48 (80)</td>
<td>0.011</td>
<td>0.088</td>
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<tr>
<td>DQB1*03</td>
<td>2 (17)</td>
<td>32 (53)</td>
<td>0.027</td>
<td>0.54</td>
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donate 40 ml of blood were selected for these studies. Their chimeric cells were isolated and stimulated with PBMCs from themselves, their mothers, and unrelated controls. Chimeric cells from three of the four JDM patients showed increased frequencies of IFN-γ-secreting T cells when stimulated with JDM cells (mean, 33.75 ± 8.4 IFN-γ-producing cells) or control cells (5.0 ± 1.25 cells) relative to stimulation with maternal cells (Fig. 1A).

We next determined whether the chimeric cells were naive cells producing a primary immunologic response or memory cells that had been previously primed. MLCs were established from cells from four JDM patients as the stimulators and chimeric cells as the responders. Cells were harvested after 18 h of a primary stimulation and 24 h after restimulation. The number of copies of IFN-γ was estimated for each sample as a measure of stimulation. The RT-PCR was performed with a fluorescently labeled primer, and the number of copies of IFN-γ were determined for each sample using computer-integrated peak areas for target and competitor; after adjusting for the product size difference, linear plots of the logs (adjusted competitor peak area/target peak area) vs logs (competitor copy number) were made. Interpolation of the linear graphs at the ordinate point zero (where target and competitor are present in a 1:1 molar ratio, or x where y = 0) was performed, and normalization was made to the amount of target total RNA (50 ng) used in each reaction. There was increased IFN-γ transcription when JDM cells were used as stimulators, with 0.24 mRNA transcripts/ng of total RNA at 18 h and 0.16 mRNA transcripts/ng of total RNA at 72 h (Fig. 1B). Control samples, including maternal cells and unrelated controls, had 0.02 and 0.016 mRNA transcripts/ng of total RNA at 18 and 72 h, respectively.

**Discussion**

In this study we demonstrated a greater frequency of chimerism in JDM patients compared with healthy children. Using a highly sensitive, PCR-based assay, we identified a noninherited maternal HLA allele (chimerism) in 85% of JDM patients and in 24% of their healthy siblings. Using a less sensitive FISH assay, we visualized maternal cells more frequently in male JDM patients (73% of PBMCs and 80% of muscle biopsies) than in their healthy brothers (31% of PBMCs) or healthy male controls (17% of PBMCs and 20% of muscle biopsies). The greater frequency of persistent fetal cells in the maternal circulation (>40% in the study by Lambert et al. (12)) compared with persistent maternal cells in the child’s circulation (<20% in this study) may reflect differences of sensitivity of the Y-specific PCR used in the Lambert study (12) compared with the HLA allele-specific assay used in our study. Alternatively, these may reflect true differences in cell transfer rates or persistence in the maternal vs the fetal circulation.

We also report for the first time an association between mother and child’s HLA alleles and the presence of chimerism in the child. The DRB1*03 and DQA1*0501 alleles were more common in both the sibling and population controls with chimerism than in those without chimerism (Tables II and III). The frequencies of these alleles were not significantly increased in the JDM patients with chimerism, but this probably reflects the fact that these alleles are present in nearly all JDM patients regardless of chimerism status. In contrast, the frequency of the DQA1*0501 was increased in the mothers of chimeric JDM patients (p = 0.011), the mothers of chimeric healthy siblings (p = 0.046), and the mothers of chimeric controls (p = 0.008). Further, among the 11 siblings and five controls with chimerism, the DQA1*0501 was either inherited or present on the chimeric cells in all cases. Thus, these combined data suggest that the HLA alleles present on the chimeric (maternal) cells are associated with transfer and/or persistence in both JDM and healthy children regardless of whether these alleles are inherited by the child. This is remarkably consistent with the results of the Lambert study, which demonstrated that the presence of DQA1*0501 on fetal cells was the most significant predictor of their presence in the circulation of healthy women and scleroderma patients (12). Taken together, these results indicate that the fate of cells transferred between the fetal and maternal circulations during pregnancy is determined largely by the HLA genotype of the “donor” cells (i.e., maternal cells in the case of children and fetal cells in the case of the mother), with the genotype of the “host” playing a lesser role. Thus, the DQA1*0501 allele may contribute to the

<table>
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<tr>
<th>HLA Allele</th>
<th>Number (%) Negative for Chimeric Cells (n = 37)</th>
<th>Number (%) Positive for Chimeric Cells (n = 11)</th>
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<th>Corrected p Value</th>
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<td>11 (30)</td>
<td>8 (73)</td>
<td>0.016</td>
<td>0.12</td>
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</tr>
<tr>
<td>DQA1*0501</td>
<td>8 (33)</td>
<td>7 (78)</td>
<td>0.046</td>
<td>0.37</td>
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* n = 24.  
** n = 9.

<table>
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<th>HLA Allele</th>
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<th>Number (%) Positive for Chimeric Cells (n = 5)</th>
<th>Uncorrected p Value</th>
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<tr>
<td>DQB1*02</td>
<td>1 (8)</td>
<td>4 (100)</td>
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</table>

* n = 12.  
** n = 4.
pathogenesis of autoimmune diseases, such as JDM and scleroderma, by facilitating the transport and/or persistence of maternal cells into the fetal circulation or of fetal cells into the maternal circulation, respectively. The DQA1*0501 allele and other allele families on this extended haplotype (DRB1*03 and DQB1*02) are associated with a large number of autoimmune diseases in addition to JDM and scleroderma, although the mechanisms for these associations are as yet unknown. These data suggest a possible mechanism related to the presence of DQA1*0501 chimeric cells. We propose that chimeric cells provide the second “hit” that triggers the onset of this disease in genetically susceptible individuals, whereby genetic susceptibility in the host is determined by the inherited HLA class II alleles (DQA1*0501 in the case of JDM). The second hit may be the loss of tolerance of the chimeric cells to the host. In most cases, the chimeric cells are also DQA1*0501.

Recently, Artlett and colleagues (22) reported a study of HLA-DQA1 alleles and chimerism in adults with scleroderma and children with heterogeneous forms of inflammatory myopathy (IM) compared with healthy controls. They found that the presence of chimerism increased in both patient groups, and the frequency of DQA1*0501 increased in the IM patients compared with controls, similar to previous reports (7, 12). However, although the frequency of the DQA1*0501 allele was increased in chimeric IM patients (70% frequency) compared with nonchimeric IM patients (frequency, 25%; uncorrected $p = 0.04$; odds ratio, 7), they did not find an association between the DQA1*0501 allele in the donor (mother) cells and chimerism in the child in an analysis of eight IM mother-son pairs and two control mother-son pairs (DQA1*0501 allele present in four of eight mothers of chimeric sons and one of two nonchimeric sons). In contrast, in our study of 72 JDM mother-son pairs the frequency of this allele was 80% in 60 mothers of chimeric sons and 40% in 12 mothers with nonchimeric sons (Table I, Mothers). The differences between our study and that of Artlett is probably due to the small number of mother-son pairs (eight IM and two controls) and the correspondingly low power to detect a difference in their study.

Although immunologic tolerance in non-HLA-identical humans has been observed (23), it is the rule rather than the exception in pregnancy (24). Tolerance to chimeric cells in both the mother and the baby after parturition is normal; chimerism was detected in ~20% of healthy children (this study) and in >40% of healthy parous women (12). The healthy children in our study with chimerism ranged in age from 6–37 years, consistent with reports of long term persistent fetal cells in the maternal circulation (2, 3). The chimeric cells represent many cell lineages (T cells, B cells, monocytes, NK cells, and neutrophils), suggesting that not only are differentiated chimeric cells persisting in the periphery from birth, but that engrafted chimeric stem cells exist as well.

In animal models the post-transplantation infusion of donor bone marrow cells induces tolerance to skin allografts, even in the presence of a full MHC mismatch. In these tolerant animals a low level of chimerism (up to 4%) was associated with clonal deletion, anergy, and an increase in suppressor cells (25). In humans evidence of circulating donor cells in patients with long-surviving solid organ transplants suggests the presence of low levels of chimerism in at least some cases of successful transplants. Therefore, in some situations, such as in organ transplantation and pregnancy, chimerism may be beneficial. Conversely, in autoimmunity, chimeric cells may switch from a tolerant state to one of active inflammation. The latter hypothesis is supported by our studies in which chimeric cells from three of four JDM patients showed high frequencies of IFN-γ-secreting T cells when stimulated with JDM.

**FIGURE 1.** A, ELISPOT demonstrating the mean number of IFN-γ-producing cells. B, Mean copies IFN-γ transcripts per nanogram of total RNA from four JDM patients and four maternal subjects.
become immunoreactive toward the child’s cells, indicating that maternally derived chimeric cells can be viewed as a double-edged sword: conferring a bene-

tice and PBMCs from patients with scleroderma (26). The pres-

ence of activated chimeric cells in JDM patients supports our pro-

posal that chimeric cells may play a direct role in the pathogenic immune response. Indeed, this immunologic mechanism of “auto”-

immune disease may be more alloimmune than autoimmune, as previously suggested (6). The presence of chimeric cells can thus be viewed as a double-edged sword: conferring a benefit as long as tolerance is maintained, such as after successful transplantation or parturition, but potentially harmful when tolerance toward the host is broken.

In summary, we have demonstrated for the first time that the chimeric cells in JDM patients are reactive against the hosts’ cells. This suggests that maternally derived T cells that normally persist in a state of tolerance or anergy can be activated, potentially leading to an ensuing anti-host inflammatory response. We further show that this response is a memory response, indicating previous reactivity against host cells. Lastly, the trafficking of maternal cells during gestation or the persistence in their children after parturition is influenced primarily by the HLA genotype of the mother’s (do-


cor) cells and, to a lesser extent, by that of the child (host). As a result, some of the risk for JDM, and perhaps other autoimmune diseases, may be determined by the HLA genotype of the mother, which influences the fate of transferred cells. In contrast, the in-

herited HLA genotype may contribute to the activation of chimeric cells, which initiates the disease process. Thus, both the inherited HLA alleles and noninherited maternal HLA alleles may interact to ultimately determine an individual’s risk for the development of autoimmune disease. The fact that the DQA1*0501 allele was asso-

ociated with both maternal chimeric cells in our study and fetal chimeric cells in the Lambert study in both healthy controls and patients with autoimmune disease suggests a more general mech-

anism for disease pathogenesis that may have implications for a broader range of immunologically mediated diseases.

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References


