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*J Immunol* 2001; 167:2619-2624; doi: 10.4049/jimmunol.167.5.2619

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The Recognition of HLA-B27 by Human CD4\(^+\) T Lymphocytes

Louise H. Boyle, Jane C. Goodall, Stephen S. Opat, and J. S. Hill Gaston

HLA-B27 transgenic animal models suggest a role for CD4\(^+\) T lymphocytes in the pathogenesis of the spondyloarthropathies, and murine studies have raised the possibility that unusual forms of B27 may be involved in disease. We demonstrate that CD4\(^+\) T cells capable of recognizing B27 can be isolated from humans by coculture with the MHC class II-negative cell line T2 transfected with B27. These CD4\(^+\) T cells recognize a panel of B27-transfected cell lines that are defective in Ag-processing pathways, but not the nontransfected parental cell lines, in a CD4-dependent fashion. Inhibition of responses by the MHC class I-specific mAb w6/32 implicates the recognition of a form of B27 recognized by both of these Abs. We suggest that B27-reactive CD4\(^+\) T cells may be pathogenic in spondyloarthropathies, particularly if factors such as infection influence expression of abnormal forms of B27.


A number of theories have been proposed to explain the association of HLA-B27 with the spondyloarthropathies (reviewed in Ref. 1), yet despite extensive research, its role in disease still remains elusive. B27 is not simply a marker of disease susceptibility because B27\(^+\) transgenic rats and mice both develop inflammatory disease (2, 3). Because the normal function of B27, associated with \(\beta_2\)-microglobulin (\(\beta_2\)-m),\(^3\) is to present peptides for recognition by CD8\(^+\) T lymphocytes, much work has concentrated on identifying B27-restricted CTL in spondyloarthropathy patients (4, 5). However, studies with B27\(^+\) transgenic mice have raised the possibility that the role of B27 in joint disease may not simply be as a restriction element for CD8\(^+\) T cells. CD8\(^+\) T cells do not appear to be pivotal in disease induction because B27\(^+\) mice lacking \(\beta_2\)-m (\(\beta_2\)-m\(^−/−\)) with extremely low levels of CD8\(^+\) T cells still develop inflammatory disease. Interestingly, \(\beta_2\)-m\(^−/−\) mice made transgenic for both B27 and human \(\beta_2\)-m also develop disease, and in these mice disease is independent of the TAP-1 gene, which is required for efficient loading of peptide onto MHC class I molecules (6). In addition, adoptive transfer studies from disease-prone B27\(^+\) transgenic rats to B27\(^−\) nude rats identified that CD4\(^+\) T cells were more efficient in transferring inflammatory disease (7). However, in some circumstances MHC class II molecules, which are conventionally recognized by CD4\(^+\) T cells, are not required for disease development because MHC class II-negative, B27\(^+\) transgenic mice still develop spontaneous disease (8), discounting the presentation of a B27-derived peptide by class II molecules. One possibility to explain these findings is that B27 itself may be recognized by CD4\(^+\) T cells.

It is also possible that forms of B27 other than the conventional H chain/\(\beta_2\)-m heterodimer may be important in disease. Support for this idea came from the finding that disease in B27\(^+\) \(\beta_2\)-m\(^−/−\) transgenic mice, which express free B27 H chains not associated with \(\beta_2\)-m, could be ameliorated by in vivo treatment with an Ab (HC10) that recognizes free H chains (8). In addition, a variety of unusual forms of B27 occur on the surface of cells in vitro; empty B27 heterodimers, not containing a peptide, are present on the surface of C1R transfected with HLA-B*2705 (C1R-B27) (9), free B27 H chains, i.e., not associated with \(\beta_2\)-m, form on tapasin-defective cell lines (10), and free B27 H chains have been shown to form disulfide-bonded homodimers, dependent on the cysteine residue at position 67 in the peptide binding groove, in TAP-deficient cell lines (11).

To investigate the possibility that CD4\(^+\) T lymphocytes might interact with B27, we have cultured highly purified human CD4\(^+\) T cells from B27\(^+\) spondyloarthropathy patients and healthy individuals with the MHC class II-negative, TAP-negative cell line T2 transfected with B27. T2 cell line transfected with HLA-B*2705 (T2-B27) expresses a variety of forms of B27 including B27 heterodimers, free B27 H chains, i.e., not associated with \(\beta_2\)-m, form on tapasin-defective cell lines (10), and free B27 H chains have been shown to form disulfide-bonded homodimers, dependent on the cysteine residue at position 67 in the peptide binding groove, in TAP-deficient cell lines (11).

Materials and Methods

Isolation of CD4\(^+\) T lymphocytes

PBMC were isolated from the blood of six B27\(^+\) ankylosing spondylitis (AS) patients and two B27\(^+\) healthy individuals by Ficoll-Paque (Pharmacia, St. Albans, U.K.) gradient centrifugation. Monocytes were removed from PBMC by adherence. Nonadherent cells were incubated with mAbs specific for CD8, CD19, CD11b, and CD16 for 30 min at 4°C (DAKO, Ely, U.K.). Labeled cells were removed using sheep anti-mouse IgG-coated magnetic beads (Dynal Biotech, Oslo, Norway). Three rounds of negative selection resulted in the isolation of CD4\(^+\) T cell populations of 95% purity, as determined by flow cytometric analysis.
determined by subtracting background [3H]thymidine incorporation by a beta plate counter (Wallace, Turku, Finland). T cell proliferation was monitored by culturing 2 × 10^5 T cells and irradiated allogenic PBMC as APCs, PHA, and 100 U/ml rIL-2.

**T cell proliferation assays**

Proliferation assays were performed at 37°C by culturing 2 × 10^5 T cells with 2.5 × 10^5 irradiated stimulator cells per well in 96-well plates. Plates were cultured for 2 days, then pulsed with [3H]thymidine (1 μCi/well; Amersham Pharmacia Biotech, Little Chalfont, U.K.). After culture for 6 h, the plates were harvested using a beta plate counter (Wallace, Turku, Finland). T cell proliferation was determined by subtracting background [3H]thymidine incorporation by stimulating pool cells from total [3H]thymidine incorporation.

The following cell lines were used: T2 (TAP1 and 2 negative, and MHC class II negative) (12); 721.220 (HLA-A and -B negative, tapasin mutant) (13, 14); C1R (hemizygous for HLA complex) (15); and the mono-cyte-derived cell line U-937 (16). This panel of cell lines was transfected with HLA-B*2705. In addition, 220 transfected with HLA-B8 was used. EBV LCLs were produced from PBMC as previously described (17).

**Coculture of CD4+ T lymphocytes with B27-specific T cell lines**

Irradiated (6 Gray) T2-B27 cells (5 × 10^3) were added to each 200-μl well of a 96-well plate in complete medium (RPMI 1640 (Life Technologies, Paisley, U.K.), 5% human serum, 25 mM HEPES, 2 mM glucose, 100 U/ml penicillin, and 100 μg/ml streptomycin sulfate). Between 10^7 and 3 × 10^8 purified CD4+ T lymphocytes were added per well to the stimulator cells. The wells were incubated for 6 days at 37°C before rIL-2 (10 U/ml) was added twice weekly thereafter (Chiron, Harefield, Middlesex, U.K.). T cells lines were restimulated with irradiated stimulator cells as required. CD4+ T cell lines were further subcloned by limiting dilution in 20 μl Terasaki wells (Nunc, Roskilde, Denmark) using 10^5/ml irradiated allogenic PBMC as APCs, PHA, and 100 U/ml rIL-2.

**Results**

CD4+ T lymphocytes can recognize B27 expressed on a panel of B27 transfectants

To investigate whether CD4+ T lymphocytes can interact with B27 we cultured highly purified CD4+ T cells, isolated from the PBMC of B27+ spondyloarthropathy patients and B27+ healthy controls, with the cell line T2-B27. T2-B27 was initially chosen as it does not express MHC class II molecules preventing the stimulation of MHC class II-reactive CD4+ T cells. In addition, this TAP-negative cell line expresses a variety of different forms of B27, including free B27 H chain monomers (shown by the weak but definite staining with HC10), homodimers, and low levels of B27 heterodimers (Fig. 1) (11, 21). Using this coculture system we isolated a CD4+ T cell clone to T2-B27 (Fig. 2b) with the same pattern of recognition (Fig. 2a). Flow cytometry analysis showed the T cell clone expressed an αβ heterodimer TCR on the cell surface. Sequencing of the TCR α- and β-chains confirmed the expression of the BV2 and AV2 TCR genes. In addition, further culture of the line resulted in the loss of CD8+ T cells, and the line was then shown to be clonal by TCR analysis.

The finding of CD4+ T cells reactive to B27+ transfectants was not unique to this individual, because additional CD4+ T cell lines showing a similar pattern of B27 cross-reactivity were subsequently isolated from two other B27+ AS patients (Fig. 2). These lines showed a lower but statistically significant response to C1R-B27; in later experiments it was found that increasing the numbers of C1R-B27 stimulator cells enhanced responses of B27-reactive T cell lines due to improved T cell-stimulator cell contact. Nevertheless, it is noteworthy that the responses were inversely correlated with B27 expression as detected by ME1, i.e., T2-B27 was most stimulatory and C1R-B27 least, suggesting that a novel form of B27 might be being recognized.

**Abs specific for class I MHC Ags inhibit the response by CD4+ T cells**

To identify the surface molecules recognized by the cross-reactive CD4+ T cells from patient 1, the ability of mAbs specific for MHC molecules to inhibit the proliferative responses of the CD4+ T cell clone to T2-B27 was tested (Fig. 3). The mAb w6/32, which recognizes MHC class I heterodimers associated with β2m and free B27 H chain homodimers, inhibited the proliferative response of CD4+ T cells to T2-B27. In addition, the response to T2-B27 was inhibited by the HLA-B7, -B27 specific mAb ME1. Because HLA-B7 is not expressed on T2-B27, B27 is the only molecule available for ME1 binding. No inhibition was observed using the HLA-DR specific mAb L243 or the free HLA-B and -C H chain specific mAb HC10. It is difficult to interpret the lack of inhibition by HC10, as the ability of this mAb to inhibit T cell responses in an in vitro system has not previously been determined. The response to 220-B27 and C1R-B27 was also inhibited by w6/32 and...
Because T2-B27 does not express MHC class II molecules and L243 did not inhibit responses of the CD4+ T cells to the MHC class II-positive cell lines 220-B27 and C1R-B27 (data not shown), MHC class II molecules do not appear to be involved in the recognition of these three cell lines, excluding the possibility of recognition of a B27-derived peptide on MHC class II.

The CD4+ T cell response to B27 is CD4 dependent

To determine whether the CD4+ T cell response to B27 was dependent on the CD4 coreceptor, as occurs with a conventional CD4+ T cell response to MHC class II molecules, the effects of a CD4-specific mAb on responses to T2-B27 were tested. The CD4+ T cell response to T2-B27 was inhibited by 64% by the CD4 mAb (Fig. 4a), similar to the inhibition demonstrated using a conventional MHC class I-specific mAb.
II-restricted CD4+ T cell clone (Fig. 4b). The inability of the CD4 Ab to inhibit a response to PHA (Fig. 4) suggests that CD4-independent responses are not affected by this mAb. A similar pattern of inhibition was observed with 220-B27 and C1R-B27 as stimulator cells (data not shown).

The form of B27 recognized is not present on B27+ EBV-transformed cell lines

To determine whether the form of B27 recognized by the CD4+ T cells was expressed in vivo and on cell lines with intact Ag-processing pathways, the ability of the CD4+ H1101 cells was expressed in vivo and on cell lines with intact Ag-processing pathways. The form of B27 recognized by the CD4+ T cells is not present on EBV LCL and cell lines with intact Ag-processing pathways.

The form of HLA-B27 recognized on T2-B27 is stabilized at 26°C

Incubation of TAP-negative cells at 26°C has been shown to stabilize expression of MHC class I on the cell surface, including expression of empty heterodimers associated with β2m. Incubation of T2-B27 at 26°C for 24 h resulted in an increase of ME1 staining and w6/32 staining compared with incubation of T2-B27 at 37°C (Fig. 6, a and b). This reduction of temperature had no stabilizing effect on MHC expression on nontransfected T2, indicating that the MHC class I molecule stabilized on T2-B27 was B27. When CD4+ T cells responses to incubation of T2-B27 at 37°C and 26°C were compared, a 2-fold increase in proliferation was observed using T2-B27 incubated at 26°C (Fig. 6c) suggesting that the form of B27 recognized is stabilized on T2-B27 by incubation at 26°C.

The form of B27 recognized is dependent on cysteine at position 67 in the peptide binding groove

To investigate whether the B27 free H chain disulfide-bonded homodimers were recognized, the ability of the CD4+ T cell to proliferate in response to C1R transfected with B27 containing a substitution of cysteine at position 67 to serine (C1R-C67S-B27) was tested. Although the chosen C67S transfectant expressed B27 at a lower level than C1R-B27, B27 expression was equivalent to levels on 220-B27 and was higher than levels found on T2-B27 (Fig. 7a). The inability of the CD4+ T cells to proliferate in response to C1R-C67S-B27 (Fig. 7a) implies that the form of B27 recognized is dependent on cysteine at position 67.

Discussion

Evidence from B27-transgenic animal models suggests the involvement of CD4+ T lymphocytes in inducing inflammatory disease (7, 22). We have demonstrated that human CD4+ T cells are

![Figure 5](image5.png)  
**FIGURE 5.** CD4+ T cells do not respond to other transfected cell lines or B27+ EBV LCLs. Proliferation of the CD4+ T cell line to a panel of B27+ and B27- stimulator cell lines, 220-B8, and EBV LCLs obtained from B27+ patients (BH, MM, SF), a B27- control (AC) and a B27+ healthy control (LB). Data shown are the means of triplicate wells ± SDs. These data are representative of at least three similar experiments.

![Figure 6](image6.png)  
**FIGURE 6.** The form of B27 recognized on T2-B27 is stabilized by incubation at 26°C. Effect of temperature on B27 expression, detected by ME1 (a) and MHC class I expression, detected by w6/32 (b), on T2 and T2-B27: T2 at 37°C (gray dotted line), T2-B27 at 37°C (black dotted line), T2 at 26°C (gray solid line), and T2-B27 at 37°C (black solid line). Lines representing staining on T2 at 37°C and 26°C overlap each other for both ME1 and w6/32. c, Proliferation of the CD4+ T cell line to T2 and T2-B27 incubated at 37°C or 26°C. T2 and T2-B27 were incubated at 26°C or 37°C for 24 h before addition of T cells. Data shown are the means of triplicate wells ± SDs. These data are representative of at least three similar experiments.

![Figure 7](image7.png)  
**FIGURE 7.** The form of B27 recognized by the CD4+ T cells is dependent on cysteine at position 67 in the peptide binding groove. a, B27 expression, as determined by ME1 staining on C1R (black dotted line), C1R-B27 (filled histogram), C1R-C67S (black solid line), 220-B27 (dashed line), T2 (gray dotted line), and T2-B27 (gray solid line). b, Proliferation of the CD4+ T cell line to B27+ and B27- stimulator cell lines a panel of stimulator cell lines and C1R-C67S-B27. Data shown are the mean of duplicate wells ± SDs. These data are representative of at least three similar experiments.
capable of recognizing B27 expressed by a panel of B27-transfected cell lines with mutations in the MHC region. Such cell lines have been isolated from three AS patients. As can be seen from comparing results of different experiments, the absolute level of proliferation (cpm) achieved in response to B27 transfectants varied substantially. It was consistently found that proliferative responses declined with time since in vitro restimulation with T2-B27, and could be restored by further rounds of restimulation. It may be speculated that interactions between TCR and B27 expressed by T cells in the absence of appropriate costimulation gradually anergize the B27-reactive T cells, necessitating restimulation.

Although it is well described that CD4+ T cells recognize peptide presented by MHC class II molecules, we have provided clear evidence that B27 is the molecule recognized by the CD4+ T cells. This is, to our knowledge, the first time that B27-reactive CD4+ T cells have been identified. In agreement to the identification of this unusual interaction between an MHC class I molecule and CD4+ T cells, several studies have previously isolated HLA-A2-restricted CD4+ T cells from humans (23, 24). Ab inhibition with w6/32 confirmed that these CD4+ T cells were MHC class I-restricted. In one case the CD4 coreceptor appeared to play a role in this interaction (23). MHC class I-restricted CD4+ T cells have also been identified in mice; one subset uses an invariant TCR and recognizes a glycolipid presented by CD1d, but others appear to be CD1 independent, including cells capable of inducing gut inflammation, a feature of some B27-associated spondyloarthropathies (25, 26).

There are a number of forms of B27 that can be expressed on the cell surface of the stimulator cells which we recognized that could interact with the CD4+ T cells; these include B27 heterodimers or free B27 H chains expressed as monomer or homodimer forms. B27 heterodimers, which are detected by the mAbs ME1 and w6/32, are expressed on all three cell lines. The pattern of responses seen could be explained if the B27 heterodimers from transfected T2, 220, and C1R cell lines contain a peptide that is absent from B27 EBV LCL or U937-B27. Alternatively, B27 heterodimers devoid of peptide, expressed on all the stimulatory APC lines (9, 10, 21, 27), may be recognized by the CD4+ T cells. These “empty” B27 molecules are also reactive with w6/32 and ME1, consistent with the strong inhibition of proliferative responses shown by these Abs. Incubation of T2 cell lines at 26°C has been shown previously to stabilize B27 molecules that do not contain an antigenic peptide (28–30). Thus, increased responses by the CD4+ T cell clone to T2-B27 incubated at this temperature may indicate that this form of B27 is being recognized.

Free B27 H chains may also be a potential target of the CD4+ T cells because they are expressed in TAP- and tapasin-negative cell lines. Because ME-1 Abs do not recognize free H chain homodimers of B27 (when prepared as a recombinant protein and tested for Ab binding by ELISA) (11), the inhibition of the response is not likely to occur by direct blocking of the CD4+ T cell-B27 homodimer interaction. However, studies have shown that free H chain forms of B27 are generated from w6/32-reactive (i.e., heterodimeric) B27 molecules on the cell surface (31). Therefore, an alternative explanation for the ability of ME1 to block the CD4+ T cell proliferative responses may be that B27 heterodimers are stabilized by the presence of ME1 so that the dissociation of the B27 heterodimer to free H chain forms of B27 is inhibited. The possibility that the homodimeric form of B27 is recognized by the CD4+ T cell clone is suggested by the lack of responses to C1R transfected with B27 in which amino acid 67 has been mutated from cysteine to serine. This substitution in the B pocket allows the normal formation of HLA-B27 heterodimers (32) and is reported not to effect its ability to be recognized by some peptide-specific CD8+ CTLs (11). However, it should also be considered that subtle changes to the peptide binding groove caused by this mutation may result in loss of the epitope recognized by these B27-reactive CD4+ T cells.

In any case, we do not propose that only one form of B27 can be recognized by CD4+ T cells because we have isolated CD4+ T cell lines from both B27+ AS and B27+ healthy individuals that exhibit different patterns of specificity for B27-transfected cell lines. For example, we have isolated CD4+ T cell lines from B27+ AS patients that recognize T2-B27 but no other B27-transfected cell line (L. H. Boyle, manuscript in preparation).

The CD4 coreceptor appears to play a role in the response of these CD4+ T cells in a manner similar to that shown for class II-restricted CD4+ T cells. We suggest that CD4 might interact with some form of B27, possibly the homodimeric form, or that CD4-associated lck may be prevented by mAb binding from associating with the TCR (33).

Although we have provided strong evidence that CD4+ T cells are capable of interacting with B27, this situation is somewhat artificial involving cell lines defective in Ag-processing pathways or containing extensive deletions in the MHC. However, in vivo it is possible that normal APCs acquire properties similar to these mutant cell lines. This has already been shown to occur following infection with CMV (34). In view of the association between spondyloarthropathy and bacterial infection it is worth considering the possibility that intracellular bacteria can subvert MHC expression in the same way as viruses, leading to expression of altered forms of B27. Indeed, normal intestinal flora are essential in the induction of arthritis in B27 transgenic rodents (3, 35).

It cannot be determined at this stage whether the B27-specific CD4+ T cells we have described have any pathogenic role in humans. In support of the role of MHC class I-restricted CD4+ T cells in pathogenesis, apparently MHC class I-restricted CD4+ T cells derived from MHC class II-deficient mice can induce aggressive inflammatory bowel disease in congenic, immunodeficient hosts (26). Future studies will determine the frequency of these B27-reactive CD4+ cells in healthy individuals and in patients with spondyloarthropathy. It may also be worthwhile to determine whether B27-reactive CD4+ T cells can be isolated from B27 transgenic rodents, and whether these interact with novel forms of B27 such as free H chains, as suggested by the occurrence of disease in B27, β2m−/− mice. Further investigations will determine whether this interaction is similar to the TCR-MHC class II interaction of conventional class II-restricted CD4+ T cells.

Acknowledgments

We thank P. Beverley for a variety of mAbs; D. Obeso and J.A. Lopez de Castro for ME1; J. Issacs for Campath-9H; S. Powis for the B27 C67S plasmid; and R. Allen and P. Lehner for cell lines, Abs, and useful discussions.

References


