Type II bare lymphocyte syndrome (BLS) or HLA class II-deficient combined immunodeficiency is an autosomal recessive disorder in which HLA class II structural genes are not transcribed due to a mutation in a gene encoded outside of the MHC (1). This lack of HLA class II expression compromises patient immunity, leading to recurrent bacterial and viral infections. Several lines of evidence suggest that the BLS phenotype results from a defect in a trans-acting factor necessary for transcription of the coordinately regulated class II genes (reviewed in reference 2).

Clustering of genes for histocompatibility antigens and accessory molecules required for antigen presentation may ensure the coordinate expression and function of these molecules. The HLA class I and class II structural genes on chromosome 6 are interspersed with genes necessary for antigen responsiveness (3). The ability of class I molecules to present antigens is dependent upon functional transporters associated with antigen processing (TAP) genes which are required to supply peptides to class I molecules in the endoplasmic reticulum (reviewed in reference 3). The TAP genes map between the HLA-DQ and -DP structural genes; in addition to this genetic proximity, they are coregulated with class I α structural genes by IFN-γ (3). Similarly, genes essential for appropriate class II-mediated antigen presentation map within this same region of the MHC (4, 5). Transcriptional regulation of genes encoded outside the MHC may also be coordinated with histocompatibility antigens to facilitate immune function. Class II antigens and their intracellular chaperone, the invariant chain, are upregulated in response to IFN-γ (6). Whether transcriptional elements are shared by additional genes required for class II-restricted antigen presentation has not been addressed.

Restoration of cellular immune responses in BLS is dependent upon the expression of functional HLA class II antigens in these patients. To determine whether stable transfection of class II DR α and β subunits would restore immunocompetency in BLS, studies of class II antigen structure and function were initiated using BLS-1, an immortalized B cell line derived from a BLS patient (7). BLS-1 cells expressing abundant amounts of cell surface DRαw4 or DR5 molecules were unable to present exogenous antigens to T lymphocytes. In addition, conformationally altered class II αβ complexes were...
detected by epitope mapping in BLS-1 cells transfected with the HLA alleles, DR3 or DR4w4. Complementation analysis indicated that a trans-acting factor was necessary to restore a wild-type APC phenotype to BLS-1 cells. Our data suggest that the mutated gene that controls class II structural gene transcription also regulates the expression of gene(s) controlling class II-mediated antigen presentation.

Materials and Methods

Cell Lines. Class II DRα (DRA1) and DRβ (DRB1*0401, DRB1*1101, DRB1*0301) cDNA encoding DR4w4, DR5w11, and DR3w17 molecules were introduced into BLS-1 using retrovirus-mediated gene transfer (8). T2 (.174×CEM.T2) is a human cell hybrid that lacks all four copies of the MHC class II region on chromosome 6 (9). T2.DR4w4 (provided by W. Kwok, Virginia Mason Research Center, Seattle, WA) and T2.DR3 and T1.DR3 (10) were generated by transfer of the DRA1 and DRB1*0401 or DRB1*0301 genes. T1 is the progenitor of T2 and retains one unmutated copy of chromosome 6. Cell lines were grown in IMDM plus 10% FCS.

Assays with T Cell Clones. The anti-HAR and BCHA59 human T cell clones (provided by J. Krieger and A. Sette, Cytel, San Diego, CA) were isolated by limiting dilution cloning after stimulation of DR5 or DR4w4 PBL with HA 307-319 (11). To measure T cell proliferation, APCs (3 × 10⁶) were pulsed for 3 h with fixed A/Mississippi/1/85 (H3N2) virus (Connaught Laboratories, Swiftwater, PA) or HA 307-319 peptide (PKYVKQNVLKLAT), washed, irradiated, plated in microdishes with an equal number of T cells, and incubated for 72 h, with [³²P]thymidine (1 µCi/well) present during the last 15 h. Live A/Bangkok/1/79 virus (200–300 HA U/ml) (provided by P. Cresswell, Yale University, New Haven, CT) was incubated with cells in serum free medium for 1 h at 37°C to allow viral adherence; nonadherent virus was washed away and cells were resuspended in complete medium for 24 h before incubation with T cells.

Assays with T Cell Hybridomas. The DR4w4-tetanus-specific T cell hybridoma 49.23.2 was generated after tetanus immunization of a DR4w4 transgenic mouse (12) as described (13). Formalin fixed tetanus toxoid protein (TT) (Wyeth Laboratories, Philadelphia, PA) was digested with N-tosyl-L-phenylalanine chloromethyl ketone (TPCK) trypsin. APC (10⁶), TT antigen (native or trypsin digested) (0.4 µM) and 49.23.2 T cells (10⁵) were incubated together for 24 h. T cell IL-2 and IL-4 production was determined by the survival of an IL-2/LPC-dependent cell line, HT2, measured using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (14). The presence of antigenic peptides in the tryptic digest of TT was confirmed by incubating the digested preparation with fixed APCs. The T cell hybridoma did not produce lymphokines in response to APC's in the absence of antigen.

Antibody Binding Assays. For flow cytometry experiments, the anti-DR, mAb L243 (15) and the anti-DR3 mAb 16.23 (16) were used at saturating concentrations and binding was detected with FITC-conjugated goat anti-mouse Ig using a FACScan® (Becton Dickinson & Co., Mountain View, CA). Binding of anti-DR4w4 mAbs was detected with a live cell enzyme-linked immunoassay (CELSIA) (17).

Cell Fusions. Fusions between BLS-1 (DR3,5) and SJO (DR5, 7) cells were carried out using 50% polyethylene glycol-1500, 5% DMSO (18). Before cell hybridization, hygromycin-resistance or neomycin genes were introduced into BLS-1 or SJO, respectively; the fused cells were selected for using hygromycin B (150 µg/ml) and G418 (1 mg/ml). Four independently fused drug-resistant populations were analyzed for cell surface expression of the endogenous DR molecules.

Results and Discussion

BLS-1 cells expressing high levels of cell surface DR molecules were assessed for their ability to process and present foreign protein antigens to T cells. The BLS-1.DR5 transfectant and a wild-type DR5 homozygous B cell line presented

![Figure 1](attachment:image.png)
the influenza hemagglutinin (HA) peptide, HA 307-319, equally well to a DR5/HA 307-319-restricted T cell clone (Fig. 1A). In contrast, BLS-1.DR5 incubated with formalin-fixed influenza virus did not stimulate proliferation of the T cell clone (Fig. 1A), suggesting that BLS-1.DR5 was unable to mediate presentation of native HA. To increase the amount of intracellular influenza HA, APCs were infected with live influenza virus before T cell addition; the T cell clone proliferated in response to the influenza-infected DR5 B cell line, yet there was no response to virally infected BLS-1.DR5 (Fig. 1A). Similarly, a BLS-1.DR4w4 transferent was unable to stimulate proliferation of a DR4w4/HA 307-319 restricted T cell clone after incubation with native influenza virus proteins, whereas the presentation of the HA 307-319 peptide by BLS-1.DR4w4 was comparable to that of a homozygous DR4w4 B cell line (Fig. 1B).

The defect in exogenous antigen presentation by BLS-1 was also observed with another native antigen, TT. Upon incubation with native TT, BLS-1.DR4w4 was ineffective in stimulating a murine T cell hybridoma that is specific for tetanus presented in the context of DR4w4 (Fig. 1C). T cell responses to BLS-1.DR4w4 and tetanus were reduced 10-fold when compared with a homozygous DR4w4 B cell line. Incubation of these APCs with proteolyzed TT (containing antigenic peptides) resulted in activation of the T cell hybridoma (Fig. 1C). Thus, BLS-1 cells expressing two different DR alleles, DR4w4 or DR5, were unable to mediate class II-restricted antigen presentation to antigen-specific T cells after incubation with the native protein antigens, TT and influenza HA; however, BLS-1.DR transferents could present preprocessed antigenic peptides derived from these proteins as efficiently as wild-type B cell lines. The inability of BLS-1 cells to present native protein antigens was not due to a defect in internalization of exogenous antigens. Infection of BLS-1 with influenza did not facilitate presentation of the viral HA antigen (Fig. 1A). In addition, BLS-1 and the wild-type B cell lines were equally able to internalize TT or influenza virus present in the culture medium (data not shown).

This defect in class II-mediated antigen presentation is similar to the phenotype of the in vitro generated mutant cell lines 9.5.3 (19), 721.174 (5), and the related cell T2 (10). These mutant cell lines each lack a gene required for the processing and presentation of native exogenous class II-restricted antigens. A comparison of BLS-1.DR4w4 and the mutant T2.DR4w4 indicated that the ability of each of these cells to present TT to T cells was similarly reduced (Fig. 1C). The mutation in 721.174 and 9.5.3 maps between the LMP2 and DNA genes in the class II region of the HLA gene complex (4, 5).

A characteristic feature of the in vitro-generated mutant APC is the expression of structurally aberrant class II αβ dimers lacking specific epitopes (5, 19). To assess the conformation of the DR molecules present on the surface of BLS-1.DR4w4 cells, BLS-1.DR3 and BLS-1.DR4w4 cells were analyzed for binding of allele-specific, conformation-dependent anti-DR3 and anti-DR4w4 mAbs using flow cytometry and CELISA (Fig. 2). The mAb 16.23, which recognizes a conformation-dependent epitope on DR3 molecules, binds to DR3 molecules on wild-type DR3 cell lines but not on BLS-1.DR3 or mutant T2.DR3 cells (Fig. 2A). Expression of the epitope bound by another anti-DR mAb, L243 is unaffected by the mutations in these cells.

A panel of anti-DR4w4-specific mAbs (20, 21) was used to probe the structure of DR4w4 on BLS-1.DR4w4 cells and a DR4w4 homozygous B cell line (Fig. 2B). Two of these mAbs, NFLD.D1 and NFLD.D10, bound to DR4w4 molecules on both cell lines equally well, as did the anti-DR mAb L243.

![Figure 2. Binding of allele-specific, conformation dependent anti-DR3 and anti-DR4w4 mAbs to BLS-1.DR3 and BLS-1.DR4w4 cells.](image-url)
mAb L243. However, mAbs NFLD.D11 and NFLD.D12 bound wild-type DR4w4 B cells but not BLS-1.DR4w4 cells; these mAbs had previously been shown to bind epitopes that are dependent on DR4w4-peptide complexes and do not bind to the DR4w4 molecules on the surface of mutant T2.DR4w4 cells (20). Binding of mAb NFLD.D2, which also recognizes an epitope dependent on class II conformation, was reduced on BLS-1.DR4w4 cells. Taken together, these data with both anti-DR4w4 and anti-DR3 mAb suggest that the DR molecules on the surface of BLS-1 cells are structurally altered and thus do not display epitopes recognized by conformationally sensitive antibodies. This defect in class II antigen structure has been linked with the impaired ability of mutant cells to present peptides derived from exogenously added proteins.

Surface expression of endogenous class II DR, DQ, and DP molecules is restored upon fusion of B cell lines isolated from different BLS patients, indicating that the defect in class II gene transcription differs among BLS cell lines and allowing the definition of four genetic complementation groups (18). BLS-1 is a member of complementation group I. SJO, a B cell line derived from a patient in complementation group IV (22), was transfected with genes encoding DR4w4 molecules and found to display a mutant APC phenotype identical to that of BLS-1.DR4w4 (data not shown). To determine if the defects in antigen presentation in BLS-1 and SJO could be complemented by trans-acting factors present in each cell line, BLS-1 was fused with SJO. BLS-1 × SJO hybrids were isolated and screened for wild-type DR3 expression using mAb 16.23. The hybrid cells were found to express the endogenous DR3 molecule from BLS-1 with a wild-type conformation (Fig. 3), indicating that a trans-acting factor present in SJO can restore the production of functional class II molecules and therefore the ability to present exogenous antigens.

Our results demonstrate that BLS-1.DR4w4 transfected cells have a defect in the class II-mediated presentation of native exogenous protein antigens to T cells. The class II αβ dimers formed in these cells also fail to express conserved epitopes as detected by conformationally sensitive antibodies. BLS-1, a cell deficient in the transcription of class II structural genes, is therefore remarkably similar in phenotype to a set of in vitro-generated APC mutants. Genetic studies indicate that

Figure 3. Fusion of class II-negative BLS-1 and SJO cells results in expression of wild-type endogenous DR3 molecules that bind mAb 16.23. Binding of anti-DR mAb L243 (A) and anti-DR3 mAb 16.23 (B) to BLS-1 (thick line), SJO (thin line), BLS-1 × SJO fusion 1 (thick dotted line) and BLS-1 × SJO fusion 2 (thin dotted line). Fluorescence intensity was determined after binding of mAbs and flow cytometry.

2020 Deficient Antigen Presentation in Bare Lymphocyte Syndrome Cells
II structural genes. To fully restore immune responsiveness in BLS patients, immunotherapeutic approaches must therefore be based on restoration of transcription factors or the expression of both class II structural genes and genes controlling antigen presentation.

We thank J. Lee for the BLS-1 cell line, J. Krieger and A. Sette for T cell clones, and J. Nettles for technical assistance. We also thank our colleagues for helpful discussions and H. Chase and N. Ducommun for help with manuscript preparation.

Supported by National Institutes of Health grants AI-31241 and AI-33418 and the Arthritis Foundation; S. Kovats is a fellow of the Leukemia Society of America.

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Received for publication 25 January 1994 and in revised form 16 March 1994.

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