Molecular Characterization of Major Histocompatibility Complex Class II Gene Expression and Demonstration of Antigen-specific T Cell Response Indicate a New Phenotype in Class II–deficient Patients

By Ilona Hauber, Heinz Gulle, Hermann M. Wolf, Maggi Maris, Heinz Eggenbauer, and Martha M. Eibl

From the Institute of Immunology, University of Vienna, A-1090 Vienna, Austria

Summary

Major histocompatibility complex (MHC) class II deficiency is an inherited autosomal recessive combined immunodeficiency. The disease is known as bare lymphocyte syndrome (BLS). BLS is characterized by a lack of constitutive MHC class II expression on macrophages and B cells as well as a lack of induced MHC class II expression on cells other than professional antigen-presenting cells (APCs) due to the absence of mRNA and protein of the human leukocyte antigen (HLA) class II molecules, designated HLA-DR, -DQ, and -DP. The defect in gene expression is located at the transcriptional level and affects all class II genes simultaneously. Here we have analyzed transcription and protein expression of class II antigens in Epstein-Barr virus (EBV)–transformed B lymphoblastoid cell lines and mononuclear cells (MNCs) of twin brothers. Whereas flow cytometric analysis failed to detect class II antigens on the cell surface of the patients' EBV-B cells and MNCs, examination of the genes coding for HLA-DR, -DQ, -DP, and the invariant chain (Ii) by reverse transcriptase-polymerase chain reaction amplification resulted in an unusual mRNA pattern in the B cell lines of the patients (HLA-DRα^+, -DRβ^-, -DQα^+, -DQβ^-, -DPα^-, -DPβ^+, Ii$^+$). In accordance with these findings no HLA-DRβ–specific protein was detected by immunoblotting, whereas low levels of HLA-DRα and normal levels of Ii were present. In contrast to EBV-B cells, the MNCs of both patients displayed a residual HLA-DRβ, -DQβ, and -DPα mRNA signal. Furthermore, HLA-DRβ–specific protein was found in addition to HLA-DRα by immunoblotting of cell lysates, even though it was clearly decreased as compared with controls. Our results indicate that the defect in class II antigen expression is not necessarily present to the same extent in B cells and cells of other lineages. mRNA levels of HLA-DRβ were found to be enriched in adherent cells within the MNC fraction. Further investigations indicated that the MHC class II expressed is functional in antigen presentation, as the two boys' CD4$^+$ T cells became activated and expressed interleukin-2R after stimulation of peripheral blood mononuclear cell cultures with recall antigen (tetanus toxoid). Furthermore, T cells tested in one of the two patients responded to both MHC class I and II allostimulation, and this response was inhibited by monoclonal antibodies of the respective specificity. Whereas the MNC population contained sufficient APCs to activate CD4$^+$ T cells in response to antigenic stimulation, the patients' EBV-B cells were unable to present recall antigen to autologous, long-term cultured, antigen-reactive T cells or to a normal, HLA-DR–compatible, antigen-specific T cell line. In contrast, the patients' EBV-B cells functioned normally as accessory cells for mitogen-induced T cell proliferation. The results obtained from the investigations of MHC class II–dependent immune functions indicate antigen presentation by a subset of cells, obviously present in the HLA-DRβ mRNA-expressing adherent MNC population, whereas the patients' EBV-B cells lack this ability.

MHC class II proteins expressed on APCs play a critical role in cellular recognition, in antigen presentation, and in the induction of the immune response. In general, many different peptides derived from naturally processed antigens bind to class II proteins and are then presented to helper T lymphocytes. MHC class II proteins are the product of immune response genes located on the short arm of chromosome 6 of the human genome (1). The class II locus

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/95/04/1411/13 $2.00
Volume 181 April 1995 1411–1423

(HLA-D) is divided into three major subregions designated HLA-DR, -DQ, and -DP (2), and each isotype is encoded by separate α and β chain genes. Conserved upstream sequence elements, termed W, X, and Y, and proteins that interact with these boxes, have been shown to mediate B cell–specific and IFN-γ–induced expression of several HLA-D locus genes (3, 4). The HLA-D locus is unusual in that both chains of the various heterodimeric proteins are products of the same genetic region (1). Before being expressed on the cell surface, all MHC class II proteins are associated with the invariant chain (Ii)[1], and it has been proposed that this chain may direct class II proteins into the endocytic pathway, where they encounter internalized antigens (5). The level of cell surface expression of class II proteins correlates closely with the level of intracellular class II mRNA (3). Thus, modulation of MHC class II surface expression is generally achieved by regulation of class II gene expression. Impaired expression of the HLA-D locus genes leads to a disease described as bare lymphocyte syndrome (BLS) (6).

A number of studies have indicated that the HLA-D locus genes and their rearrangement are intact in reported MHC class II–deficient patients and that regulatory mutations are responsible for the lack of stable mRNA (6–8). The regulatory mutations involved affect class II expression at the transcriptional level (9). Analysis of MHC class II transcriptional mutant B cell lines that were either derived from BLS patients or produced in vitro has provided a clue to the role of *cis*- and *trans*-acting regulatory factors. Fusion experiments of mutant cell lines to healthy B cell lines resulted in reconstituted expression of all class II genes (3, 10–12). The same experiments carried out between various class II–negative B cell lines have defined four separate complementation groups, suggesting that multiple regulatory defects can cause this disease (3, 10–12). The consequences of all regulatory defects are the lack of RNA encoding MHC class II molecules.

In this paper, we present the molecular and immunological characterization of a new phenotype of MHC class II deficiency in twin brothers. Flow cytometric analysis of their mononuclear cells (MNCs) and EBV-B cells isolated and established from peripheral blood of both children failed to detect class II antigen on the cell surface. Therefore, we analyzed transcription and protein expression of MHC class II genes by reverse transcriptase (RT)–PCR and immunoblotting techniques. Our results show an unusual mRNA pattern in the patients' MNCs and EBV-B cells (reduced or undetectable mRNA levels of HLA-DRβ, -DQβ, and -DPα, and normal mRNA levels encoding HLA-DRα, -DQα, and -DPβ). By fractionating MNCs into nonadherent (lymphoid cell population) and adherent cells (monocyte/macrophage lineage), an enrichment of HLA-DRβ gene expression was found in cells of the adherent MNC fraction. Supporting the molecular results, the patients' T cells were capable of responding to recall antigen (tetanus toxoid [Tet Tox]) after vaccination. In response to stimulation of the MNCs with recall antigen, in both patients CD4$^+$ and TCR-α/β^+ cells were the major lymphocyte phenotype that became activated and expressed IL-2R (CD25). However, patients' EBV-B cells were not able to present antigen to autologous, long-term cultured, antigen-reactive T cells or to an HLA-DR–compatible, antigen-specific T cell line from a healthy individual, whereas they functioned normally as accessory cells in the mitogen–response. These results indicate that cells within the monocyte/macrophage lineage, obviously present in the HLA-DRβ mRNA-expressing adherent MNC population, are likely to be APCs in these two boys. In comparison to already known class II–deficient cell lines, we describe here a new in vivo phenotype, suggesting the existence of a new complementation group, and provide evidence for the functional equivalent of the molecular characteristics.

Materials and Methods

Patients. The children are histoidentical twin brothers born prematurely at 34 wk of gestation to unrelated healthy parents of Turkish origin. The boys had low birth weight (KEN: 1,020 g, KER: 2,030 g), and were delivered by cesarian section because of premature rupture of membranes. The detailed clinical description of the two boys is reported elsewhere (13). One boy (KEN) was first admitted to the hospital at the age of 10 wk because of febrile seizures, and again with pneumonia at the age of 4 mo. Immunological examination revealed hypogammaglobulinemia, normal lymphoproliferative responses to mitogens, and a normal distribution of lymphocyte subpopulations (CD3$^+$ 62%, CD4$^+$ 43%, CD8$^+$ 23%, and CD19$^+$ 27%). The diagnosis of MHC class II deficiency was based on flow cytometric analysis, which revealed a lack of HLA-DR expression on the patient's resting peripheral blood leukocytes. The other boy (KER) was asymptomatic, and his physical and mental development was uneventful since birth. Immunological studies revealed a defect in MHC class II expression comparable with his twin brother, whereas the distribution of lymphocyte subsets (CD3$^+$ 75%, CD4$^+$ 48%, CD8$^+$ 29%, and CD19$^+$ 14%) and the lymphoproliferative responses to mitogens were within the normal range. Serum IgG and IgA levels were normal, but serum IgM was decreased (20 mg/dl).

Isolation of Mononuclear Cells, Establishment of Lymphoblastoid B Cell Lines and Long-term Cultures of T Cells. MNCs were isolated from heparinized peripheral blood (7.5 IU/ml) by buoyant density gradient centrifugation (14, 15). After enrichment by multiple rounds of polystyrene adsorption, nonadherent cells were fractionated into T-enriched cells and non-T cells by rosetting with sheep erythrocytes treated with AET (2-aminoethylisothiouronium bromide; Sigma Chemical Co., St. Louis, MO) as described elsewhere (16). Non-T cells were transformed with EBV using the supernatant from the B 95-8 marmoset cell line (American Type Culture Collection, Rockville, MD) according to a standard protocol (17). Growing cells were expanded in RPMI 1640 medium supplemented with 20% heat-inactivated FCS (Hyclone Laboratories, Logan, UT), 2 mM L-glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin (Gibco, Paisley, Scotland). To establish an antigen-specific T cell line from a healthy, HLA-DR–compatible, control individual or long-term cultured T cells from one (KEN) of the two patients, MNCs from the patient or the control were stimulated with Tet Tox for 1 wk and were then cultured for several weeks in the presence of highly purified human IL-2 (10 IU/ml, Lymphocult T-HPLyo.-

[1] *Abbreviations used in this paper:* BLS, bare lymphocyte syndrome; β_2m, β_2-microglobulin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Ii, invariant chain; LF, Loeffler flocculation units; MNC, mononuclear cell; Tet Tox, tetanus toxoid.

Biotest-No.811040, Dreieich, Germany) without (patient) or with (control individual) repeated restimulation using antigen and autologous macrophages. All cell cultures were maintained at 37°C in 5% CO_2 humidified air.

One-way Mixed Lymphocyte Reaction. To examine MHC class I- and II-specific alloresponses in the patient KEN, MNCs from this patient or an unrelated healthy control individual were stimulated for 7 d with irradiated (10,000 rad) Daudi cells or EBV-transformed B cells from another unrelated patient with MHC class II deficiency (BCH). Triplicate cultures of responder cells (2.5×10^5/ml) and stimulator cells (2.5×10^5/ml) were set up in flat-bottomed microtiter plates (Falcon 3070, Microtest III; Becton Dickinson Labware, Lincoln Park, NJ) in culture medium as previously described (18) supplemented with mouse ascites (1:50) containing irrelevant mAbs (control ascites) or mouse ascites containing the MHC class II-specific mAb 9-49 (kindly provided by Dr. R. F. Todd III, University of Michigan Medical School, Ann Arbor, MI) (19) or MHC class I-specific mAb PA2.6 (kindly provided by Dr. J. L. Strominger, Harvard Medical School, Boston, MA) (20). The resulting cell proliferation was assessed by [^{3}H]thymidine incorporation measured with a Tri-Carb liquid scintillation counter (model 4640; Packard Instruments, Meriden, CT).

B Cell Accessory Cell Function for Recall Antigen- and Mitogen-induced T Cell Response. To examine B cell accessory cell function for antigen- and mitogen-induced T cell proliferation, a Tet Tox-specific T cell line from a healthy individual (HLA-DR compatible to the patients) or long-term cultured, antigen-reactive T cells from the patient KEN (2.5×10^5 T cells/ml) were stimulated for 3 d with Tet Tox (10 Loeffler flocculation units (LF)/ml; Swiss Serum and Vaccine Institute, Berne, Switzerland) or PWM (1:1,000, GIBCO BRL, Gaithersburg, MD) in the presence of autologous EBV-B cells or HLA-DR-compatible EBV-B cells from the patients or healthy control individuals (2.5×10^5 B cells/ml) as accessory cells. As a control, T cells were cocultured with EBV-B cells in medium alone. Antigen- or mitogen-induced T cell proliferation was determined by measuring [^{3}H]thymidine incorporation.

Flow Cytometry. Flow cytometric analysis of surface membrane expression of MHC class I and II antigens and detection of intracytoplasmic expression of Ii was carried out by indirect immunofluorescence using the mAbs Mon 1013 (clone 7.5.10.1, directed against HLA-DR, -DP, and -DQ; Monosan, Uden, The Netherlands) (21), IOT2 (clone B9.12.1, detecting a monomorphic determinant of MHC class I antigen associated with β_2-microglobulin (β_2m); Immunotech, Marseilles, France) (22), and VIC-Y1 (directed against Ii, kindly provided by Dr. O. Majdic, Institute of Immunology, University of Vienna, Vienna, Austria) (23). Isotype-matched mouse monoclonal Ig was used as a control for unspecific staining. For intracytoplasmic staining, cells were fixed with 0.25% paraformaldehyde and permeabilized with 0.2% Tween in PBS.

Surface expression of the IL-2R on CD3- or CD4-positive cells within the MNC fraction was examined by dual-color flow cytometry after stimulation with antigen or mitogen. Therefore, MNCs (10^6/ml) were cultured in 24-well tissue culture plates for 7 d in the presence of antigen (Tet Tox, 10 LF/ml), mitogen (PWM, 1:1,000), or culture medium alone. Cells were stained using directly labeled mAbs Leu-4 (CD3), Leu-3a, (CD4), and IL-2R (anti-Tac, CD25) (all purchased from Becton Dickinson & Co., Mountain View, CA) and immunofluorescence staining was evaluated with a cytofluorograph (FACScan®, Becton Dickinson & Co.).

Isolation of RNA and Specific Amplification of cDNA. Total cellular RNA was isolated from nonstimulated MNCs and EBV-transformed B cells of the patients (KER, KEN) and healthy controls (HC, MOTHER, FATHER) according to the method of Chomczynski (24). Equal amounts of total RNA, quantified at 260 nm, were reverse transcribed into cDNA by first and second strand synthesis employing avian myeloblastosis virus (AMV) RT (Boehringer Mannheim Biochemicals, Mannheim, Germany). The cDNA was directly amplified (25, 26) on a thermocycler (model 60; Biomed Instruments Inc., Fullerton, CA) using AmpliTaq DNA polymerase (Perkin Elmer Cetus, Norwalk, CT) and oligonucleotide primer pairs specific for HLA-DRα (exon1, sense: ATGGCCATAAGTGGAGTCCCTGTGC; exon4, antisense: CCTGCGTTCTGCTGCATTGCTTTTGCGCACTCC) (27), HLA-DRβ_1 (sense: GTCATTTCTTCAATGGGACGGAGCG, antisense: CGCCGCTGCACTGTGAAGCTCTC) (28), HLA-DQα (RS151 and RS152), HLA-DQβ (AmpA and AmpB), HLA-DPα (AmpA and AmpB) (Genset, Paris, France), HLA-DPβ_1 (sense: CCTTGCAGCACCACAACCTGCTTGTC, antisense: CCTGTGCATGAAGATGCCCACTCCAC) (29), HLA-Ii (sense: GGATGACCAGCGCGACCTTATCTCC, antisense: CCAGATCCTGCTTGGTCACACCCAG) (30), β_2m (sense: CTCGCGCTACTCTCTCTTTCTGG, antisense: GCTTACATGTCTCGATCCCACTTAA) (31), TNF-β and M-CSF (Clontech Lab., CA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (sense: CCACCCATGGCAAATTCCATGGCA, antisense: TCTAGACGGCAGGTCAGGTCCACC) (32), and S14 ribosomal protein (sense: GGCAGACCGAGATGAATCCTCA, antisense: CAGGTCCAGGGGTCTTGGTCC) (33) were used as internal controls. The amplification profile involved 30 cycles (35 cycles for TNF-β and M-CSF) of denaturation at 95°C for 1 min, primer annealing at 60°C for 2 min (Genset primer pairs were annealed at 55°C), and primer extension at 72°C for 3 min. Aliquots of PCR-generated products were fractionated on 1.5% EtBr-agarose gels, validated by the predicted size, and blotted onto Hybond-N filter membranes according to the manufacturer's protocol (Amersham International, Buckinghamshire, UK).

Hybridization and Analysis of Data by Densitometry. Blotted filter membranes were validated by hybridization with internal probes. Therefore oligonucleotides specific for HLA-DRα (exon3: CCTCAGTTGAGGGCAGGAAGGGGAGATAGTGG) (27), HLA-DRβ_1 (CTCCCCCACGTCGCTGTCGAAGCG)(28), HLA-DQα (exon 2, RH54) (Genset), HLA-DQβ (CTGGTAGTTGTGTCTGCACAC), HLA-DPα (Aso: AAGATGAGATGTTCTATG or AAGATGAGCAGTTCTATG) (Genset), HLA-DPβ_1 (CCAGCTCCCGTCAATGTCTTACTCCG) (29), HLA-Ii chain (CAGGAGCCAATGGTGCATCCAGCTCTC) (30), β_2m (CACTTTTTCAATTCTCTCTCCATTCTTCAG) (31) and GAPDH (TCTAGACGGCAGGTCAGGTCCACC) (32) were 3′ end labeled using the enzyme terminal transferase (Boehringer Mannheim Biochemicals) and α[^{32}P]deoxyadenosine 5′-triphosphate (Amersham International). The unincorporated nucleotides were removed on an NAP-5 column (Pharmacia, Uppsala, Sweden). Prehybridization of the filter membranes was carried out for 4 h at the corresponding annealing temperature (55–60°C) in 5× SSC, 20 mM $NaPO_4$, pH 7, 10× Denhardt's solution, 7% SDS, 100 μg/ml sonicated salmon sperm DNA, and 100 μg/ml poly(A). Hybridization was performed at 55–60°C in the prehybridization buffer containing 10% dextran sulphate plus added radiolabeled-specific oligonucleotides for 16 h. The blots were washed once in 3× SSC, 20 mM $NaPO_4$ pH 7, 10× Denhardt's solution, 5% SDS for 30 min, followed by a second wash in 1× SSC, 1% SDS at hybridization temperature for 40 min. Air-dried filters were exposed to Kodak XDS films using Kodak X-OMATIC regular intensifying screens (Kodak, Rochester, NY).

The relative density of the fragments was determined by image-analyzing densitometry (OD*MM) (Pharmacia LKB Biotech., pdi Quantity One, Uppsala, Sweden). The densitometric reading of

the indicated genes in a particular test was related to the corresponding densitometric reading for the internal controls S14 ribosomal protein and/or GAPDH.

Antibodies. The anti-HLA-class II α chain mAbs DA6.147 (34) and 5F2.3 (35) were a generous gift of Dr. C. M. Steel (Western General Hospital, Edinburgh, Scotland) and the anti-HLA-class II α chain mAb TAL-1B5 (36) was kindly provided by Dr. J. G. Bodmer (Imperial Cancer Research Fund, Lincoln's Inn Fields, London, UK). The anti-HLA-DRβ mAb, clone CR3/43, was obtained from Boehringer Mannheim.

Immunoblotting. MNCs were lysed in 1× SDS sample buffer and boiled for 5 min before SDS-PAGE. EBV-B cells were lysed in NP-40 lysis buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% deoxycholic acid, 1 mM PMSF, 10 μg/ml aprotinin, 10 μg/ml leupeptin) and boiled for 5 min in 5× SDS sample buffer or, alternatively, directly lysed and boiled in 1× SDS sample buffer. After electrophoresis, gels were equilibrated in blotting buffer (48 mM Tris, 39 mM glycine, 20% methanol) and proteins were transferred to 0.2 μm nitrocellulose sheets (Schleicher and Schuell, Dassel, Germany) using a Trans-Blot SD semi-dry electrophoretic transfer cell (Bio-Rad Laboratories, Vienna, Austria). The nitrocellulose sheets were blocked in 5% skim milk/TBS (Tris-buffered saline) containing 0.1% Tween-20, and then probed with the primary antibodies. Specific binding was visualized either by autoradiography at −70°C using ^{35}S-labeled anti-mouse Ig (0.2 μCi/ml; Amersham International) and Kodak XAR films or with the ECL Western blotting analysis system (Amersham International) in combination with horseradish peroxidase–conjugated anti-mouse Ig (Amersham International) and Kodak XDS films.

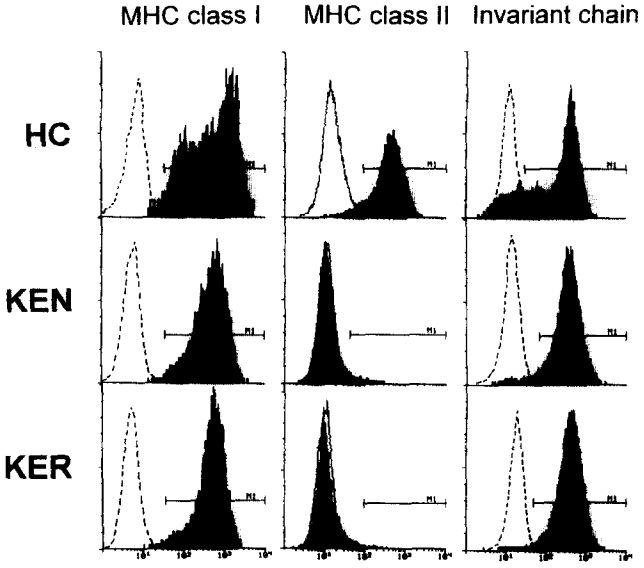

Figure 1. Surface membrane expression of MHC class I and II antigens and intracytoplasmic expression of invariant chain in EBV-transformed peripheral blood B cells. EBV-B cells from both patients (*KEN*, *KER*) and a healthy, unrelated control (*HC*) were examined for surface membrane expression of MHC class I and II antigen and intracytoplasmic expression of invariant chain, using indirect immunofluorescence evaluated by flow cytometry. Results are depicted as fluorescence intensity displayed on the horizontal axis (logarithmic scale) versus relative cell number given on the vertical axis. Black shading indicates staining with the specific mAb, whereas broken lines indicate binding of an unspecific isotype-matched control mouse Ig.

Results

Lack of Class II Antigen Expression on EBV-B Cells from Patients with BLS. Analysis of the patients' EBV-B cells by flow cytometric analysis revealed a lack of MHC class II antigens, whereas Ii chain and class I antigen expression was normal and comparable to a healthy control subject (Fig. 1, KEN, KER versus HC). Since the mAbs used do not distinguish polymorphism of class II molecules, it is likely that the observed lack of HLA-DR, -DQ, and -DP expression in both children reflected an absence of cell surface class II antigens, each consisting of distinct α and β chains (Fig. 1).

Uncoordinated Expression of α and β Chain Genes of the HLA-D Region in the Patients. Having demonstrated the lack of class II antigen on the surface of the MNCs and EBV-B cells (Fig. 1) of the patients, we examined class II–specific gene expression. Total RNA from the patients and from three healthy control individuals was subjected to RT-PCR analysis. The PCR-generated products for the genes encoding HLA-DR, -DQ, and -DP α and β chains, HLA-associated Ii chain, β_2m, and GAPDH were detected by Southern blotting and specific hybridization under conditions in which no cross-hybridization occurs (for details see Materials and Methods). As shown in Fig. 2, mRNAs encoding DRα, DQα, and DPβ_1 clearly accumulated in the MNCs and EBV-B cells of the patients as well as the control subjects. However, no DRβ_1-, DQβ-, or DPα-specific transcripts were seen in the EBV-B cells, and very low levels could be detected in the MNCs of the patients (Fig. 2, KER and KEN). For accurate quantification, we subjected the Southern blot–obtained signals to densitometric image analyzing. The densitometric readings were normalized to the expression of the constitutive control mRNA encoding GAPDH. In agreement with the results shown in Fig. 2, DRβ_1-, DQβ-, and DPα-specific mRNA could not be detected in the patients' EBV-B cells, whereas low levels were present in their MNCs (Fig. 3, *b*, *d*, and *e*). Control experiments demonstrated that mRNA expression of the Ii chain (Fig. 3 *g*) and expression of the β_2m gene, which is associated with the class I molecule (Fig. 3 *h*), were normal in all individuals tested.

Presence and Distribution of HLA-DR Proteins in Cellular Lysates of Class II–Deficient Patients. To analyze in which way the defective transcription of one of the chains specific for HLA-DR will affect translation of the observed mRNA (Fig. 2), we examined the occurrence of intracellular HLA-DR proteins and Ii chain by immunoblotting techniques. As shown in Fig. 4, despite normal mRNA levels strongly reduced amounts of HLA-DRα protein were found in the cellular lysates of EBV-B cells of both patients, whereas, as was to be expected, no HLA-DRβ was detected in the same lysates, even after extensive exposure time. On the other hand, low levels of HLA-DR α and β proteins were observed in the cellular lysates of patients' MNCs (Fig. 4). In agreement with the results obtained by flow cytometric analysis (Fig. 1), normal amounts of Ii chain were also found by immunoblotting (data not shown).

Enrichment of HLA-DRα Protein in the Adherent Cell Population of One Patient Tested. Since reduced amounts of HLA-

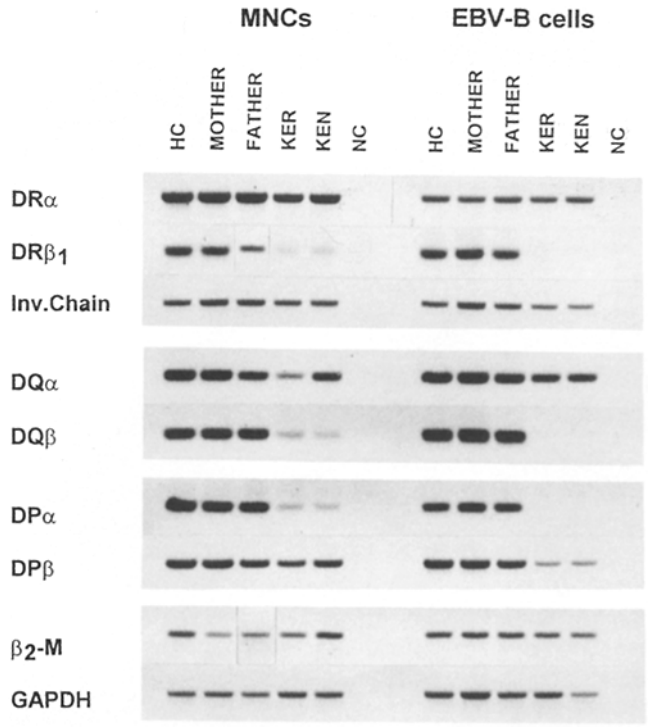

Figure 2. Southern blot analysis of amplified cDNA from unstimulated MNCs and EBV-B cells. RNA from three healthy donors (*HC, MOTHER, FATHER*) and two patients (*KER, KEN*) was isolated, reverse-transcribed, and amplified with the indicated specific primer pairs (*HLA-DRα, -DRβ1, Inv. Chain, -DQα, -DQβ, -DPα, -DPβ1, β2m*, and as internal control, *GAPDH*) as described in Materials and Methods. RT-PCR assayed without cDNA template served as a negative control (*NC*). Amplified products were fractionated on 1.5% agarose gels, blotted onto filter membranes, and hybridized with internal probes (see Materials and Methods).

DR α and β proteins were present in MNC lysates, we wanted to identify the cells within the MNC fraction expressing HLA-DR molecules. Therefore, MNCs from one patient (KEN) were divided into nonadherent and adherent cell populations by polystyrene adsorption and the cell lysates were examined by immunoblotting. In good agreement with the results obtained from EBV-B cells, a low level of HLA-DRα protein was found in the nonadherent cell population, consisting mainly of T and B lymphocytes (Fig. 5). However, HLA-DRα strongly accumulated in the adherent cell population (Fig. 5). Image-analyzing densitometry of the depicted bands revealed a nearly fourfold increase of HLA-DRα in the adherent cells, reaching 42% of the protein level present in the same cell population of a healthy control. Because of the small number of MNCs available from our patient and the constitutively lower amount of HLA-DRβ present in all cells expressing MHC class II molecules, we were unable to detect HLA-DRβ by immunoblotting in either the nonadherent or the adherent cells. However, the observed increase of HLA-DRα protein in the adherent cell population, together with our mRNA data presented in Fig. 6, strongly suggest that the cells expressing both α and β chains are a subset of the monocyte/macrophage lineage.

HLA-DR mRNA Expression in the Monocyte/Macrophage Lineage of One Patient Tested. Here we investigated the origin of the HLA-DRβ1 message of one patient's MNCs after separating the nonadherent cells from the adherent cell populations by multiple rounds of polystyrene adsorption. Total RNA was isolated from the various cell fractions and subjected to RT-PCR analysis. Our results show that the mRNA specific for HLA-DRβ1 was enriched in the adherent cell fraction as compared to the mRNA seen in the nonadherent cells, whereas EBV-B cells of the same patient lacked any detectable HLA-DRβ1-specific mRNA (Fig. 6 *a*).

To demonstrate that the HLA-DRβ1-specific transcript seen in the adherent cell population originates from a monocyte/macrophage lineage, we investigated the expression pattern of the cytokines TNF-β and M-CSF in the same cell fractions. Whereas TNF-β was constitutively produced by human EBV-B cells, the cytokine M-CSF was clearly increased in adherence-activated macrophages. As shown in Fig. 6 *b*, no TNF-β message, indicative of the presence of lymphoid cells, was found in the adherent cell fraction. However, mRNA encoding M-CSF could easily be detected (Fig. 6 *c*). These findings indicate that the HLA-DRβ1 gene expression observed in the MNCs of the patient is derived from cells of the monocyte/macrophage lineage.

Patients' CD4+ T Cells Respond to Recall Antigen. Flow cytometric analysis of peripheral blood MNCs after antigenic stimulation was carried out to investigate whether the patients' T cells are capable of responding to recall antigen (Tet Tox), and to examine the phenotype of the antigen-responsive cells. MNCs were stimulated with Tet Tox for 7 d, and the expression of IL-2R (CD25) on activated lymphocyte subpopulations was then assessed by dual-color flow cytometry. The results depicted in Fig. 7 demonstrate that despite the fact that the patients' MNCs lack detectable MHC class II antigen on the cell surface, their T cells responded to recall antigen. In two previously vaccinated controls (HC 1 and HC 2) tested in parallel as well as in our two patients, the majority of activated cells after Tet Tox stimulation were $CD3^+$ T cells [(percent $CD25^+CD3^+$ cells: controls, HC1 21.5, HC2 37.3; patients, KEN 42.4, KER 24.9; Fig. 7) (percent $CD25^+CD3^-$ cells: controls, HC1 5.7, HC2 17.4; patients, KEN 8.1, KER 9.9; Fig. 7)]. However, the patients' IL-2R–expressing T cells activated in response to antigenic stimulation were still negative for HLA-DR (data not shown). In antigen-stimulated cell cultures of controls and patients, a significant subset of the activated T cells expressed the CD4 phenotype, even though the portion of $CD4^-$ activated cells appeared to be higher in the patients' cell cultures [(percent $CD25^+CD4^+$ cells: controls, HC1 17.0, HC2 32.8; patients, KEN 23.4, KER 15.1) (percent $CD25^+CD4^-$ cells: controls, HC1 5.1, HC2 8.1; patients, KEN 25.0, KER 14.8)]. In addition, the majority of the activated cells within the antigen-stimulated MNC cultures expressed the TCR-α/β complex in the patients as well as in the controls (data not shown).

Patients' EBV-B Cells Are Unable to Present Recall Antigen. The above described findings clearly demonstrate that $CD4^+$ T cells become activated and express IL-2R after

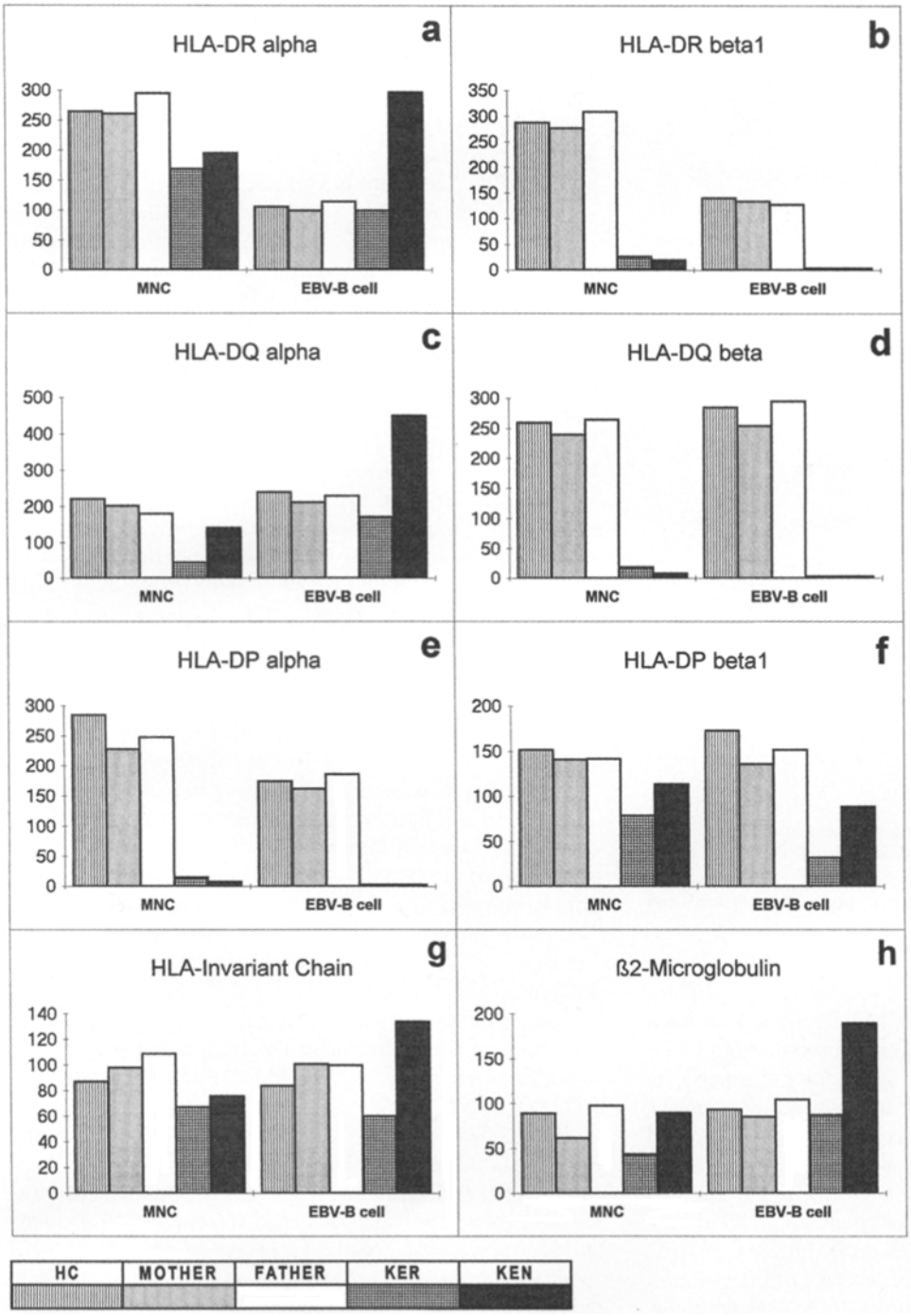

Figure 3. Densitometric quantification of HLA-DRα (*a*), -DRβ_1 (*b*), -DQα (*c*), -DQβ (*d*), -DPα (*e*), -DPβ_1 (*f*), invariant chain (*g*), and β_2-microglobulin (*h*) signals obtained from patients' and controls' unstimulated MNCs and EBV-B cells. The relative density of the signal obtained by Southern blot analysis of the PCR-generated products (depicted in Fig. 2) was measured by image-analyzing densitometry. In both patients (*KER*, *KEN*) and three healthy control individuals (*HC*, *MOTHER*, *FATHER*) the densitometric reading of each assay was related to the corresponding densitometric reading of the internal control GADPH. Relative levels of mRNA are presented as optical density × mm [OD*MM].

stimulation of the patients' MNCs with recall antigen. This observation indicates that cells within the MNC population must be capable of presenting recall antigen in the context of MHC class II molecules. In addition to cells of the monocyte/macrophage lineage and dendritic cells, B cells have the capacity to act as APCs. To investigate whether B cells functioned as APCs in inducing T cell responses to recall antigen in the two boys, we examined the antigen-presenting capacity of the patients' EBV-B cells. The results presented in Table 1 demonstrate that in contrast to healthy controls, the EBV-B cells from both patients were unable to induce antigen-specific stimulation of a normal, HLA-DR4–restricted (i.e., HLA-DR compatible to the patients). Tet Tox–specific T cell line, whereas the patients' EBV-B cells behaved normally with respect to accessory cell function for PWM-induced T cell proliferation. In addition, the EBV-B cells from one patient tested were unable to present antigen (Tet Tox) to autologous, long-term cultured, antigen-reactive T cells (data not shown).

Lymphocytes from One Patient Tested Mount an MHC Class I–

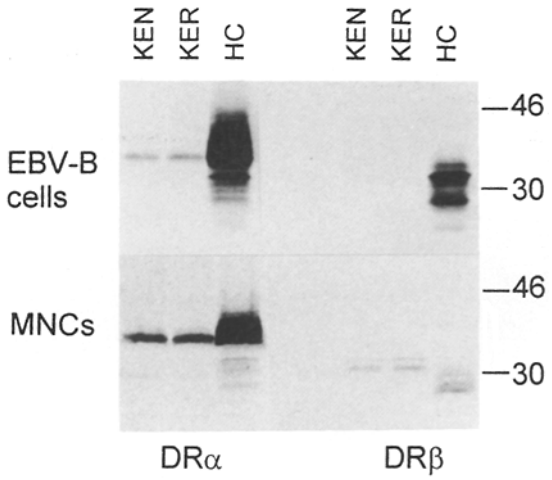

Figure 4. Occurrence of HLA-DR α and β proteins in whole lysates of EBV-B cells and MNCs. Cell lysates from two patients (*KEN*, *KER*) and a healthy control (*HC*) were separated by SDS-PAGE and the proteins were transferred onto nitrocellulose sheets. The separated proteins were probed with a pool of three monoclonal anti-HLA-DR α antibodies (DA6.147, 5F2.3, and TAL-1B5) (*left lanes*), or with a β chain–specific monoclonal anti-HLA-DR antibody CR3/43 (*right lanes*). ^{35}S-conjugated anti–mouse Ig was used as a secondary antibody and the x-ray film was exposed for 14 d at −70°C. Molecular weights in kD are indicated on the right.

and II–specific Alloresponse, which Can Be Inhibited by mAbs. MHC class I– and II–specific alloresponses have been examined in one patient (KEN) in a one-way mixed lymphocyte reaction (MLR), applying irradiated Daudi cells (MHC class II$^+$, MHC class I$^-$) and irradiated EBV-B cells from another class II–deficient patient ([BCH] MHC class II$^-$, MHC class I$^+$) as stimulator cells. The data depicted in Table 2 show that MNCs from our patient responded to both allogeneic class I and II antigens, although alloresponse to MHC class I was lower than in the control (the patient's responder cells and the BCH-stimulator cells shared one class I determinant, HLA-A2). The patient's cellular response to MHC class II antigens could be inhibited by adding the mAb 9-49 to the system, known to block class II–specific alloresponses (19). Addition of the MHC class I–specific mAb PA2.6 inhibited MHC class I–specific allostimulation, whereas it had no effect on MHC class II.

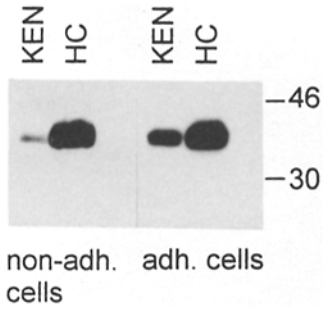

Figure 5. Distribution of HLA-DR α protein in adherent and nonadherent cell populations. MNCs of one patient (*KEN*) and a healthy control (*HC*) were fractionated into nonadherent (*left lanes*) and adherent cells (*right lanes*) by polystyrene adsorption. Cell lysates were separated by SDS-PAGE and transferred onto nitrocellulose as described and the proteins were probed with the anti-HLA-DR α mAb pool. Specific binding was visualized with horseradish peroxidase–conjugated anti–mouse Ig in combination with the ECL Western blotting detection system. Molecular weights in kD are indicated on the right.

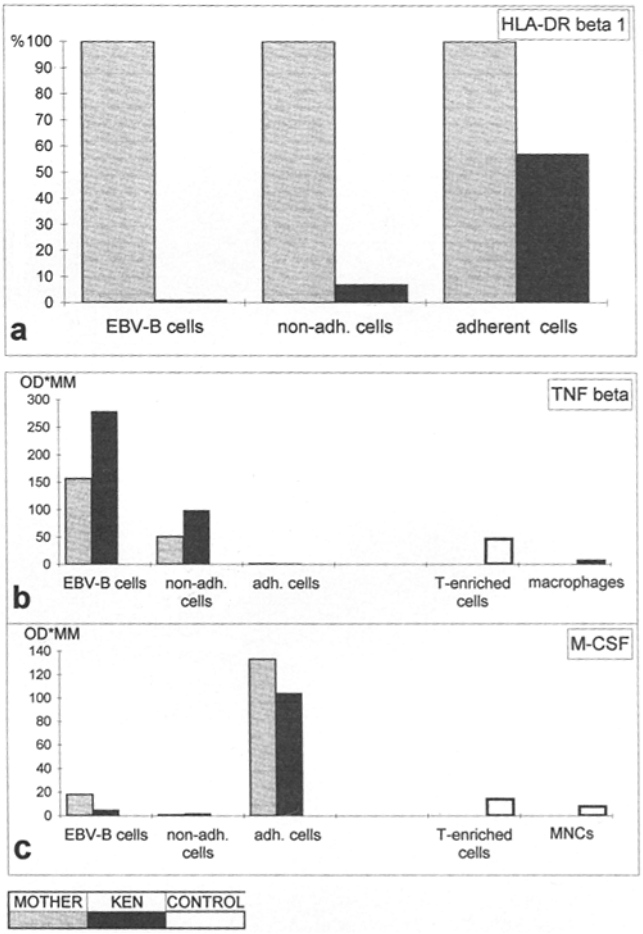

Figure 6. Densitometric quantification of Southern blots analyzed for amplified cDNAs encoding HLA-DRβ$_1$ (*a*), TNF-β (*b*), and M-CSF (*c*). Total RNA of the indicated cells from one class II–deficient patient (*KEN*) and a healthy control individual (*MOTHER*) was isolated and analyzed by RT-PCR as described in Materials and Methods. The amplified products were fractionated on 1.5% agarose gels and the corresponding bands were quantified by image-analyzing densitometry. In each lane the densitometric reading of HLA-DRβ$_1$, TNF-β, and M-CSF was related to the corresponding reading for the control ribosomal protein S14 which was coamplified in each reaction. In *a*, the relative DRβ$_1$ mRNA levels in the patient's cells are presented as percentage of his mother's levels (100%). In *b* and *c*, the relative levels of TNF-β and M-CSF are presented as optical density × mm [OD*MM]. RNA derived from the T-enriched cells, elutriated macrophages, and MNCs of two Red Cross healthy donors served as assay controls in *b* and *c* (*open boxes*).

Discussion

MHC class II antigens are expressed on B cells and cells of the monocyte/macrophage lineage and are inducible on many other cell types by certain cytokines such as IFN-γ and IL-4 (3). Defective MHC class II surface expression as well as the absence of the respective mRNA characterize a genetic disease known as BLS. The diagnosis of BLS in the twin

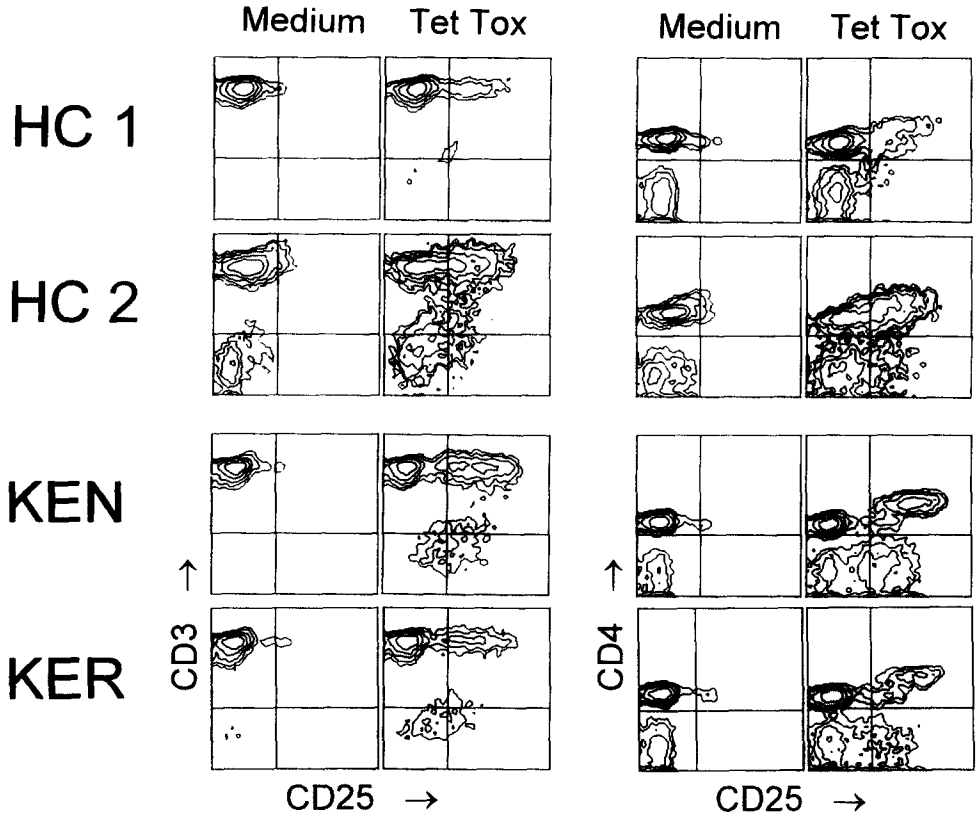

Figure 7. Peripheral blood MNCs from the patients (*KEN, KER*) and unrelated healthy controls (*HC 1, HC 2*) were cultured for 7 d in RPMI 1640 medium with or without tetanus toxoid (*Tet Tox*, 10 LF/ml). After activation, the surface expression of CD25 on $CD3^+$ and $CD4^+$ cells was examined by dual-color flow cytometry, as described in Materials and Methods.

brothers presented in this study was based on flow cytometric analysis of MNCs. FACS® analysis of their EBV-B cells failed to detect MHC class II antigens, whereas class I and Ii chain surface expression was comparable to the controls. Since the antibodies used recognize monomorphic class II determinants, the observed lack of HLA-DR, -DQ, and -DP surface expression in the patients' EBV-B cells most likely reflects an actual absence of all class II molecules. Similar results have been reported from other laboratories on cell lines derived from MHC class II–deficient patients as well as on laboratory-mutant cell lines (12).

Whereas IFN-γ treatment could not restore class II ex-

Table 1. *Patients' EBV-B Cells Function Normally as Accessory Cells for Mitogen-induced T Cell Proliferation, but Are Unable to Present Recall Antigen to a Normal HLA-DR–restricted, Antigen-specific T Cell Line*

EBV-B cells	HLA-DR typing	Medium	Tet Tox	PWM
–		112 ± 60	117 ± 141	106 ± 42
Autologous control	4	854 ± 130	16,854 ± 720	40,326 ± 602
HLA-DR compatible controls				
HWG	4	487 ± 11	19,977 ± 1,802	–
MYD	4	3,419 ± 292	62,975 ± 4,569	58,962 ± 3,280
HWE	4,7	1,680 ± 38	36,649 ± 3,554	18,824 ± 3,275
DE	4,7	3,593 ± 218	28,730 ± 6,310	–
Patient KEN (HLA-DR compatible)	2,4	593 ± 91	437 ± 24	41,390 ± 3,604
Patient KER (HLA-DR compatible)	2,4	618 ± 220	622 ± 266	42,872 ± 3,837

To examine B cell accessory cell function for antigen- and mitogen-induced T cell proliferation, a Tet Tox–specific normal T cell line (2.5×10^5/ml) was stimulated for 3 d with Tet Tox (10 LF/ml) or PWM (1:1,000) in the presence of autologous or HLA-DR–compatible EBV-B cells from control individuals or the patients (2.5×10^5/ml). As a control, T cells were cocultured with EBV-B cells in medium alone. Antigen- or mitogen-induced T cell proliferation was determined by measuring [^{3}H]thymidine incorporation and results are expressed as dpm [^{3}H]thymidine uptake (mean ± SD of triplicate cultures).

Table 2. *Patients' T Cells Mount an MHC Class I- and II-specific Alloresponse*

Stimulus	Proliferative Response of MNCs ([^{3}H]thymidine incorporation, dpm, mean ± SD of triplicate cultures)	
	Control	Patient KEN
Medium	505 ± 251	153 ± 19
Daudi + control ascites	31,343 ± 12,242	34,978 ± 7,078
Daudi + 9-49 (MHC class II)	3,154 ± 521	6,697 ± 2,545
Daudi + PA2.6 (MHC class I)	nt	22,523 ± 4,796
BCH-B + control ascites	103,518 ± 16,426	9,399 ± 591
BCH-B + 9-49 (MHC class II)	93,022 ± 20,290	10,072 ± 390
BCH-B + PA2.6 (MHC class I)	nt	969 ± 472

MHC class I- and II-specific alloresponses were examined by coculturing MNCs from patient KEN or a healthy, unrelated control with irradiated Daudi cells or EBV-B cells from another class II-deficient patient BCH for 7 d in the presence of control mouse ascites (diluted 1:50 in medium) as a source of irrelevant mouse mAbs, mouse ascites containing the MHC class II-specific mAb 9-49, or the MHC class I-specific mAb PA2.6. MNCs cultured without Daudi cells were included as a control. Background [^{3}H]thymidine uptake of irradiated Daudi cells was 1,104 ± 420 dpm (medium control) and 1,136 ± 758 dpm after cultivation in the presence of PWM, background [^{3}H]thymidine uptake of BCH-B cells was 869 ± 73 and 449 ± 109, respectively.

pression in most BLS patients' B cell lines (6, 9, 37, 38), IL-4 was able to enhance class II antigen expression on B and T cells of MHC class II-deficient CID patients (39, 40). Though IFN-γ and IL-4 are strong inducers of class II genes in a variety of cells, neither cytokine was able to restore class II-specific mRNA levels in our patients' EBV-B cells (data not shown).

Analysis of the transcription of HLA-D locus genes by RT-PCR revealed an uncoordinated expression of the class II isotype α and β chains in the EBV-B cells derived from the twin brothers. Whereas HLA-DRα, -DQα, and -DPβ chain-specific transcripts were readily detected, no message was observed for HLA-DRβ, -DQβ, or -DPα. Only after extremely extended exposure time, was a faint signal for HLA-DQβ observed. Importantly, gene expression of the Ii chain was normal or even increased in both patients' EBV-B cells. Furthermore, proteins for HLA-DRα and the Ii chain were present as visualized by immunoblotting of cell lysates, indicating that the observed transcripts were functional and that a translational defect could be ruled out. The uncoordinated expression of the class II isotype α and β chains detected differs from all other published results concerning regulation of HLA-D locus genes. Four levels of regulation have been characterized on the basis of the defects observed in MHC class II gene expression. Level one comprises cells from BLS patients responsive to cytokine treatment (3). The second level of gene regulation involves combined class II and Ii chain expression, as illustrated by defects in class II-negative mouse mutants (41). A third level includes defective expression of all HLA-D locus genes, whereas expression of the Ii chain is not affected, as described for several MHC class II-negative patients (9), for the EBV-B cell lines BLS-2 and BCH (8, 12), and for the laboratory mutants RJ2.2.5 and 6.1.6 (12, 42). The fourth and last level concerns rare cases with dissociation in gene regulation of the class II isotypes. DePreval et al. (9) reported a class II-deficient, patient-derived B cell line expressing low amounts of HLA-DR α and β chain-specific mRNA, whereas the other two isotypes were absent. This mRNA pattern (DR$\alpha^+\beta^+$, DQ$^-$, DP$^-$) distinguishes this B cell line from other class II-negative patients tested (9). Furthermore, a subclone of a human erythroleukemia cell line (HEL-DR$^+$) expressing HLA-DR and -DP, but no -DQ mRNA, has been described (43). In another BLS patient-derived B cell line (BLS-1) a very low level of HLA-DQα mRNA was present, whereas -DR, -DP, and -DQβ transcripts were not detected (8). Although the defect seen in our patients' EBV-B cells is closest to the fourth level with heterogeneous defects in gene regulation (DR$\alpha^+\beta^-$, DQ$\alpha^+\beta^-$, DP$\alpha^-\beta^+$), their class II isotype mRNA pattern clearly distinguishes them from other members of that group, e.g., HEL-DR$^+$ cells (DR$\alpha^+\beta^+$, DQ$^-$, DPα^+), BLS-1 cells (DR$^-$, DQ$\alpha^+\beta^-$, DP$^-$), and the laboratory mutant clone 13 (DR$^-$, DQ$\alpha^+\beta^+$, DP$^-$) (7, 8, 37).

Fusion experiments between different class II-defective cell lines from BLS patients and laboratory mutant cell lines have shown that genetic defects in *trans*-activating factors can lead to a failure in transcription and expression of class II genes (3, 12). Because of these fusion experiments, complementation groups were defined: most class II-negative patients' B cell lines (DR$^-$, DQ$^-$, DP$^-$) and the BLS-1 cell line (described above) belong to complementation group I. Binding of a protein (RF-X) to the X box sequences on the HLA-DRα promotor was deficient in the cells of this group I (44). Whereas nearly all members (RJ2.2.5, BLS-2, BCH, etc.) of the complementation group II did not express any of the HLA-D locus genes, clone 13 was negative for DR and DP,

but does express DQ. Benichou et al. (12) suggested that the cell lines of this group II lack the activity of a gene that can differentially regulate DR/DP and DQ promotors (12). Steimle et al. (45) identified a splicing mutation in a gene coding for a *trans*-activator protein (CIITA) necessary for MHC class II expression. CIITA is responsible for the class II regulatory defect in the cell lines of the patients and mutants belonging to complementation group II (38, 45). The defects responsible for the complete lack of class II gene expression in cell lines belonging to complementation groups III (6.1.6) and IV (SJO and TF) are still unknown. The differential expression of α and β chains of the individual HLA-isotypes observed in our patients' EBV-B cells differs from all other B cell lines of class II–deficient patients and laboratory mutants described. The results clearly demonstrate an uncoordinated expression of HLA-D locus genes and reflect regulatory mechanisms unrecognized up to now. We therefore suggest the existence of a new level of regulation of the MHC class II molecules. It cannot be differentiated whether the regulatory mechanism is chain specific or represents a coordinated regulation of several chains, e.g., DRβ, DQβ, and DPα. However, the results indicate that the B cells of our patients represent a new complementation group.

Whereas no message for DRβ, DQβ, or DPα could be observed in the patients' EBV-B cells, trace amounts of specific mRNA were found in their MNCs. Gene expression of DRα, DQα, and DPβ was normal in the MNCs as well. Quantification revealed that in one patient's MNCs (KER) 7–8% and in the other patient (KEN) 3–6% of the mRNAs encoding HLA-DRβ, -DQβ, and -DPα were detectable. Moreover, the trace amounts of mRNA found in both patients' MNCs were confirmed by the detection of protein for HLA-DRβ. These results indicate that regulation of the class II genes vary depending on the cell type.

Class II–expressing cells were further characterized by fractionating MNCs from one child (KEN) and his mother into nonadherent and adherent cells. We analyzed the expression of the HLA-D locus gene DRB found to be defective in the patients' EBV-B cells. Interestingly, HLA-DRβ–specific mRNA could be detected in the adherent cell population in about half of the quantity (57%) expressed by cells of the mother. To further characterize these cells, we examined the expression pattern of certain cell-specific cytokines, e.g., TNF-β and M-CSF. Whereas TNF-β is predominantly a product of lymphocytes and constitutively produced by human EBV-B cell lines, M-CSF is strongly expressed by macrophages, especially after activation by adherence to plastic and during differentiation (46). As expected, TNF-β mRNA levels were high in EBV-B cells and in the nonadherent fraction, indicative of an enrichment of lymphoid cells. Whereas no transcripts for TNF-β were detectable in the adherent cell fraction, the M-CSF mRNA level was increased, confirming enrichment of cells of the monocyte/macrophage lineage. These results indicate that HLA-DR–expressing cells are obviously cells of the monocyte/macrophage lineage present in the adherent cell fraction of the patients' MNCs. This finding supports the assumption of a "fine tuning" of the regulatory mechanisms of the MHC class II genes (3, 47).

MHC class II molecules play a key role in the regulation of antigen-induced immune responses due to their pivotal role in antigen presentation. Peptides bound to class II molecules on the surface of APC are the ligand for specific TCRs on $CD4^+$ T cells (48). In addition, class II expression on thymic epithelial cells is critical for the generation of mature $CD4^+$ T cells (49). The majority of previously described MHC class II–deficient patients showed a marked reduction in peripheral blood $CD4^+$ T cell counts (50). The CD4 lymphopenia was independent of the clinical status of the patients, and thus appeared to be in consequence of MHC class II deficiency (51). A similar reduction or complete absence of $CD4^+$ T cells in the periphery has been found in MHC class II–deficient mice that lacked cell surface expression of class II molecules due to disruption of the class II gene in embryonic stem cells (52, 53). Treatment of the mice with MHC class II–specific antibody in vivo prevented the differentiation of immature thymocytes into mature $CD4^+$, $CD8^-$ T cells (54).

The two boys described here lacked MHC class II antigen on the surface of their peripheral blood leukocytes, but in contrast to previously described patients, presented with normal numbers of $CD4^+$ T cells in the periphery (13, 50). Furthermore, specific antibodies were found after vaccination in vivo (13). In agreement with the patients' observed antibody response, the present study shows that $CD3^+$ T cells became activated and expressed IL-2R (CD25) in response to stimulation of MNCs with recall antigen (Tet Tox). $CD4^+$ TCR-α/β^+ cells were the major activated phenotype within these T cell populations.

The results seen on the transcriptional level in the two patients clearly indicated that regulation of MHC class II expression was different in nonadherent cells (e.g., B cells) compared to a subpopulation of cells observed in the adherent MNC fraction. Further investigations showed that the patients' B cells were also different from other APCs (e.g., monocytes, macrophages, dendritic cells) with respect to their capacity to present recall antigen, which is known to require functional MHC class II molecules. Whereas APCs present within the boys' MNC population were capable of inducing antigen-specific activation of $CD4^+$ T cells, our patients' EBV-transformed B cells were unable to function as APCs in the activation of autologous antigen-reactive T cells or a normal, HLA-DR–compatible, antigen-specific T cell line derived from a healthy individual. The data show that cells contained within the MNCs other than B cells function as APCs in inducing recall antigen-specific T cell responses in the two boys. Supporting these data, a subpopulation of cells acting as APCs within the adherent MNC fraction, but not patients' B cells, expressed DRβ-specific mRNA and DRα protein.

Although expression of MHC class II molecules on the surface of the patients' MNCs was not detected by flow cytometry, cells of the monocyte/macrophage lineage might still express very low levels of class II antigens on their surface, sufficient to stimulate antigen-reactive $CD4^+$ T cells. In accordance with this hypothesis, it is known that expression of very low numbers of antigen/MHC class II complexes

on an APC, probably as few as 200–300 complexes, are sufficient to stimulate a T cell (55).

In a previous study, we found that T cells from a patient with MHC class II deficiency were able to respond to class I alloantigens, but failed to recognize allogeneic MHC class II determinants. The lack of the MHC class II–specific T cell repertoire was accompanied by a substantially decreased number of circulating $CD4^+$ lymphocytes (56). Although this has not been examined directly, both findings might have been due to defective expression of MHC class II antigen on thymic epithelial cells.

In contrast, T cells tested in one of the two patients described in the present paper could respond to MHC class I and II allostimulation, and this response could be inhibited by mAbs of the respective specificity. Compared to the previously described patient by Mannhalter et al. (56), the T cells of the patient examined in this study were capable of recognizing both MHC class I and II antigens. Whereas direct examination of MHC class II expression in the thymus of the two boys is not possible, a normal class II–specific T cell response might be due to a residual expression of class II antigens in the thymus, which also enabled the maturation of normal numbers of $CD4^+$ T cells as present in our patient.

On the basis of the above considerations, it is tempting to speculate that B cells and monocytes/macrophages may acquire the capacity to regulate class II genes in a distinct fashion, pointing to cell type–specific factors involved in regulatory pathways of the class II molecules. In this sense, the biological role of MHC class II antigens should not only be seen within their structural characteristics, but also in the context of a developmentally controlled program of differentiation of various cell types.

We wish to thank Mr. E. Vogel and Mrs. E. Schaffer for performing the flow cytometry analysis; Mr. P. Breit for photographic assistance; Mrs. M. Pfunder for secretarial support; and Drs. M. B. Fischer and V. Thon for helpful discussions and their steady encouragement during the course of this study.

Address correspondence to Dr. Martha M. Eibl, Institute of Immunology, University of Vienna, Borschkegasse 8A, A-1090 Vienna, Austria.

Received for publication 23 May 1994 and in revised form 6 October 1994.

References

1. Gregersen, P.K. 1989. Biology of disease. HLA class II polymorphism: implication for genetic susceptibility to autoimmune disease. *Lab. Invest.* 61:5–19.
2. Trowsdale, J., J.A.T. Young, A.P. Kelly, P.J. Austin, S. Carson, H. Meunier, A. So, H.A. Erlich, R.S. Spielman, H. Bodmer, and W.F. Bodmer. 1985. Structure, sequence and polymorphism in the HLA-D region. *Immunol. Rev.* 85:5–43.
3. Glimscher, L.H., and C.J. Kara. 1992. Sequences and factors: a guide to MHC class II transcription. *Annu. Rev. Immunol.* 10:13–49.
4. Benoist, C., and D. Mathis. 1990. Regulation of the major histocompatibility complex class II-genes: X, Y and other letters of the alphabet. *Annu. Rev. Immunol.* 8:681–715.
5. Sant, A.J., and J. Miller. 1994. MHC class II antigen processing: biology of invariant chain. *Curr. Opin. Immunol.* 6:57–63.
6. Lisowska-Grospierre, B., D. Charron, C. DePreval, A. Durandy, C. Griscelli, and B. Mach. 1985. A defect in the regulation of major histocompatibility complex class II gene expression in human HLA-DR negative lymphocytes from patients with combined immunodeficiency syndrome. *J. Clin. Invest.* 76: 381–385.
7. DePreval, C., B. Lisowska-Grospierre, M. Loche, C. Griscelli, and B. Mach. 1985. A trans-acting class II regulatory gene unlinked to the MHC controls expression of HLA class II genes. *Nature (Lond.).* 318:291–293.
8. Hume, C.R., L.A. Shookster, N. Collins, R. O'Reilly, and J.S. Lee. 1989. Bare lymphocyte syndrome: altered HLA class II expression in B cell lines derived from two patients. *Hum. Immunol.* 25:1–11.
9. DePreval, C., M.R. Hadam, and B. Mach. 1988. Regulation of genes for HLA class II antigens in cell lines from patients with severe combined immunodeficiency. *N. Engl. J. Med.* 318:1295–1300.
10. Yang, Z., R.S. Accolla, D. Pious, B.J.M. Zegers, and J.L. Strominger. 1988. Two distinct genetic loci regulating class II gene expression are defective in human mutant and patient cell lines. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:1965–1972.
11. Hume, C.R., and J.S. Lee. 1989. Congenital immunodeficiencies associated with absence of HLA class II antigens on lymphocytes result from distinct mutations in trans-acting factors. *Hum. Immunol.* 26:288–309.
12. Benichou, B., and J.L. Strominger. 1991. Class II-antigen-negative patient and mutant B-cell lines represent at least three, and probably four, distinct genetic defects defined by complementation analysis. *Proc. Natl. Acad. Sci. USA.* 88:4285–4288.
13. Wolf, H.M., I. Hauber, H. Gulle, V. Thon, H. Eggenbauer, M.B. Fischer, S. Fiala, and M.M. Eibl. 1995. Twin boys with major histocompatibility complex class II deficiency but inducible immune responses. *N. Engl. J. Med.* 332:86–90.
14. Bøyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest. Suppl.* 97:77–89.
15. Mannhalter, J.W., G.J. Zlabinger, R. Ahmad, C.C. Zielinski, W. Schramm, and M.M. Eibl. 1986. A functional defect in the early phase of the immune response observed in patients with haemophilia. *Clin. Immunol. Immunopathol.* 38:390–397.

16. Pellegrino, M.A., S. Ferrone, and A.N. Theofilopoulos. 1976. Isolation of human T and B lymphocytes by rosette formation with 2-aminoethylisothiouronium bromide (AET)-treated sheep red blood cells and with monkey red blood cells. *J. Immunol. Methods.* 11:273–279.
17. Aman, P., B. Ehlin-Henriksson, and G. Klein. 1984. Epstein-Barr virus susceptibility of normal human B lymphocyte populations. *J. Exp. Med.* 159:208–220.
18. Wolf, H.M., M. Pum, R. Jager, L. Istvan, J.W. Mannhalter, and M.M. Eibl. 1992. Cellular and humoral immune responses in haemophiliacs after vaccination against tick-borne encephalitis. *Br. J. Haematol.* 82:374–383.
19. Todd, R.F., S.C. Meuer, P.L. Romain, and S.F. Schlossman. 1984. A monoclonal antibody that blocks class II histocompatibility-related immune interactions. *Hum. Immunol.* 10:23–40.
20. Brodsky, F.M., P. Parham, C.J. Barnstable, M.J. Crumpton, and W.F. Bodmer. 1979. Monoclonal antibodies for analysis of the HLA system. *Immunol. Rev.* 47:3–61.
21. Koning, F., I. Schreuder, M. Giphart, and H. Bruning. 1984. A mouse monoclonal antibody detecting a DR-related MT2-like specificity: serology and biochemistry. *Hum. Immunol.* 9:221–230.
22. Malissen, B., N. Rebai, A. Liabeuf, and C. Mawas. 1982. Human cytotoxic cell structures associated with expression of cytolysis. I. Analysis at the clonal level of the cytolysis-inhibiting effect of 7 monoclonal antibodies. *Eur. J. Immunol.* 2:739–747.
23. Quaranta, V., O. Majdic, G. Stingl, K. Liszka, H. Honigsmann, and W. Knapp. 1984. A human Ia cytoplasmic determinant located on multiple forms of invariant chain (τ, $\tau 2$, $\tau 3$). *J. Immunol.* 132:1900–1905.
24. Chomczynski, P. 1993. A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. *BioTechniques.* 15:532–536.
25. Saiki, R.K., S. Scharf, F. Faloona, K.B. Mullis, G.T. Horn, H.A. Erlich, and N. Arnheim. 1985. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science (Wash. DC).* 230:1350–1354.
26. Mullis, K.B., and F. Faloona. 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods Enzymol.* 155:335–350.
27. Das, H.K., S.K. Lawrance, and S.M. Weissman. 1983. Structure and nucleotide sequence of the heavy chain gene of HLA-DR. *Proc. Natl. Acad. Sci. USA.* 80:3543–3547.
28. Long, E.O., C.T. Wake, J. Gorski, and B. Mach. 1983. Complete sequence of an HLA-DR β chain deduced from a cDNA clone and identification of multiple non-allelic DR β chain genes. *EMBO (Eur. Mol. Biol. Organ.) J.* 2:389–394.
29. Tonnelle, C., R. DeMars, and E.O. Long. 1985. DOβ: a new β chain gene in HLA-D with a distinct regulation of expression. *EMBO (Eur. Mol. Biol. Organ.) J.* 4:2839–2847.
30. Claesson, L., D. Larhammar, L. Rask, and P.A. Peterson. 1983. cDNA clone for the human invariant gamma chain of class II histocompatibility antigens and its implications for the protein structure. *Proc. Natl. Acad. Sci. USA.* 80:7395–7399.
31. Güssow, D., R. Rein, I. Ginjaar, F. Hochstenbach, G. Seemann, A. Kottman, and H.L. Ploegh. 1987. The human $\beta 2$-microglobulin gene: primary structure and definition of the transcriptional unit. *J. Immunol.* 139:3132–3138.
32. Maier, J.A.M., P. Voulalas, D. Roeder, and T. Maciag. 1990. Extension of the life-span of human endothelial cells by an interleukin-1α antisense oligomer. *Science (Wash. DC).* 249: 1570–1574.
33. Foley, K.P., M.W. Leonard, and J.D. Engel. 1993. Quantitation of RNA using the polymerase chain reaction. *TIG (Trends Genet.).* 9:380–385.
34. Guy, K., V. Van Heyningen, B.B. Cohen, D.L. Deane, and C.M. Steel. 1982. Differential expression and serologically distinct subpopulations of human Ia antigens detected with monoclonal antibodies to Ia alpha and beta chains. *Eur. J. Immunol.* 12:942–948.
35. Cohen, B.B., D.N. Crichton, and C.M. Steel. 1987. A new set of monoclonal antibodies to human MHC class II α chains demonstrate that most α epitopes are inaccessible on the living cell surface. *Immunology.* 61:255–260.
36. Adams, T.E., J.G. Bodmer, and W.F. Bodmer. 1983. Production and characterization of monoclonal antibodies recognizing the α-chain subunit of human Ia alloantigens. *Immunology.* 50:613–624.
37. Ono, S.J., V. Brazil, M. Sugawara, and J.L. Strominger. 1991. An isotype-specific trans-acting factor is defective in a mutant B cell line that expresses HLA-DQ, but not -DR or -DP. *J. Exp. Med.* 173:629–637.
38. Steimle, V., C.A. Siegrist, A. Mottet, B. Lisowska-Grospierre, and B. Mach. 1994. Regulation of MHC class II expression by interferon-γ mediated by the trans-activator gene CIITA. *Science (Wash. DC).* 265:106–109.
39. Rousset, F., R. De Waal Malefijt, B. Slierendregt, J.P. Aubry, J.Y. Bonnefoy, T. Defrance, J. Banchereau, and J.E. De Vries. 1988. Regulation of Fc receptor for IgE (CD23) and class II MHC antigen expression on Burkitt lymphoma cell lines by human IL-4 and IFN-γ. *J. Immunol.* 140:2625–2632.
40. Boothby, M., E. Gravallese, H.C. Liou, and L. Glimscher. 1988. A DNA binding protein regulated by IL-4 and by differentiation in B cells. *Science (Wash. DC).* 242:1559–1562.
41. Polla, B.S., A. Poljak, S.G. Geier, S.G. Nathenson, J. Ohara, W.E. Paul, and L.H. Glimscher. 1986. Three distinct signals can induce class II gene expression in a murine pre-B-cell line. *Proc. Natl. Acad. Sci. USA.* 83:4878–4882.
42. Stimac, E., S. Urieli-Shoval, S. Kempin, and D. Pious. 1991. Defective HLA-DRA X box binding in the class II transactive transcription factor mutant 6.1.6 and in cell lines from class II immunodeficient patients. *J. Immunol.* 146:4398–4405.
43. Symington, F.W., F. Levine, M. Braun, S.L. Brown, H.A. Erlich, and B. Torok-Storb. 1985. Differential Ia antigen expression by autologous human erythroid and B lymphoblastoid cell lines. *J. Immunol.* 135:1026–1032.
44. Reith, W., S. Satola, C. Herrero-Sanchez, I. Amaldi, B. Lisowska-Grospierre, C. Griscelli, M.R. Hadam, and B. Mach. 1988. Congenital immunodeficiency with a regulatory defect in MHC class II gene expression lacks a specific HLA-DR promotor binding protein, RF-X. *Cell.* 53:897–906.
45. Steimle, V., L.A. Otten, M. Zufferey, and B. Mach. 1993. Complementation cloning of an MHC class II transactivator mutated in hereditary MHC class II deficiency (or Bare lymphocyte syndrome). *Cell.* 75:135–146.
46. Thomson, A. 1992. The Cytokine Handbook. Chapter 6. Academic Press Limited, London. pp. 119–148.
47. Maffei, A., L. Scarpellino, M. Barnard, G. Carra, M. Jotterand-Bellomo, J. Guardiola, and R.S. Accolla. 1987. Distinct mechanisms regulate MHC class II gene expression in B cells and macrophages. *J. Immunol.* 139:942–948.
48. Rosenthal, A.S., and E.M. Shevach. 1973. Function of macrophages in antigen recognition by guinea pig T lymphocytes. I. Requirements for histocompatible macrophages and lymphocytes. *J. Exp. Med.* 138:1194–1211.

49. Hugo, P., J. Kappler, and P.C. Marrack. 1993. Positive selection of TCR$\alpha\beta$ thymocytes: is cortical thymic epithelium an obligatory participant in the presentation of major histocompatibility complex protein? *Immunol. Rev.* 135:133–155.
50. Klein, C., B. Lisowska-Grospierre, F. LeDeist, A. Fischer, and C. Griscelli. 1993. Major histocompatibility complex class II deficiency: clinical manifestations, immunologic features, and outcome. *J. Pediatr.* 123:921–928.
51. Griscelli, C., B. Lisowska-Grospierre, and B. Mach. 1993. Combined immunodeficiency with defective expression in MHC class II genes. *In* Immunodeficiencies. F.S. Rosen and M. Seligmann, editors. Harwood Academic Publishers, Switzerland. 141–154.
52. Cosgrove, D., D. Gray, A. Dierich, J. Kaufman, M. Lemeur, C. Benoist, and D. Mathis. 1991. Mice lacking MHC class II molecules. *Cell.* 66:1051–1066.
53. Grusby, M.J., R.S. Johnson, V.E. Papaioannou, and L.H. Glimscher. 1991. Depletion of CD4$^+$ T cells major histocompatibility complex class II-deficient mice. *Science (Wash. DC).* 253:1417–1420.
54. Kruisbeek, A.M., J.J. Mond, B.J. Fowlkes, J.A. Carmen, S. Bridges, and D.L. Logo. 1985. Absence of the Lyt-2$^-$, L3T4$^+$ lineage of T cells in mice treated neonatally with anti-I-A correlates with absence of intrathymic I-A-bearing antigen-presenting cell function. *J. Exp. Med.* 161:1029–1047.
55. Harding, C.V., and E.R. Unanue. 1990. Quantitation of antigen-presenting cell MHC class II/peptide complexes necessary for T-cell stimulation. *Nature (Lond.).* 346:574–576.
56. Mannhalter, J.W., H.M. Wolf, I. Hauber, M. Miricka, H. Gadner, and M.M. Eibl. 1994. T cell differentiation and generation of the antigen-specific T cell repertoire in man: observations in MHC class II deficiency. *Clin. Exp. Immunol.* 97: 392–395.