

## BRIEF REPORT: TWIN BOYS WITH MAJOR HISTOCOMPATIBILITY COMPLEX CLASS II DEFICIENCY BUT INDUCIBLE IMMUNE RESPONSES

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**T**HE major histocompatibility complex (MHC) class II molecules are composed of two polypeptide chains ( $\alpha$  and  $\beta$ ). In humans the three distinct isotypes are designated HLA-DR, DP, and DQ. The different isotypes serve similar functions, and their expression on cells is regulated in coordinated fashion.<sup>1</sup> MHC class II molecules are expressed on the surface of certain cells of the immune system, such as monocytes and macrophages, B cells, and dendritic cells, where they play an important part in the immune response.<sup>2</sup>

MHC class II deficiency is an autosomal recessive disease characterized by cellular and humoral immunodeficiency.<sup>3</sup> Family studies show that the disease locus segregates independently of the MHC class II gene loci,<sup>4</sup> so the defect is not in the MHC class II genes themselves. A regulatory defect at the level of MHC class II gene transcription has been identified.<sup>4-7</sup> Despite the fact that affected families have different transcription defects,<sup>7,8</sup> phenotypic expression of the disease has been uniform in all the patients described so far. A regulatory defect in the transcription of MHC class II genes leads to the absence of messenger RNA (mRNA) and protein for all three isotypes.<sup>4,9,10</sup> Typically, patients have hypogammaglobulinemia and severe failure to thrive in the first year of life. They have severe bacterial, fungal, and viral infections. Since the disease is associated with a high rate of early mortality, bone marrow transplantation is considered the only possible treatment.<sup>3,11</sup>

We report the case of identical twin brothers with a defect in constitutive and inducible surface expression of MHC class II molecules on B cells, monocytes, and activated T cells. Unlike previously reported patients, the two infants did not have a regulatory defect in the coordinated control of MHC class II gene transcription. Low levels of HLA-DR  $\alpha$ -chain and  $\beta$ -chain protein were detected and, most surprisingly, cellular and humoral immune responses were induced in both infants after tetanus vaccination. For more than 18 months the two boys have had a benign clinical course.

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They appear to represent a previously unrecognized phenotype of this rare genetic disease.

### CASE REPORT

Twin brothers were born at 34 weeks of gestation to unrelated healthy parents of Turkish origin. Their HLA haplotypes were identical. Cesarean section was performed because of premature rupture of the membranes, and both infants had low birth weight (Twin 1 weighed 1020 g, and Twin 2 weighed 2030 g). At 10 weeks of age the first twin was admitted to the hospital because of febrile seizures. Cultures of blood and cerebrospinal fluid were negative. He was admitted to the hospital again at the age of four months with pneumonia confirmed by radiologic examination. Immunologic examination revealed severely decreased serum IgG levels (132 mg per deciliter), low or nearly absent serum IgA (<3 mg per deciliter), and serum IgM levels within the normal range for age (80 mg per deciliter). The distribution of lymphocyte subgroups was normal for the infant's age: CD4 cells, 43 percent (1080 per cubic millimeter); CD8 cells, 23 percent (580 per cubic millimeter); CD3 cells, 62 percent (1560 per cubic millimeter); and CD19 cells, 27 percent (680 per cubic millimeter). The lymphoproliferative responses to mitogens were also normal for age (data not shown). The expression of HLA-DR on his peripheral-blood lymphocytes, however, was  $\leq 1$  percent (mean [ $\pm$ SD] value in 14 age-matched controls,  $27 \pm 6$  percent). Intravenous treatment with immune globulin (400 mg per kilogram of body weight every two weeks) was begun. The infant's clinical condition was uneventful thereafter.

Twin 2 has had an uneventful clinical course from birth. Immunologic examination at the age of seven months revealed a defect of HLA-DR expression comparable to that in his brother. He had lymphocytosis (9470 lymphocytes per cubic millimeter), but the relative distribution of lymphocyte subgroups (CD4 cells, 48 percent; CD8 cells, 29 percent; CD3 cells, 75 percent; and CD19 cells, 14 percent) and the lymphoproliferative responses to mitogens (data not shown) were within the normal ranges. Serum IgG and IgA levels were normal, but the serum IgM level was low (20 mg per deciliter).

### METHODS

The expression of surface-membrane antigens on peripheral-blood leukocytes, T cells activated with phytohemagglutinin, and mononuclear cells incubated for 48 hours in the presence of recombinant human interferon gamma (500 IU per milliliter; Genzyme, Cambridge, Mass.) was examined by flow cytometry (FACScan, Becton Dickinson, San Jose, Calif.) with the following monoclonal antibodies used in direct two-color immunofluorescence as specified by the manufacturer (Becton Dickinson): HLA-DR (L243), CD19 (Leu-12), CD3 (Leu-4), CD14 (Leu-M3), and CD25 (interleukin-2 receptor). HLA-DR, DP, and DQ molecules were examined on B-cell lines transformed by Epstein-Barr virus by indirect immunofluorescence with the monoclonal antibodies MID3 (Sera-Lab, Crawley Down, Sussex, United Kingdom), B7/21 (Becton Dickinson), and SPVL3 (Immunotech, Marseilles, France), respectively. We measured the proliferative response of isolated peripheral-blood mononuclear cells to stimulation with antigen (10 Lf units of tetanus toxoid per milliliter; Swiss Serum and Vaccine Institute, Berne) or mitogen (pokeweed mitogen, 1:1000; GIBCO, Grand Island, N.Y.) as previously described.<sup>12</sup>

Immunoblotting of lysates of peripheral-blood mononuclear cells from the patients and a healthy control subject was performed after proteins separated by sodium dodecyl sulfate-polyacrylamide-gel electrophoresis were transferred to nitrocellulose filters. We used a pool of monoclonal antibodies against the  $\alpha$  chain of HLA-DR (DA6.147,<sup>13</sup> 5F2.3,<sup>14</sup> and TAL-1B5<sup>15</sup>) or the monoclonal antibody CR3/43, which is specific for the HLA-DR  $\beta$  chain. Horseradish-peroxidase-conjugated antimouse immunoglobulin (Amersham Life Science, Amersham Little Chalfont, Buckinghamshire, United Kingdom) was used as secondary antibody, and specific binding was visualized by Western blotting (ECL kit, Amersham).

We analyzed HLA-DR mRNA in peripheral-blood mononuclear

cells by the reverse-transcribed polymerase chain reaction as previously described,<sup>16</sup> using oligonucleotide primer pairs specific for HLA-DR $\alpha$  (exon 1, sense ATGGCCATAAGTGGAGTCCCTGTGC; exon 4, antisense CTGCGTTCTGCTGCATTGCTTTTGGCG-CTCC<sup>17</sup>) and HLA-DR $\beta_1$  (sense GTCATTTCTCAATGGGACGGAGCG, antisense CGCCGCTGCACTGTGAAGCTCTC<sup>18</sup>). Primer pairs specific for glyceraldehyde-3-phosphate dehydrogenase (sense CCACCCATGGCAAATTCATGGCA, antisense TCTAGACGGCAGGTCAGGTCCACC<sup>19</sup>) were included as an internal control. The products generated by the polymerase chain reaction were fractionated on 1.5 percent ethidium bromide-agarose gels, validated by their conformity to the predicted size, blotted onto Hybond-N filter membranes, and hybridized with synthetic oligonucleotides specific for HLA-DR $\alpha$  (exon 3, CCTCAGTTGAGGGCAGGAA-GGGGAGATAGTGG<sup>17</sup>), HLA-DR $\beta_1$  (CTCCCCACGTCGCTG-TCGAAGCG<sup>18</sup>), and glyceraldehyde-3-phosphate dehydrogenase (TCTAGACGGCAGGTCAGGTCCACC<sup>19</sup>). The internal probes were labeled at the 3' end with DNA deoxynucleotidyltransferase (Boehringer-Mannheim Biochemica, Mannheim, Germany) and [ $\alpha^{32}$ P]deoxyadenosine triphosphate (Amersham), and the hybridization signals were examined by autoradiography at  $-80^{\circ}\text{C}$ .

### RESULTS

The most prominent immunologic abnormality in the twin brothers was undetectable or inducible surface expression of MHC class II molecules on peripheral-blood mononuclear cells. Dual-color immunofluorescence staining demonstrated a lack of

HLA-DR expression on the infants' B cells, monocytes, and activated T cells (Fig. 1). However, the activated T cells expressed normal levels of interleukin-2 receptor (CD25) (Fig. 1). Furthermore, HLA-DR expression could not be induced on the infants' monocytes or lymphocytes by interferon gamma (Fig. 2). In both infants, B cells transformed by Epstein-Barr virus revealed defective expression of all three MHC class II isotypes (Fig. 2), whereas the surface expression of MHC class I antigens on their lymphocytes and transformed B cells was normal (data not shown).

Unexpectedly, both infants had the capacity to produce specific antibodies and to develop antigen-specific T-cell reactivity after vaccination. Before the second diphtheria and tetanus vaccination, serum antibodies specific for tetanus toxoid were undetectable or at very low levels ( $<0.02$  IU per milliliter) in both infants. After the second vaccination, substantial antibody titers developed against tetanus toxoid, which were further increased by a booster vaccination at the age of 14 months (Fig. 3). Even though the dosage of the IgG-substitution therapy in Twin 1 remained unchanged, antibody titers against tetanus toxoid increased significantly after the second vaccination. In

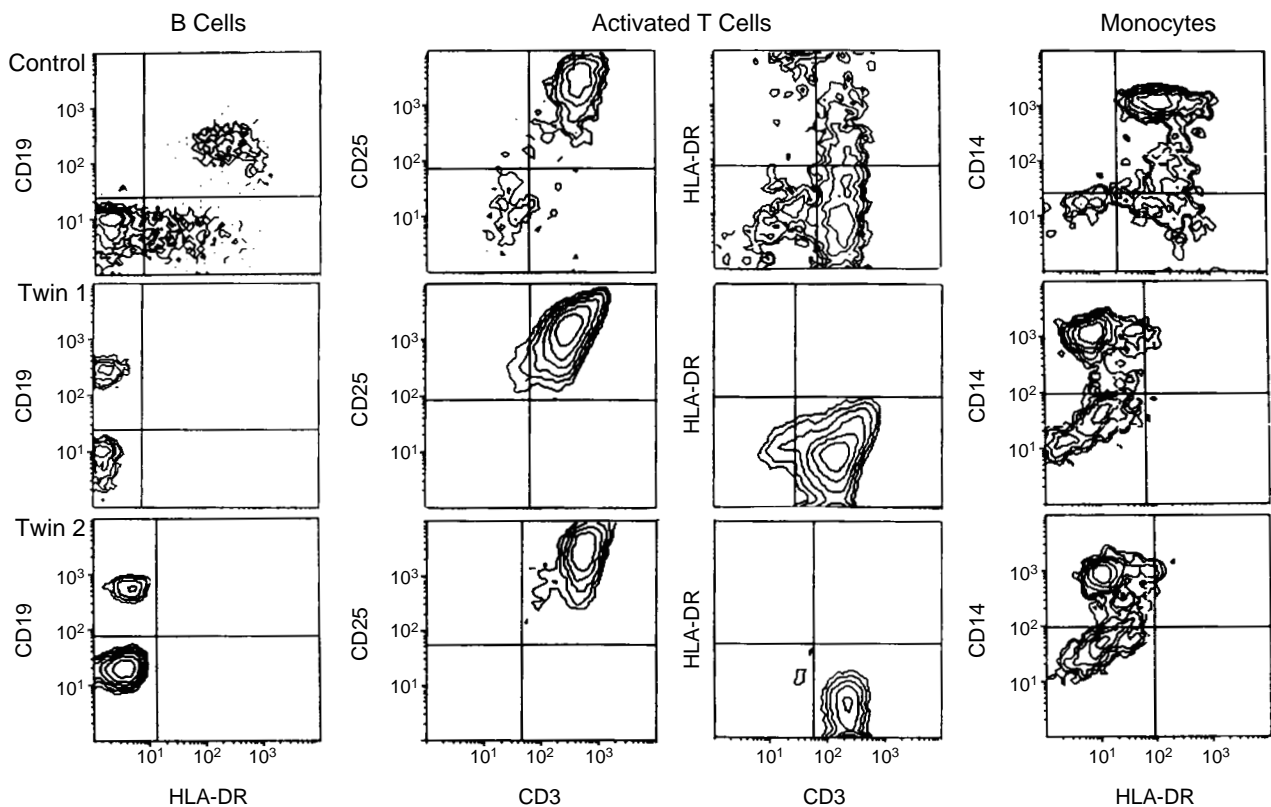


Figure 1. Defective Surface Expression of HLA-DR on B Cells, Activated T Cells, and Monocytes.

HLA-DR expression was examined by dual-color flow cytometry on peripheral-blood B cells (CD19 cells), phytohemagglutinin-activated T cells (CD3/CD25 cells), and peripheral-blood monocytes (CD14 cells in the peripheral-blood mononuclear-cell fraction with a characteristic profile of forward scatter as opposed to 90-degree side scatter) in the two twins and in a healthy control subject. Results are expressed in arbitrary units of fluorescence.

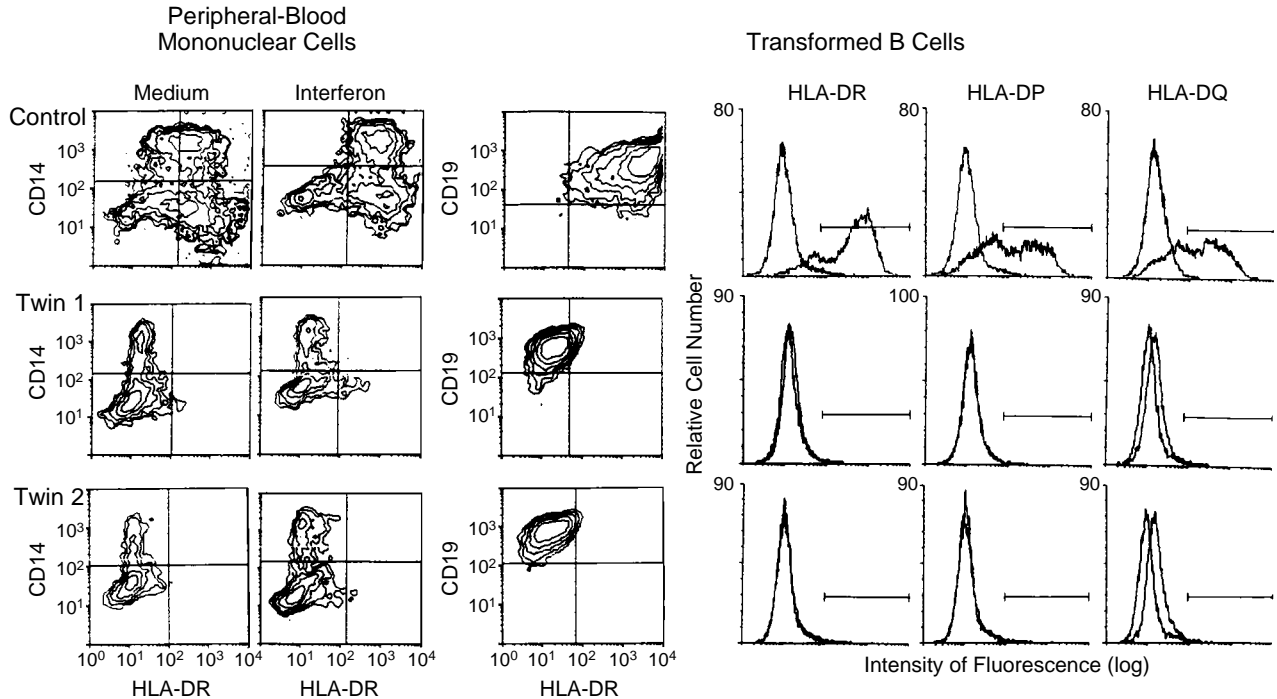


Figure 2. Defective Surface Expression of MHC Class II Molecules on Peripheral-Blood Mononuclear Cells Treated with Interferon Gamma and B Cells Transformed by Epstein-Barr Virus.

The two panels on the left show surface expression of HLA-DR and CD14 as determined by flow cytometry on peripheral-blood mononuclear cells after a 48-hour incubation with recombinant human interferon gamma (500 IU per milliliter) or without it (Medium) in the two twins and a healthy control. The number of monocytes was increased by preselection of a gate on the forward-angle as opposed to the right-angle side-scatter histogram. The four panels on the right show the results of flow cytometry measuring the expression of HLA-DR, DP, and DQ on B-cell lines transformed by Epstein-Barr virus (>96 percent CD19). The horizontal bars indicate the numbers of cells that showed specific staining for the respective monoclonal antibodies.

addition, both infants had substantial serum levels of antibodies against diphtheria toxin after vaccination (>0.3 IU per milliliter), and antibodies against poliovirus were also detectable after vaccination with inactivated-poliovirus vaccine (titer in Twin 1, 1:31; in Twin 2, 1:20).

Although no substantial antigen-specific T-cell responses could be detected after the first two diphtheria and tetanus vaccinations (administered at the ages of 3 and 8 months), levels of tetanus-toxoid-specific T-cell proliferation after the third diphtheria and tetanus vaccination (administered at the age of 14 months) were similar to those in healthy adults (Fig. 3). A monoclonal antibody (9-49)<sup>20</sup> against a determinant expressed on HLA-DR, DP, and DQ completely blocked the tetanus-toxoid-specific proliferation of lymphocytes from the two infants. The inhibitory effect was seen in the antigen-induced proliferation of T cells, whereas mitogen-induced proliferation of T cells was unaffected (Fig. 3).

Immunoblotting of protein extracts from the infants' peripheral-blood mononuclear cells demonstrated that the cells in both infants were capable of producing HLA-DR  $\alpha$ -chain and  $\beta$ -chain protein. However, substantially less protein was detected than

in a healthy control subject (Fig. 4A). In the two infants, normal expression of mRNA for the  $\alpha$  chain of HLA-DR could be demonstrated. In contrast, only trace amounts of mRNA were detected for the HLA-DR  $\beta$  chain (Fig. 4B).

## DISCUSSION

In previously described patients with MHC class II deficiency, the lack of expression of MHC class II molecules in cell surfaces has been ascribed to a defect in transcription that affects all three class II isotypes.<sup>4,9,10</sup> The histologically identical twin brothers we describe have an MHC class II deficiency in which trace amounts of mRNA for the HLA-DR  $\beta$  chain and normal levels of HLA-DR $\alpha$  mRNA can be found in blood cells.

The transcriptional defect in HLA-DR  $\beta$ -chain expression clearly accounts for the substantially decreased levels of HLA-DR $\beta$  protein. However, the levels of HLA-DR $\alpha$  protein were decreased as well, although the levels of HLA-DR $\alpha$  mRNA were normal. The likely reason for the decreased amounts of  $\alpha$ -chain protein is accelerated degradation of free  $\alpha$  chain in the absence of normal  $\beta$ -chain production. Incomplete MHC class II complexes that lack any chains are not

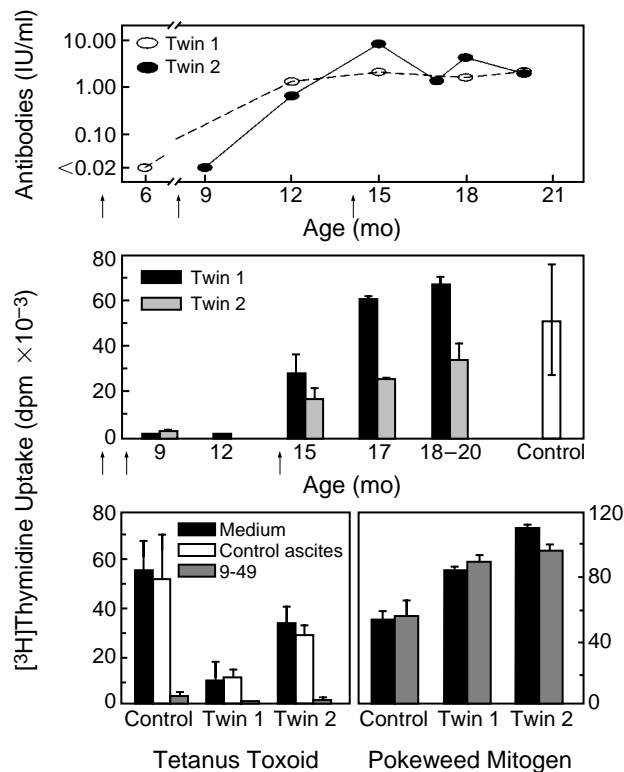


Figure 3. Humoral and Cellular Immune Responses after Vaccination with Tetanus Toxoid.

Serum levels of tetanus-toxin–neutralizing antibodies (top panel) and proliferative responses in peripheral-blood mononuclear cells induced by tetanus toxoid (mean  $\pm$ SD) results of triplicate cultures, middle panel) were examined in the twins after diphtheria and tetanus vaccination at the ages of 3, 8, and 14 months (arrows). Background proliferative responses in the absence of antigen were always below 2800 dpm. The control value indicates the mean ( $\pm$ SD) proliferation in peripheral-blood mononuclear cells induced by tetanus toxoid in nine healthy controls (background response,  $845 \pm 626$  dpm). Data were not available for Twin 2 at 12 months. The bottom panel shows the mean ( $\pm$ SD) results of triplicate cultures of peripheral-blood mononuclear cells from the two infants and a healthy control that were stimulated with tetanus toxoid or pokeweed mitogen in the presence of medium alone, medium containing control mouse ascites, and medium containing the MHC class II–specific monoclonal antibody 9-49.

expressed on the cell surface but are retained in the cell and degraded there.<sup>21</sup>

Previously described patients with MHC class II deficiency had no ability to present antigen on MHC class II molecules, leading to a complete lack of T-cell responses to recall antigens and severe impairment in the production of specific antibodies.<sup>3,11,22</sup> In contrast, the two infants we describe had the capacity to mount a specific antibody response after vaccination. Substantial serum levels of antibodies to a variety of different antigens could be detected in both infants.

In addition, the infants had a normal antigen-specific T-cell response after vaccination with tetanus toxoid, and this response was completely inhibited by the ad-

dition of a monoclonal antibody specific for MHC class II. Thus, the T-cell response to recall antigen was restricted to MHC class II. Amounts of MHC class II molecules too low to be detected by flow cytometry but sufficient to induce substantial T-cell memory are probably expressed on the surface of the infants' cells. T cells are known to be very sensitive and can recognize low concentrations of a specific antigen presented on MHC class II molecules, since probably fewer than 100 peptide–MHC complexes are required to trigger T-cell activation.<sup>23,24</sup>

The expression of MHC class II molecules on thymic stromal cells is important for the maturation of CD4

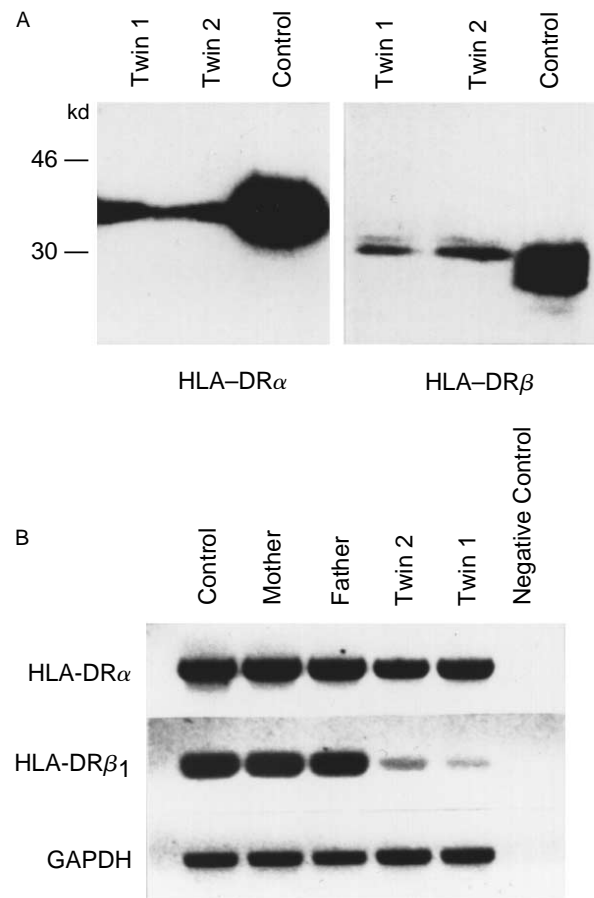


Figure 4. Immunoblotting of HLA-DR $\alpha$  and HLA-DR $\beta$  and Polymerase-Chain-Reaction Analysis of mRNA Expression.

Immunoblotting of lysates of peripheral-blood mononuclear cells obtained from the twins and an unrelated healthy control was performed with monoclonal antibodies directed against the HLA-DR $\alpha$  chain and a monoclonal antibody directed against the HLA-DR $\beta$  chain (Panel A). Reverse-transcribed polymerase-chain-reaction analysis of HLA-DR $\alpha$  and  $\beta_1$  mRNA expression in peripheral-blood mononuclear cells from three healthy control subjects (an unrelated subject and the twins' mother and father) and the twins was performed as described in the Methods section (Panel B). An assay without the complementary DNA template served as the negative control. GAPDH denotes glyceraldehyde-3-phosphate dehydrogenase.

T cells.<sup>25</sup> Although the usual phenotype of MHC class II deficiency presents with decreased CD4 T cells in peripheral blood,<sup>3,11</sup> the two infants had normal levels of CD4 cells. It appears probable that, like their antigen-presenting cells, their thymic epithelial cells express MHC class II antigens in low quantities that are nonetheless sufficient to induce the maturation of normal numbers of CD4 T cells.

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