

Genetic Modulation of Antigen Presentation by HLA-B27 Molecules

By Laszlo Pazmany,* Sarah Rowland-Jones,* Stephane Huet,* Ann Hill,* Julian Sutton,* Ruth Murray,† Jill Brooks,‡ and Andrew McMichael*

From the *Molecular Immunology Group, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DU; and the †Department of Cancer Studies, University of Birmingham, Medical School, Birmingham B15 2TJ, United Kingdom

Summary

In studies of antigenic peptide presentation, we have found a healthy volunteer whose lymphoblastoid cells were unable to present three different virus-derived epitopes to cytotoxic T lymphocytes (CTL) despite expressing the correct restricting HLA-B27 molecules on the cell surface. B cell lines were established from other members of the donor's family, including individuals suffering from ankylosing spondylitis and related diseases, and were tested for their ability to function as target cells in the same assay. None of the eight B cell lines that expressed HLA-B27 presented a known peptide epitope to CTL. However, cells from a family member that expressed HLA-B8 could present an epitope peptide restricted by that molecule. The B27 molecule in this family proved to be the B2702 subtype on isoelectric focusing gels, appearing in exactly the same position as B2702 from other cell lines that did present the peptide. To exclude mutations resulting in noncharged amino acid substitutions, cDNA coding for B2702 was cloned from the proband's cell line and sequenced. No coding changes were found. The cloned cDNA was transfected into HLA-A- and B-negative HMy/C1R cells, and the B2702 molecules generated in this environment rendered these cells, after incubation with peptide, susceptible to lysis by peptide-specific CTL. These data are compatible with the presence of a factor(s), possibly HLA linked, interfering with antigen presentation by otherwise normal B2702 molecules in this family.

HLA class I molecules, comprised of a polymorphic heavy chain and an invariant molecule, β_2 -microglobulin, are present on almost all nucleated cells. The function of these molecules is to present peptide antigens to CTL (1-4). After translation and translocation into the endoplasmic reticulum, the heavy chains form trimolecular complexes with β_2 -microglobulin and those peptides that are able to bind specifically (5-7). These complexes are transported through the Golgi apparatus to the cell surface. Here they can be recognized, if they contain foreign peptides, by the antigen receptors of specific CTL.

Although numerous studies have indicated that any cell line expressing an HLA class I molecule should be able to present peptide epitopes restricted by that allele to the appropriate CTL (3, 4, 8, 9), recently some exceptions to this rule have been found. Mutant cell lines were generated in vitro that, although synthesizing normal amounts of class I heavy chains, fail to form the trimolecular complexes with β_2 -microglobulin and endogenously derived peptides. Surface expression could be restored if the cells were incubated with peptides known to be epitopes (10, 11). Although the defects in these cell lines have not been fully elucidated, at

least one of the genes involved is located within the MHC (11) and may be a peptide transporter (12). The possibility that MHC-linked genes affect antigen processing was raised by Livingstone et al. (13), who identified a rat strain in which a gene in the MHC class II region modified the function of a rat class I molecule, altering its recognition by alloreactive T cells and interfering with its ability to present minor histocompatibility antigens to CTL. Again, the ATP-binding cassette transporter gene family members that map in this region are candidates (14-17). Cell lines have also been described that were unable to present peptides known to be specific for the HLA class II molecules on their surface (18); these cell lines may lack some of the enzymes that trim the peptides to the right size to fit into the peptide binding groove (18). All of these findings imply that there are genetic factors, some of which map to the MHC, that are able to modify antigen presentation.

Here we describe cells from a healthy human donor that were unable to present peptide epitopes to CTL via HLA-B27, despite expressing normal amounts of this molecule on the cell surface. This phenotype was not caused by a mutation of the HLA molecule itself, and cells from all HLA-

B27-positive members of this individual's family showed the same behavior. These findings may be explained by the presence of a gene or genes linked to the MHC complex. The implications for HLA and disease associations are discussed.

Materials and Methods

Propagation of CTL Lines. HLA-B27-restricted influenza A nucleoprotein peptide-specific CTL lines were grown as previously described (9). Briefly, PBMC, at 2×10^6 /ml, taken from two donors, HF (HLA-A2, -A3, -B7, -B2705) and GR (HLA-A3, -A31, -B2705), were incubated with influenza A virus X31 (titer 10^3 hemagglutination units) at a dilution of 10^{-3} , for 1 h at 37°C in the absence of serum and then in RPMI 1640 with 10% FCS for 7 d at 37°C .

CTL lines were grown from this bulk culture by mixing the T cells with equal numbers of autologous B-lymphoblastoid cells (BCL¹) that had been incubated for a period of 1 h with the influenza A nucleoprotein peptide 380-393 (synthesized by J. Rothbard, Immunologic, Palo Alto, CA) at $50 \mu\text{g}/\text{ml}$, and then irradiated at 3,000 rad. These lines were grown in medium supplemented with 10 U/ml rIL-2 (Cetus Corp., Emeryville, CA). Each line was shown to be specific only for the stimulating peptide presented by HLA-B27 before being used in these experiments. An HLA-B8-restricted CTL line was grown on the nucleoprotein (NP) peptide 379-402, which is an epitope presented by HLA-B8 (Sutton et al., manuscript in preparation); this was also highly specific.

The method used to generate HLA-B27-restricted HIV gag-specific CTL lines has been fully described (19). Briefly, lymphocytes from a B27 HIV-seropositive donor were stimulated with 1-d-old autologous PHA (Wellcome Diagnostics, Greenville, NC)-activated lymphocytes. After 7 d, cells were restimulated with irradiated autologous BCL preincubated with gag peptide 265-279 at $50 \mu\text{M}$. T cell growth factor (Lymphocult T; Biotest AG, Dreieich, Germany) at 10 U/ml was added to the culture. EBV-specific CTL lines were generated exactly as described previously (20).

Cytotoxicity Assay. Effector cells were harvested and tested for their ability to lyse ^{51}Cr -labeled target cells in a 4-h assay. Effectors and targets were mixed together in wells of a 96-well round-bottomed plate in $150 \mu\text{l}$ of RPMI 1640/10% FCS. $50 \mu\text{l}$ of peptide at various dilutions was added to appropriate wells. Control wells had either medium or Triton X-100 in place of effector cells added to estimate spontaneous and maximal ^{51}Cr release. After a 4-h incubation, $20 \mu\text{l}$ of supernatant was harvested from each well onto a filtermat, dried, scintillant fluid added and the radioactivity measured in a Beta-plate counter (LKB Instruments, Inc., Gaithersburg, MD). The results are expressed as percent specific lysis and calculated as follows: percent specific lysis = $100 \times (\text{experimental counts} - \text{spontaneous release}) / (\text{detergent control} - \text{spontaneous release})$.

In most of the experiments, three control cell lines were used: LY is an EBV-transformed B lymphocyte cell line expressing the HLA-B2702 molecule. It was established from a healthy donor and made available to us by Dr. A. Rickinson (University of Birmingham). P815-B2702 is a mouse mastocytoma cell line transfected with the HLA-B2702 molecule, kindly donated by Dr. E. Weiss (University of Munich). HMy/C1R-B2702 cells transfected with the same molecule were a gift from Dr. J. Lopez de Castro (Fundacion Jimenez Diaz, Madrid, Spain). C1R cells, which are identical

to HMy/C1R cells, were given to us by Dr. J. Frelinger (University of North Carolina) and used for the transfection experiments.

Flow Cytometry. 10^5 cells were stained with 1:50 to 1:500 dilutions of the anti-HLA-B27 mAb ME1 (21) for 30 min. After washing the cells in PBS-0.1% BSA, FITC-labeled rabbit anti-mouse Ig antibody (Sigma Chemical Co., St. Louis, MO) was added to a dilution of 1:100 for 20 min on ice, followed by another wash in PBS-0.1% BSA. Finally, the cells were resuspended in 0.5 ml PBS containing 1% FCS and 1% formaldehyde. 5,000 cells were counted on a Facscan[®] flow cytometer (Becton Dickinson & Co., Mountain View, CA) using Consort 30 software.

Pulse Chase Labeling. 10^8 cells in log phase of growth were spun down and resuspended in 2 ml methionine-free RPMI 1640 with 5% FCS and incubated at 37°C in a small flask for 40 min. ^{35}S -Methionine (ICN, Irvine, CA), 0.5 mCi, was then added. After 15 min, labeling was stopped by the addition of $300\text{-}\mu\text{l}$ aliquots to either 20 ml ice-cold PBS (0 time point sample) or to 5 ml RPMI with 10% FCS and 2 mM cold methionine at 37°C (other time points). These were removed from the incubator at the intervals shown and immediately added to 20 ml ice-cold PBS. The cells in PBS were centrifuged at 4°C , and the pellet lysed in 1 ml lysis buffer (0.5% NP-40, 20 mM Tris, pH 8, 10 mM EDTA, pH 8, 0.1 M NaCl, 1 mM PMSF). After 30 min on ice, the nuclei were sedimented at 14,000 rpm for 5 min at 4°C . The lysates were stored on ice until all samples were ready for preclearing. Lysates were precleared with $150 \mu\text{l}$ 10% Pansorbin (Calbiochem Corp., San Diego, CA) for 1 h, centrifuged, and the supernatant was divided into two equal aliquots for immunoprecipitation with $3 \mu\text{l}$ HC10 (22) or W6/32 (23) ascites. These aliquots were incubated with antibody for 1 h at 4°C , then $100 \mu\text{l}$ 10% protein A-Sepharose (Sigma Chemical Co.) in lysis buffer was added and samples mixed end over end for 30 min at 4°C . Protein A-Sepharose beads were then washed at 4°C twice with 20 mM Tris, pH 8, 10 mM EDTA, pH 8, 0.1 M NaCl, 0.5% NP-40, 0.1% SDS, 1% BSA, once with 10% lysis buffer in 0.5 M NaCl, and once in lysis buffer. They were then resuspended in $60 \mu\text{l}$ sample buffer (9.5 M urea, 2% NP-40, 2% ampholines, pH 3.5-10, 5% 2-ME) and incubated at room temperature for 30 min. The beads were sedimented by spinning briefly in a microcentrifuge, and $40 \mu\text{l}$ of the supernatant was removed for analysis on isoelectric focusing gel.

Isoelectric Focusing. One-dimensional IEF was performed according to the method of Neeffes et al. (24) in 4.5% polyacrylamide gels containing monoacrylamide/bisacrylamide (Bio-Rad Laboratories, Richmond, CA) (ratio 30:1.6), 9 M urea, 2% NP-40, 4% ampholyte pH5-7 (LKB Instruments, Inc.), and 1% ampholyte pH 3.5-10 (LKB Instruments Inc.). To polymerize 30 ml gel solution, $60 \mu\text{l}$ freshly prepared 10% (wt/vol) ammonium persulfate, and $30 \mu\text{l}$ TEMED were used. 16-cm gel plates were run on a Protean II apparatus (Bio-Rad Laboratories). The upper buffer was 50 mM NaOH, and the lower buffer 20 mM H_3PO_4 . A constant voltage of 880 V was set, with the current limited to 12 mA per gel and power to 8 W, allowing the maximum voltage to be achieved over several hours. The run was continued for 13-16 h, the gels fixed in 10% acetic acid for 30 min, then treated with Amplify for 30 min, dried, and exposed to Kodak XAR film. The position of bands corresponding to individual HLA class I alleles was determined by comparison with cell lines of known HLA type and published information (22, 24, 25).

DNA Cloning and Sequencing. Total cellular RNA was isolated from 10^8 cells by a single-step method (26). First-strand cDNA was synthesized using the Red Module kit (Invitrogen Corporation, San Diego, CA) according to the manufacturer's instructions. cDNA coding for B locus HLA class I products was amplified using

¹ Abbreviations used in this paper: BCL, B-lymphoblastoid cells; NP, nucleoprotein.

the following primers: 5'GCC CGT CGA CGG ACT CAG AAT CTC CCC AGA CGC CGA 3' for the 5' untranslated region, and 5' CCG CAA GCT TCT GGG GAG GAA ACA CAG GTC AGC GGA AC 3' for the 3' untranslated region. Standard PCR buffer was used (27) with the Mg^{2+} concentration at 1.5 mM. After 3 min at 94°C initial denaturation, the samples were amplified for 25 cycles of 1 min at 94°C, 1 min at 67°C, and 1.5 min at 72°C in a temperature cycler (Grant Instruments, Cambridge, UK). The 1.3-kb amplified product was purified on SeaPlaque (FMC Bio-products, Rockland, ME) low melting point agarose gel. The amplified band was excised from the gel, melted and cut sequentially with HindIII and SalI (Promega Biotec, Madison, WI). After another purification step on low melting point agarose, the band was excised and mixed at a ratio of 3:1 with M13mp19, which was previously cut with the same enzymes and then gel purified. The melted gel mixture was kept at 37°C, T4 ligase buffer and ligase (Promega Biotec) were added, and the ligation was carried out at room temperature for 5 h. The gel was remelted and 10 μ l of the gel was mixed with 10 μ l water. *Escherichia coli* JM109-competent cells were transformed according to the manufacturers instructions (Promega Biotec). White plaques were picked, grown, and sequenced using T7 polymerase and Deaza GTP sequencing mixes (Pharmacia Fine Chemicals, Piscataway, NJ). The sequencing primers used were published previously (28).

cDNA Expression. The eukaryotic expression vector pKG5 (29) was a gift from Dr. Pita Braddock (Imperial Cancer Research Fund, Institute of Molecular Medicine, Oxford, UK). The HLA-B2702 cDNA grown in M13mp19 was subcloned using the purification of DNA fragments in SeaPlaque agarose gels according to the protocol described above. *E. coli* HB 1061-competent cells were transformed, grown with standard methods (27). The plasmid was isolated using a scaled-up version of the protocol described by He et al. (30). After RNase digestion (RNase; Stratagene, La Jolla, CA) and ethanol precipitation the DNA was cut with Scal (Promega Biotec). The precipitated DNA was dissolved at 1,000 μ g/ml in water, and 10^7 C1R cells were mixed with 50 μ l DNA and electroporated using a GenePulser (Bio-Rad Laboratories) set to 230 V and 250 μ F. Cells were resuspended in 25 ml RPMI containing 10% FCS and antibiotics and plated into 24-well plates (Costar, Cambridge, MA). After 2 d, selective medium containing 1.8 mg/ml G418 (Sigma Chemical Co.) was added. 4 wk later, cells from the wells containing living cells were stained with the mAb ME 1.

The cells from individual wells expressing the highest amounts of B27 were expanded and used as targets in CTL assays.

Results

Inability to Present Peptide Epitopes via HLA B27. The influenza virus NP peptide 380-393 is an epitope presented by HLA-B2705 and recognized by the CD8-positive peptide-specific HLA-B27-restricted cytotoxic T cell lines HF and GR. As we have previously shown, all B2705-positive cell lines tested presented this peptide. Several NP 380-393-specific sublines of the HF and GR CTL were generated from different donations of peripheral blood; some were able to recognize this peptide when it was presented by the cell lines expressing another B27 subtype, B2702, which differs from B27005 at three amino acid residues at positions 77, 80, and 81 (31). As shown in Fig. 1, the HLA-B2702-positive lymphoblastoid cells LY and transfectant cell lines P815-B2702 and HMy/C1R-B2702, both expressing the HLA-B2702 gene, were able to present this peptide to an HF CTL line. However, neither BCL nor PHA-activated T cell blasts from B2702-positive donor NW presented this peptide. Shorter and longer peptides based on the same region of the NP (9) were also tested but none was recognized (data not shown).

Similar results were obtained with CTL specific for the HIV virus gag peptide 265-279 also restricted by HLA-B27. Again, NW cells invariably failed to present the added peptide, while in the same experiments the HMy/C1R-B2702 and P815-B2702 cells presented this peptide (Fig. 2).

The BCL NW expressed HLA-B8 as well as -B2702 and was tested for its ability to present an HLA-B8-restricted peptide, NP 379-402, to highly specific CTL. As shown in Fig. 3, this peptide was presented efficiently to the B8-restricted CTL, which indicates that in this cell line there is no generalized defect in peptide presentation to CTL.

HLA-B2702 Restricted EBV-specific CTL from LY Fail to Lyse NW BCL. It was not possible to generate influenza-specific CTL from HLA-B2702-positive donors LY and NW. However, four HLA-B2702-restricted CTL lines specific for EBV

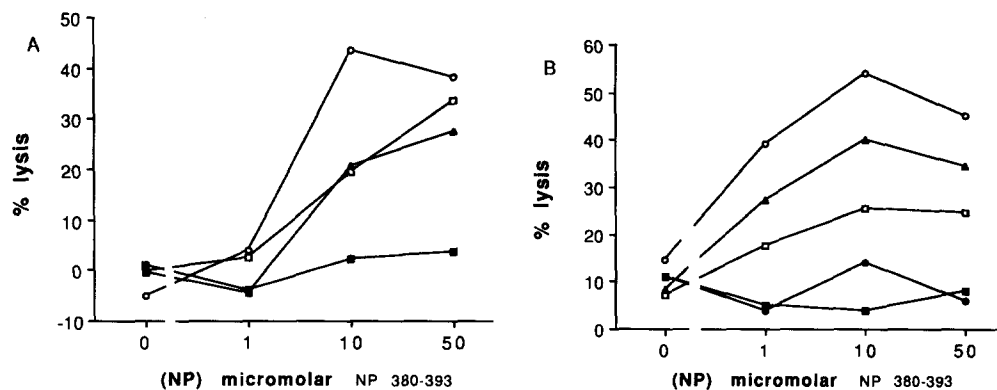


Figure 1. Peptide NP 380-393 presentation by HLA-B2705- and B2702-positive cell lines to an HLA-B27-restricted peptide-specific CTL line HF at an E/T ratio of 4:1. (A) The B2705 autologous BCL (○) and the B2702-transfected P815-B2702 cells (△) and HMy/C1R-B2702 cells (□) could present the peptide to the effector cells while the B2702-positive NW cell line (■) did not. (B) The same peptide tested with peptide-specific B27-restricted CTL line from donor GR at an E/T ratio of 6:1 lysing peptide-treated autologous cells (○), B2702-positive LY BCL (□), and B2702-transfected P815-B2702 cells (△), but not recognizing B2702-positive NW BCL (■) or NW PHA blasts (●).

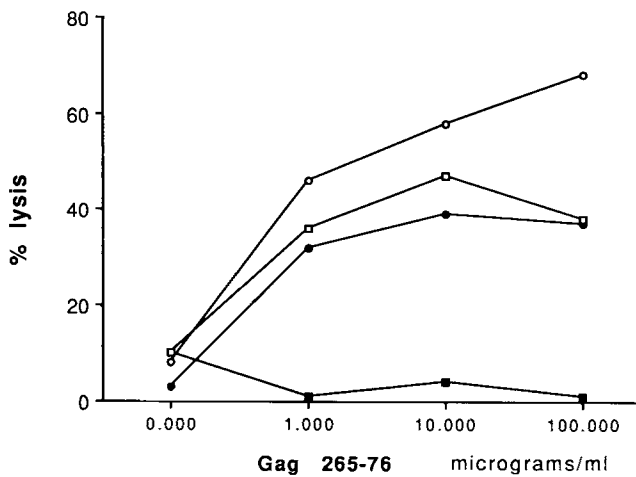


Figure 2. Failure of NW cells to present the HIV gag peptide 265–276. Peptide-specific B27-restricted CTL were derived from a HLA-B2705-positive, HIV-1 seropositive donor 007. NW B2702 BCL (■) did not present the peptide, but B2705-positive HF BCL (●) and transfectants HMy/C1R-B2705 (○) and HMy/C1R-B2702 (□) did.

were generated from donor LY. All of the four CTL lines lysed the autologous LY cells, but not NW BCL. This contrasted with the ability of three of the CTL lines to lyse B2705-positive SC cells. This result supports the observations made with the influenza NP and HIV gag-specific CTL from HLA-B2705-positive donors, that B2702 and B2705 molecules can present the same peptides, but again, NW cells failed to present the expected epitope (Fig. 4).

The Nonpresenting Phenotype Is Inherited. We established EBV-transformed BCL from relatives of the donor NW. As shown in the family tree (Fig. 5), the cell lines came from three generations. Several family members were B27 positive and three have had some form of HLA-B27-associated disease. The nine B27-positive cell lines were tested as targets

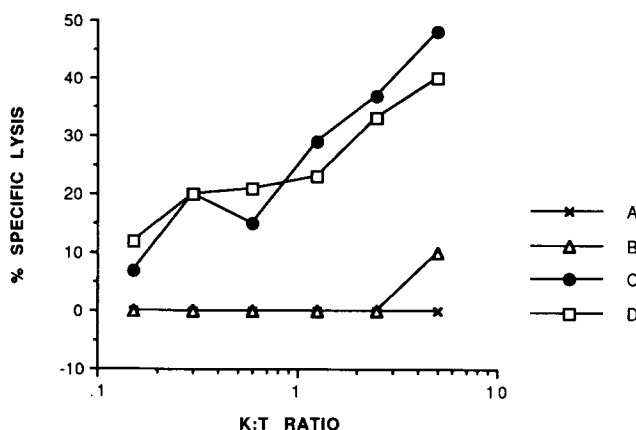


Figure 3. Peptide presentation by HLA-B8 is intact in NW BCL. An influenza NP 380–393-specific HLA-B8 (but not B27)-restricted CTL line recognized the autologous CM BCL (●) and NW BCL (□) equally well in the presence of peptide at 20 μ M; neither CM BCL (X) nor NW BCL (Δ) was killed in the absence of peptide. Killer/target (K/T) ratios were as shown.

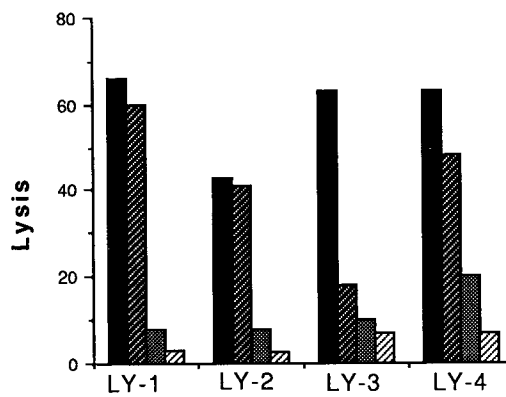


Figure 4. LY CTL lines specific for EBV presented by HLA-B27 could distinguish between autologous target cells (■) and NW BCL (▨). Three out of four lines (LY-1, LY-2, and LY-3) reacted with HLA-B2705-positive SC BCL (■), while none recognized B2702-positive NW BCL (■) and the HLA-mismatched BCL IB (▨).

with HLA-B27-restricted peptide-specific CTL lines, using the influenza A NP peptide 380–393; all failed to present this peptide epitope (Table 1). Cells from six family members were also tested with the HLA-B27-restricted HIV gag peptide 265–279. The CTL line used recognized LY cells and the HMy/C1R-B2702 transfectants treated with the gag 265–279 peptide, but did not kill peptide-treated cells from B2702-positive family members (Table 1). Furthermore, when presentation of antigen by cells infected with influenza virus was tested, autologous and LY cells presented the epitope derived from virus while BCL from a family member did not (Fig. 6).

Expression of HLA-B2702 by NW and LY BCL. When B cell lines from NW and family members were tested for surface expression of HLA-B27 with the ME1 mAb, it was found that they expressed similar amounts of B27 molecules to the B2702 cell line LY, which could present the peptides (Fig. 7).

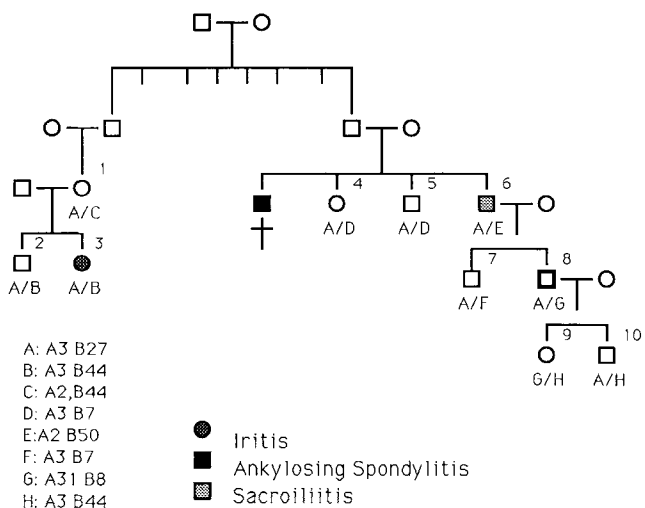


Figure 5. The family W. HLA class I haplotypes of the individuals studied are given on the left. Three individuals have had AS or related disorders, as indicated. The proband, NW, is member 8.

Table 1. Failure of BCL from Members of Family NW to Present NP 380-393 to Specific CTL

Exp.	B2705 [†]	B2702 [‡]	8(NW)	Targets*							
				1	2	3	4	5	6	7	10
1 NP	36 [¶]	20	4	2			9	10			
2 NP	30	15	0	0	0	0	0		0	0	1
3 gag	34	16	7	3	0			0	6	0	

* Family members are designated by their numbers as given in Fig. 5.

[†] HLA-B2705-matched target cells were HMy/C1R in Exp. 1, autologous (GR) in Exp. 2, and HH BCL in Exp. 3.

[‡] HLA-B2702-matched target cells were HMy/C1R in Exps. 1 and 2, and LY BCL in Exp. 3.

^{||} Peptide NP 380-393 was tested at concentrations between 1.0 and 50 μ M; results at 10 μ M are shown.

[¶] Results are shown as percent specific lysis. CTL were HF CTL in Exp. 1, GR CTL in Exp. 2, and 007 CTL in Exp. 3. All three lines were previously shown to be highly specific for the peptide and restricted by HLA-B27. Killer target ratios were 10:1, 9:1, and 10:1 in Exps. 1, 2, and 3, respectively.

To examine the assembly and glycosylation of HLA class I molecules in NW compared with LY cells, BCL from each donor were pulse labeled with ³⁵S-methionine, chased with cold methionine, and HLA class I molecules were immunoprecipitated at intervals thereafter. By using the mAbs HC10 and W6/32, specific for unfolded and folded heavy chains, respectively (22), it was possible to follow assembly and sialation in IEF gels, which clearly distinguishes between different class I types (21, 23, 24, 31-33). As shown in Fig. 8, HLA-B2702 associated with β_2 -microglobulin more rapidly in NW cells compared with LY cells, but subsequent sialation occurred at about the same rate. Folding of B27 was markedly slower than HLA-A24 and -B35 in the LY cells and faster than HLA-B8 in the NW cells.

The NW HLA-B2702 Molecule Is Structurally Normal. Side by side comparison in IEF gels, which can distinguish six HLA-B27 subtypes (32), indicated that B2702 from LY and NW ran in the same position. To exclude mutations resulting

in noncharged amino acid substitutions, cDNA coding for the B2702 molecule was cloned from the NW BCL. Sequencing of the 1.3-kb insert revealed two noncoding differences from the published B2702 DNA sequence (31). One was in the $\alpha 3$ domain at position 708 and the other was located in the 3' untranslated region at position 1067. Neither would result in changes in the amino acid sequence.

The B2702 from the Cell Line NW Presents Peptide in a Different Cellular Environment. The cloned cDNA used for the sequencing was subcloned into the expression vector pKG5 and transfected into C1R cells. As shown in Fig. 8, these cells expressed amounts of B2702 molecules on the surface that were comparable with those on BCL. The transfectant cell line was lysed by the HF influenza-specific CTL line in the presence of the influenza NP peptide 380-393 (Fig. 9).

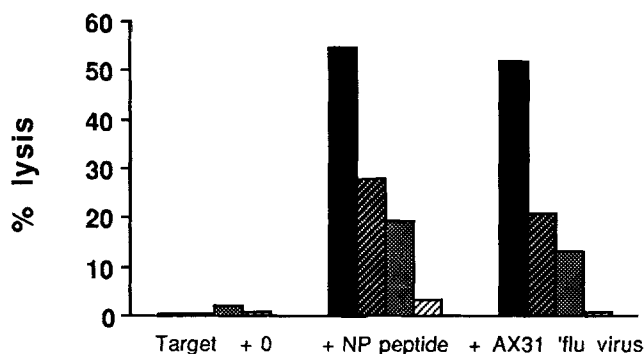


Figure 6. Failure to present epitope peptides generated during viral infection. (Left) Untreated cells; (middle) cells sensitized with peptide; and (right) the same cells after viral infection, showing similar patterns of lysis. The killer CTL were an HF peptide 380-393 B27-restricted line used at a K/T ratio of 5:1. Target cells were B2705-positive HF BCL (■), HMy/C1R-B2702 (▨), B2702-positive LY BCL (▧), and family member 10 (see Fig. 5) (▧).

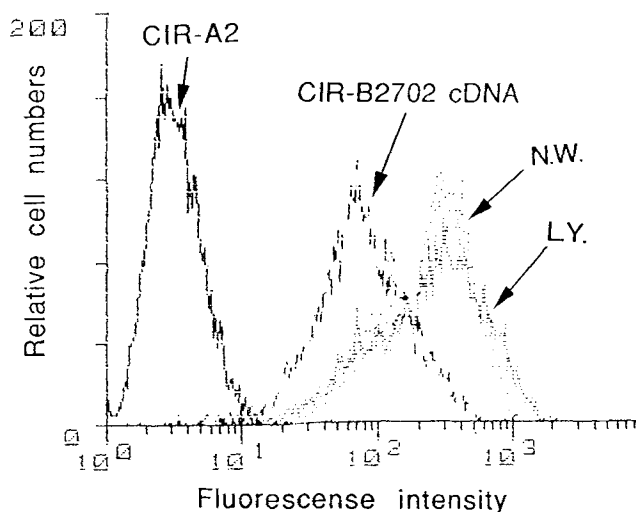


Figure 7. Histograms after staining the cell lines with the B27-specific monoclonal antibody ME1. The four cell lines tested are indicated: CIR-A2 (HMy/C1R cells transfected with HLA-A2), HMy/C1R-B2702 cDNA (HMy/C1R cells transfected with B2702 cDNA prepared from NW BCL), NW BCL, and LY BCL.

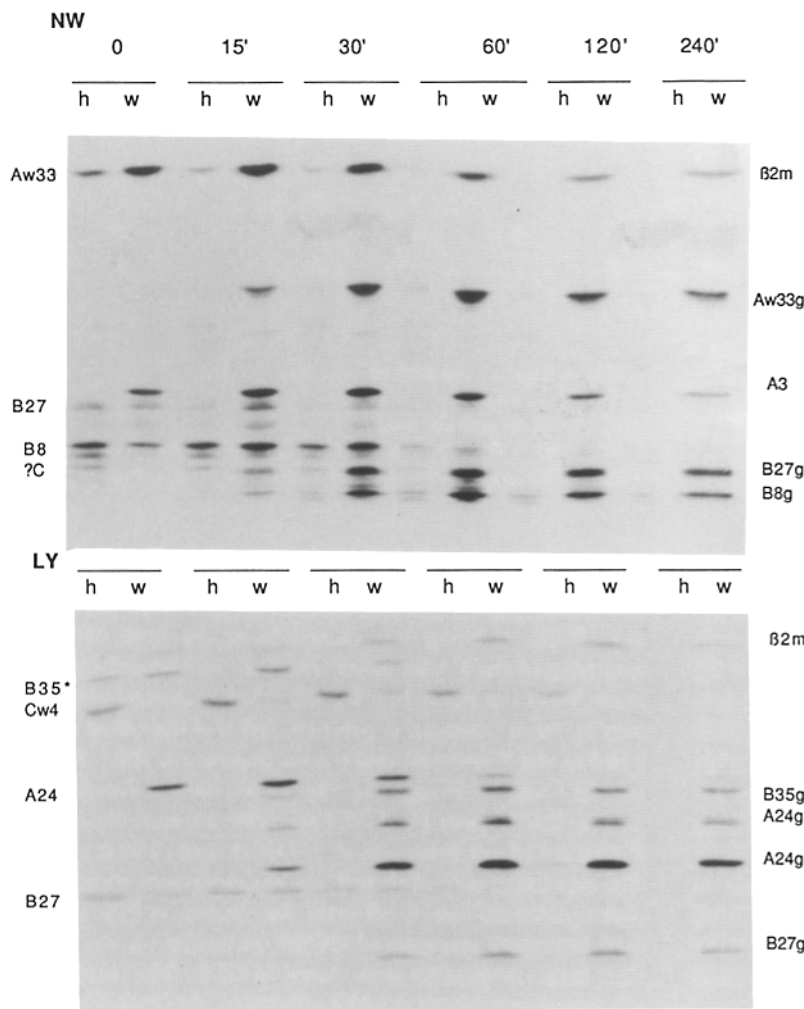


Figure 8. Processing of HLA class I molecules in NW BCL and LY BCL. The two cell lines were pulse labeled with ^{35}S methionine and then chased with cold methionine for the times shown (0–240 min). Labeled class I molecules were immunoprecipitated with antibodies HC10 (*h*), specific for free heavy chain, and W6/32 (*w*) specific for β_2 -microglobulin-associated heavy chain. Precipitates were run on two isoelectric focusing slab gels and the autoradiographs (NW and LY) are shown, with basic pH at the top. The positions of each of the class I molecules agrees with previously published results (21, 23, 24, 31, 32), except for the band marked B35*, which probably represents a B35 variant molecule, and ?C, which probably represents a C locus product. Immunoprecipitates were not treated with neuraminidase and the emergence of the sialated products is indicated by g.

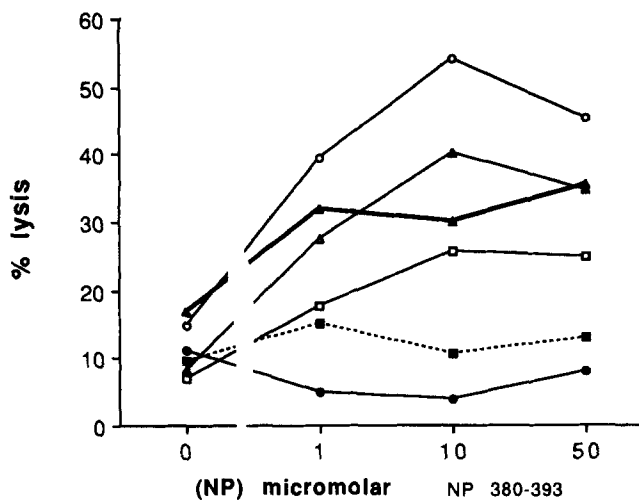


Figure 9. Presentation of peptide by HLA-B2702 from NW was restored by transfection into HMMy/C1R cells. GR CTL peptide, NP 380-393 specific and HLA-B27 restricted, were tested at a K/T ratio of 6:1 with peptide treated (at the concentrations shown) autologous GR BCL (○), B2702-positive LY BCL (□), NW BCL (●), B2702 transfectant P815-B2702 (Δ), HMMy/C1R transfectant with B2702 from NW (▲), and a control of HMMy/C1R transfectant with HLA B35 (■).

Discussion

Here we describe several EBV-transformed B cell lines from members of a family, in which two cases of radiologically confirmed ankylosing spondylitis have occurred and a third individual has had an episode of anterior uveitis. In the experiments described here, all B27-positive B cell lines from this family failed to present two peptide epitopes via the HLA-B2702 molecule, irrespective of whether the peptides were added exogenously or generated during viral infection. In contrast, the unrelated BCL LY and two transfectant cell lines, all expressing the same B2702 restriction element, were able to present the peptides and the virus. An effect caused by transformation by EBV was unlikely because, although PHA blasts can readily present peptides to CTL (2), peptide-treated NW PHA blasts were not lysed. Conversely, HLA-B2702 expressed in both the EBV-negative mouse cell line P815 and the EBV-positive HMMy/C1R line could present antigen. When EBV was tested as an antigen, it was found that four CTL lines recognizing EBV presented by B2702 could distinguish between the BCL of NW and LY. When the B2702 molecule was cloned from one family member and expressed in HLA-A- and -B-negative HMMy/C1R cells, these transfectants could present the peptide epitope.

The CTL lines recognizing the influenza NP and HIV gag peptides were from HLA-B2705 donors and were primed with this B27 subtype *in vivo*. HLA-B2702 differs from B2705 at three amino acids: aspartic acid replaced by asparagine at position 77, threonine by isoleucine at 80, and leucine by alanine at 81. All three side chains point into the peptide binding groove of HLA-B27, but are sited at one of its ends. It is clear from these results that B2702 can bind the two peptides used in these experiments and that each can be recognized by some of the CTL clones that normally respond to peptide presented by HLA-B2705. Three of the four CTL lines specific for EBV from the B2702-positive donor LY recognized EBV-transformed B2705-positive cells, supporting the idea that there is an overlap in the peptides presented by these two closely related HLA molecules. Because HLA-B8-positive cells from NW presented a peptide epitope via this restriction element, a general defect in these cell lines, for example, the lack of accessory molecules such as CD11a-CD18, was excluded.

The prerequisite for recognition and killing of a target cell by CTL is the presence of a structurally intact MHC molecule on the target cell that is able to bind the viral peptides in the peptide binding groove and can interact with the TCR. Although unrelated peptides can bind to the same MHC molecule with relatively low affinity (34, 35), epitope peptides bind with high affinity and the interaction is very specific (6, 7, 36–38). Since single amino acid substitutions at certain parts of the MHC molecule can abrogate recognition by CTL by interfering with either peptide binding or interaction with the TCR (8, 39, 40), it was important to show that the B2702 molecule on the cell lines from the studied family did not have any additional changes in the amino acid sequence. This was excluded by sequencing of cDNA cloned from the proband, NW; the derived amino acid sequence showed no coding changes from that published for HLA-B2702 (31).

There is considerable current interest in the series of events that leads to the intracellular formation of the heavy chain- β_2 -microglobulin-peptide complexes. This was stimulated by the identification of the mutant cell lines RMA-S (10), 721.174 (11), and 721.134 (12), which show defects in this assembly process. These cells produce normal amounts of structurally intact MHC heavy chain and β_2 -microglobulin molecules but their assembly is impaired and they express very few stable class I molecules on the cell surface. Peptide-specific CTL fail to recognize internally derived antigen when these cells are infected with influenza virus but do lyse cells when they have been incubated in the epitope peptide (10, 11). These results imply that the peptide induces MHC class I molecule assembly or stabilizes assembled molecules on the cell surface (5, 10, 11, 38, 41). The cell lines described here are different. They expressed normal amounts of HLA-B2702 molecules on the surface, and the expression did not increase after incubation in the presence of peptides (data not shown). Exogenously added peptides, as well as virus antigens, were not presented.

Given the sequence identity of the B2702 in cells that did or did not present peptides, we reasoned that the cellular environment must influence antigen presentation by this mole-

cule. To test this hypothesis we cloned the cDNA encoding the HLA-B2702 from the NW cell line and expressed it in the HLA-A- and -B-negative HMy/C1R cell line. In this cellular environment, the cloned B2702 molecule functioned differently, presenting the peptide. This implies the presence of one or more factors that influence peptide presentation by this molecule in the original host cell. BCL from nine individuals, representing three generations in the NW family, were established. All those with HLA-B2702 failed to present peptide to CTL lines that could recognize peptide presented by LY, HMy/C1R-B2702, and P815-B2702. The occurrence of the described phenotype in every B27-positive member of this family can be explained by two mechanisms. It is possible that the frequency of the gene or genes responsible for this effect is very high in the population, in which case a recessive or dominant gene could account for the observations. The other possibility is that the trait is dominant and is linked to the HLA-B2702 haplotype.

The findings described here are reminiscent of those in a congenic rat strain in which a gene mapping to the MHC class II region modified antigen presentation by the otherwise normal class I RT1.A^a molecules, rendering them unrecognizable both for alloreactive T cell clones and CTL specific for the HY antigen (13). The members of the ABC transporter superfamily, located in the class II region (14–17), are obvious candidate genes. Phenotypically, the deficit in antigen presentation by this rat allele shows some similarity to our findings. However, in the rat, the RT1.A^a molecule was retained within the endoplasmic reticulum or *cis*-Golgi complex for an unusually long period in the PVG.R1 and R8 congenic lines, and rapid processing was dominant in F₁ cells (42). This differs in important detail from our preliminary study of processing of HLA-B2702 in NW and LY cells; association between B2702 heavy chain and β_2 -microglobulin, measured by loss of reactivity with HC10 antibody and acquisition of the W6/32 epitope, was delayed in LY cells compared with NW. The difference was not large but was definite and reproducible; subsequent passage through the Golgi complex, measured by increasing negative charge of the B2702 band due to addition of sialic acid, occurred at a similar rate in both cell lines. The way antigenic peptides are generated and transported into the endoplasmic reticulum is far from understood. The faster assembly of the B2702 molecules in NW could be due to a more efficient peptide-generating or -transporting system. Another possibility is that the peptides reaching the endoplasmic reticulum in NW cells are different and some of them may have a very high affinity for the HLA-B2702 molecule. This could explain both faster assembly and the apparent inability of externally added peptides to bind to the B27 molecules in these cells. If the phenotype is caused by a rare dominant gene (or genes) and the responsible element(s) is linked to the MHC, a possibility compatible with the presented findings, the ABC transporter genes mapping here will be candidates if they are found to be polymorphic. These possibilities are currently under detailed investigation.

HLA-B27 is one of the most widely investigated human HLA class I molecules because of its strong association with

ankylosing spondylitis, reactive arthritis, juvenile spondyloarthritis, psoriatic arthritis, and enteropathic arthropathy (43). More than 90% of Caucasian patients who suffer from ankylosing spondylitis carry the B27 allele, compared with 7% of controls, making this the strongest known HLA disease association (44). However, the probability that a B27-positive individual will develop the disease depends on the family history. While in general only 2% of the HLA-B27-carrying population is affected, if there is a family history of the disease the incidence may be as high 20% (45), as illustrated by the occurrence of three cases in the family investigated here. These observations suggest that inherited ele-

ments, other than the B27 molecule itself, play an important role in the pathophysiology. These need not necessarily be linked to the MHC although our observations may suggest that this is the case. The association of the unusual behavior of the NW B2702 molecule with the history of ankylosing spondylitis, and related disorders, in this family argues that the nonpresenting phenotype might be associated with increased risk of developing the disease. If such a linkage can be demonstrated it will support the view that the peptide-presenting function of HLA-B2702 is important in the pathogenesis of ankylosing spondylitis.

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Address correspondence to Laszlo Pazmany, Molecular Immunology Group, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DU, UK.

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