Brief Definitive Report

Spontaneous Inflammatory Arthritis in HLA-B27 Transgenic Mice Lacking β_2-Microglobulin: A Model of Human Spondyloarthropathies

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Summary

Human class I major histocompatibility complex allele HLA-B27 is associated with a group of human diseases called "spondyloarthropathies." Studies on transgenic rats expressing HLA-B27 and human β_2-microglobulin have confirmed the role of HLA-B27 in disease pathogenesis. Here we report spontaneous inflammatory arthritis in HLA-B27 transgenic mice lacking β_2-microglobulin ($B27^+\beta_2m^{-/-}$). In the absence of β_2-microglobulin, $B27^+\beta_2m^{-/-}$ animals do not express the HLA-B27 transgene on the cell surface and have a very low level of $CD8^+$ T cells. Most of the $B27^+\beta_2m^{-/-}$ male mice showed nail changes, hair loss, and swelling in paws, which leads to ankylosis. The symptoms occur only after the $B27^+\beta_2m^{-/-}$ mice are transferred from the specific pathogen-free mouse colony. These results suggest that aberrant assembly, transport, and expression of the HLA-B27 molecule may predispose an individual for development of the disease when exposed to an appropriate environmental trigger.

One of the strongest linkages known to date between the presence of an HLA allele and disease susceptibility is that of HLA-B27 to inflammatory spondyloarthropathies in humans (1). Although it is clear that the presence of the HLA-B27 allele predisposes humans to the disease, its role in disease pathogenesis is unclear. Studies on genetic polymorphism suggest that B*2705 and B*2702 alleles predispose humans for disease development, while B*2703 is not associated (2). Recent knowledge about the three-dimensional structure of the HLA-B27 molecule and peptide elution studies suggest that peptides approximately nine amino acids long with arginine at position 2 bind to HLA-B27 (3, 4). Several hypotheses for the association of HLA-B27 with the human disease have been proposed, including (*a*) presentation of arthritogenic peptide by this MHC class I molecule, (*b*) molecular mimicry of HLA-B27 with certain microorganisms, (*c*) altered self, and (*d*) the possible role of closely linked genes (5).

Transgenic rats expressing several copies of the HLA-B27 and the human β_2-microglobulin genes develop a spontaneous inflammatory disease that has many similarities to human spondyloarthropathy (6). We have previously demonstrated expression of the HLA-B27 gene in transgenic mice (7). Until now, no arthritis, either spontaneous or induced, has been observed in these transgenic mice. To determine whether processing, assembly, transport, and expression of the HLA-B27 molecule may play a role in the disease process, we introduced the HLA-B27 transgene into the β_2-microglobulin–deficient mice. Spontaneous arthritis was observed in HLA-B27 transgenic mice lacking β_2-microglobulin ($B27^+\beta_2m^{-/-}$) compared with $B27^+\beta_2m^{+/-}$ full sibs. An environmental trigger for the development of disease is involved, since mice housed in the specific pathogen-free barrier colony are unaffected. Similar to HLA-B27–related human spondyloarthropathies, male animals were primarily affected. Our results suggest that aberrant assembly and/or expression of HLA-B27 may perturb the T cell repertoire and cause disease development.

Materials and Methods

Mice. The HLA-B27 (B*2705) transgenic mice used in this study have been described elsewhere (7). Mutated $\beta_2m^{-/-}$ mice were the kind gift of Dr. B. Koller (8). HLA-B27–positive mice from ($\beta_2m^{-/-}$ × HLA-B27)F_1 crosses were intercrossed to obtain $B27^+\beta_2m^{-/-}$ animals. The presence of the HLA-B27 transgene in the F_2 population was identified by PCR using 3′ (CTC TGC CTT GGC CTT GCA GA) and 5′ (CCA CTC CAT GAG GTA TTT CCA) oligonucleotide sequences. The homozygous mutation in β_2-microglobulin gene was identified as previously described by Koller et al. (8). The $B27^+\beta_2m^{+/-}$ full sibs, $B27^-\beta_2m^{+/-}$ full sibs, and $B27^-\beta_2m^{-/-}$ full sibs were used as controls. All the mice in our study were bred in the specific pathogen-free barrier facility.

Flow Cytometric Analysis. Analysis of expression of the HLA-B27 transgene was performed by flow cytometry. Isolated mononuclear cells from peripheral blood were incubated with either purified or biotinylated ME-1 (American Type Culture Collection, Rockville, MD), HC10 (heavy chain specific; 9) or Ye-2

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(MHC plus peptide; 10) or B27M1 (cross-reactive with bacterial proteins; American Type Culture Collection) mAbs for 30 min. After washing with BSA (1%) and sodium azide (0.1%) containing PBS, cells were incubated with fluorescein-labeled secondary antibody (IgG goat anti-mouse Fab'_2; Accurate Chemical & Scientific Corp., Westbury, NY) or Streptividin-R-phycoerythrin (Tago, Inc., Burlingame, CA). Cell-surface expression was analyzed on 10,000 gated lymphocytes on forward and side scatter by flow cytometry.

Cytotoxicity Assay. Target Con A blast of spleen cells were labeled with 150 μCi ^{51}Cr (Amersham Corp., Arlington Heights, IL) for 90 min and placed at 1×10^4 per well in 96-well U-bottomed plates (Corning Inc., Corning, NY) in triplicates. Effector anti-B27 CTLs used in this study were the kind gift of Dr. Kaufman and Dr. Liebson (Mayo Clinic, Rochester, MN). These CTLs were generated from PBLs of a normal healthy individual by stimulating them with irradiated C1RB27 cells. Anti-CD14 mAb and rabbit complement were used to lyse possible NK cells. Effector cells (10^5) (10:1 E/T ratio) were added and incubated at 37°C for 4 h. The supernatant was harvested and counted in a gamma counter (LKB Instruments Inc., Gaithersburg, MD). The percent specific ^{51}Cr release was calculated as follows: % Cytolysis = (Sample − Spontaneous)/(Maximum − Spontaneous) × 100.

Quantification of Arthritis. The severity of arthritis was measured three times a week for a period of 6 mo as previously described (11). Briefly, the severity of arthritis was measured on a scale of 0–3 for each paw (0, no arthritis; 1, mild inflammation in one or two fingers; 2, moderate inflammation in three or more fingers; and 3, ankylosis). Thus, the score for a single animal could vary from 0–12 depending on the severity of arthritis.

Results and Discussion

Generation of $B27^+\beta_2m^{-/-}$ Mice. As described in Materials and Methods, $B27^+\beta_2m^{-/-}$ animals were identified from the ($\beta_2m^{-/-}$ × HLA-B27)F_2 population and intercrossed to produce the line (Fig. 1, *A* and *B*). The presence of the HLA-B27 transgene in the $\beta_2m^{-/-}$ background was confirmed by PCR. Cell-surface expression of the transgene product either on splenocytes or on PBLs was not detected by using several HLA-B27–specific antibodies (Fig. 2). Metabolic labeling of the spleen cells by ^{35}S and immunoprecipitation showed monomers of heavy chains (HCs)

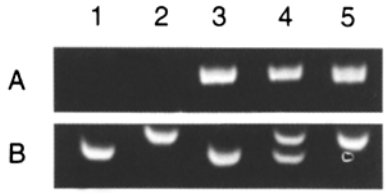

Figure 1. Generation of $B27^+\beta_2m^{-/-}$ mice. Transgenic HLA-B27 mice were mated with $\beta_2m^{-/-}$ mice, and B27 positive offspring were intercrossed to produce $B27^+\beta_2m^{-/-}$ mice. In this figure, *A* and *B* gels show PCR analysis for the identification of HLA-B27 transgene and homozygous mutant β_2-microglobulin, respectively (6, 8). Lane *1*, nontransgenic control B10 mice show normal wild-type β_2m and no B27; lane *2*, mutated β_2m from $\beta_2m^{-/-}$ mice; lane *3*, HLA-B27 transgenic mice show presence of B27 gene and wild-type β_2m; lane *4*, $B27^+\beta_2m^{+/-}$ offspring from (HLA-B27 × $\beta_2m^{-/-}$)F_1 cross show the B27 gene with normal and mutated β_2m; lane *5*, $B27^+\beta_2m^{-/-}$ animals from the F_2 population show the B27 gene and mutated β_2m.

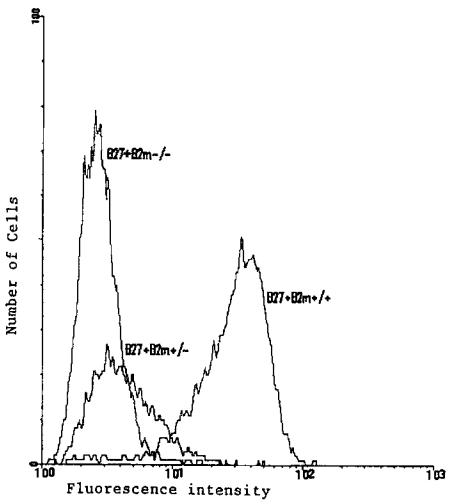

Figure 2. HLA-B27 expression on PBL in $\beta_2m^{+/+,+/-}$ and $^{-/-}$ mice by using ME-1 mAb by fluorocytometry. Cells from $B27^+\beta_2m^{-/-}$ mice show expression similar to nontransgenic B10 controls (overlapped on $B27^+\beta_2m^{-/-}$). Cells from $B27^+\beta_2m^{+/-}$ animals show intermediate expression of HLA-B27. Cell-surface expression was also undetectable by using HC-10, Ye-2, and B27M1 mAb on PBLs from $B27^+\beta_2m^{-/-}$ animals (data not shown).

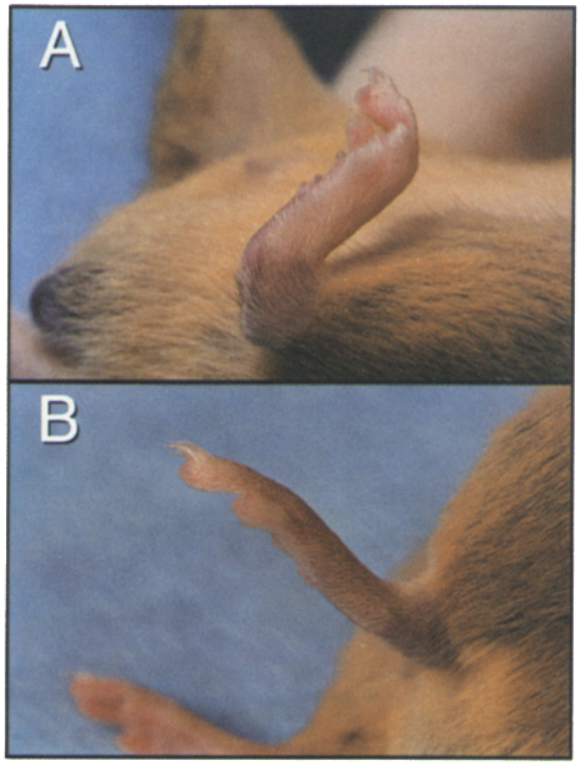

Figure 3. Lateral views of the peripheral joint of arthritic $B27^+\beta_2m^{-/-}$ (*A*) and nonarthritic B27-$\beta_2m^{-/-}$ (*B*) full sibs. Mild arthritis with swelling in one toe of the left hind paw in $B27^+\beta_2m^{-/-}$ mice started 3 wk after transferring from a barrier facility to a conventional colony. 17 d after the first symptom of arthritis, joint ankylosis was noticed. At this time, the right hind paw was also affected with arthritis. Other male $B27^+\beta_2m^{-/-}$ mice from the same cage also exhibited similar symptoms.

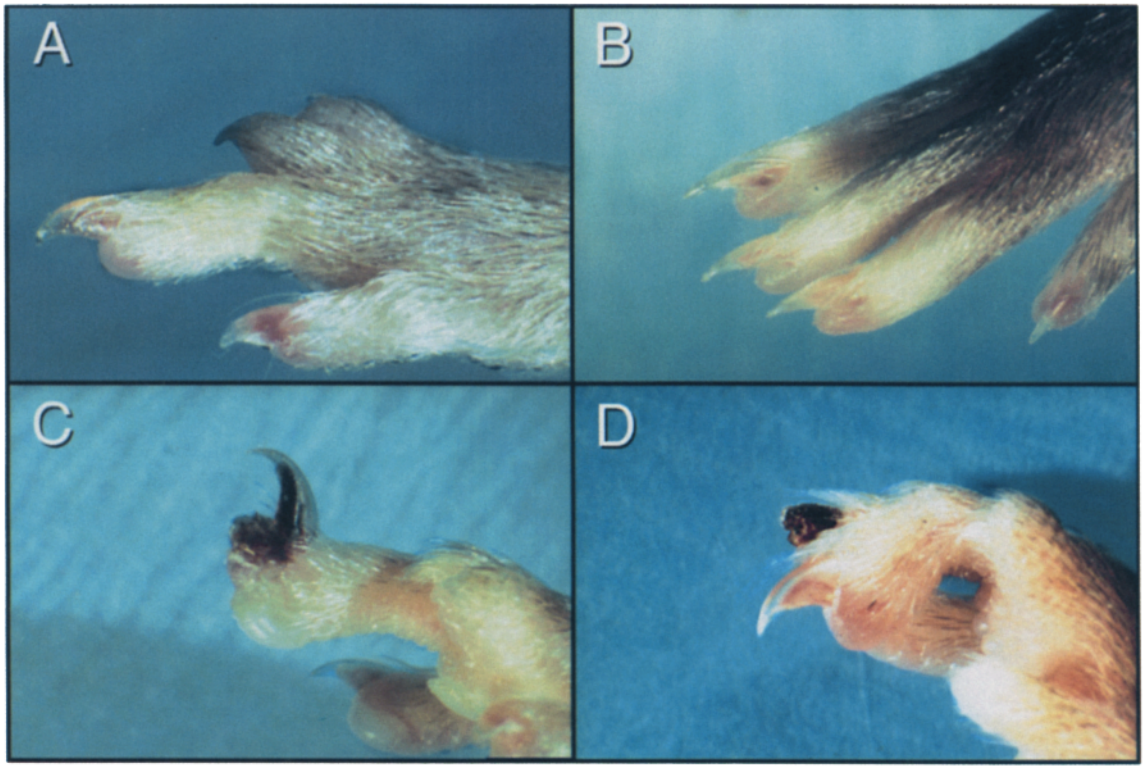

Figure 4. Nail changes in a $B27^+\beta_2m^{-/-}$ animal. Normal nails (*A*) and digits (*B*) compared with nails of $B27^+\beta_2m^{-/-}$ male mice showing vascular dilatation (*C* and *D*) and hyperkeratosis. 10 d after transferring from the barrier facility to a conventional colony, a vascular dilatation and darkness in a nail of the rear paw was noticed. Within 5–7 d, this darkness in the nail changed to hyperkeratosis, and ultimately the nail fell off.

of HLA-B27 (data not shown). The copy number of the HLA-B27 gene was found to be between 4 and 8. Similar to β_2m-deficient mice (8), $B27^+\beta_2m^{-/-}$ mice have a negligible number of $CD8^+$ T cells but a normal repertoire of $CD4^+$ T cells.

Spontaneous Inflammatory Disease in $B27^+\beta_2m^{-/-}$ Mice. The mice were healthy and normal as long as they stayed in our pathogen-free colony. Within 2–4 wk after transfer to a conventional mouse colony, spontaneous development of nail and joint changes was observed in $B27^+\beta_2m^{-/-}$ mice but not in $B27^+$ ($\beta_2m^{+/-, +/+}$) or $B27^-$ ($\beta_2m^{+/-, -/-}$) littermates. Clinical arthritis began with redness and swelling in a single toe of the rear paw. This progressed to involve both hind paws with swelling, deformity, and ankylosis (Fig. 3). Nail changes were observed primarily in male mice at the onset of clinical arthritis (Fig. 4). 33 of 44 (75%) male mice, 4 mo or older, developed spontaneous arthritis in comparison to 7 of the 23 (30%) female mice (Table 1). Female mice developed milder arthritis with a delayed onset. About 40% of arthritic male animals showed ankylosis in at least one of their rear paws. The other mice developed a milder form of arthritis involving swelling in one or both rear paws. Front paws were unaffected in most of the diseased animals. These findings are similar to HLA-B27–related arthropathies in humans (12). Histological changes in arthritic mice were characterized by synovial cell proliferation, cartilage and subchondral bone erosions, and mononuclear cell proliferation (Fig. 5). Preliminary data show no occurrence of such symptoms in $HLA\text{-}Cw3^+\beta_2m^{-/-}$ mice. Cultures for tested microorganisms inside and outside the barrier facility showed no difference (data not shown). Preliminary analysis for the presence of arthritis causing *Mycoplasma* strains such as *Mycoplasma arthritidis* and *M. pulmonis* were negative (courtesy of Dr. B. Cole, University of Utah, Salt Lake City, UT).

Table 1. *Spontaneous Arthritis in $B27^+$ $\beta_2m^{-/-}$ Mice*

Mice	Sex	No. of Mice	No. of arthritic mice	Mean arthritis severity	Nail changes
$\beta_2m^{-/-}$	M	38	–	–	–
	F	29	–	–	–
$B27^+$ $\beta_2m^{+/+}$	M	43	–	–	–
	F	34	–	–	–
$B27^+\beta_2m^{-/-}$	M	44	33 (75%)	3.0 ± 1.7	22
	F	23	7 (30%)	1.6 ± 0.5	2
$B27^-\beta_2m^{-/-}$	M	19	1(?)	–	–
littermates	F	13	–	–	–

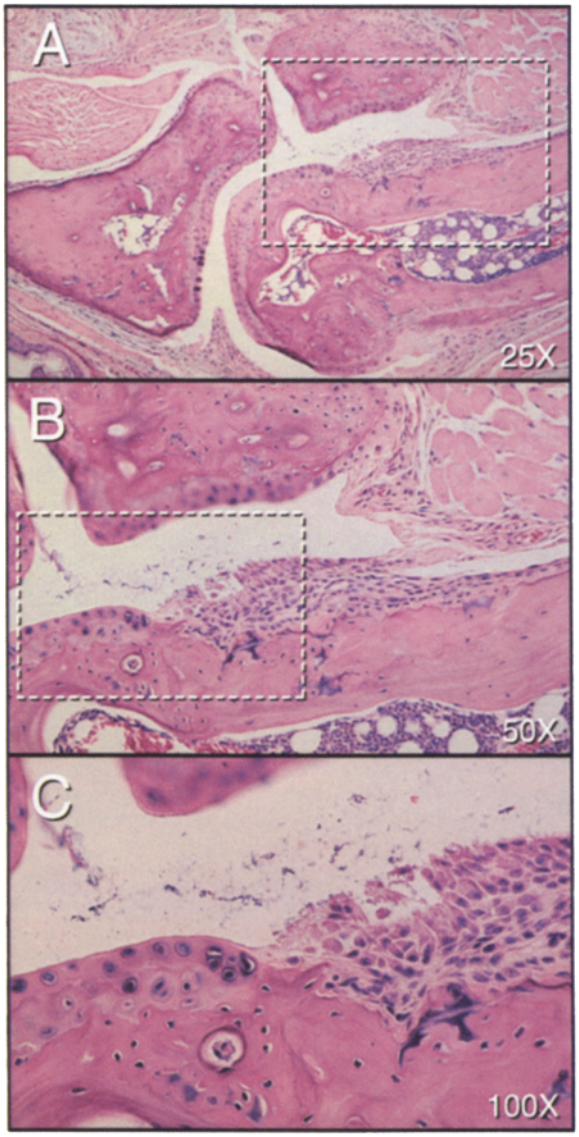

Figure 5. Histology of the arthritic hind paw of the B27$^+\beta_2m^{-/-}$ mice 3 wk after transferring from the pathogen-free barrier facility to a conventional colony. Cellular proliferation in the synovium with exudation into the joint cavity are shown. The presence of pannus eroding the cartilage and subchondral bone is highlighted. Hematoxylin and eosin staining, ×25 (*A*), ×50 (*B*), and ×100 (*C*).

Potential Mechanism for the Development of Disease. The occurrence of spontaneous arthritis only in the B27$^+\beta_2m^{-/-}$ mice suggests several potential mechanisms in the disease process. With few exceptions (13), the HC of MHC class I molecules binds to β_2m and peptides in the endoplasmic reticulum, and the trimeric complexes are transported to the cell surface (14). β_2m-deficient mice do not express class I molecules on the cell surface and have a negligible number of CD8$^+$ T cells because of a lack of positive selection (8). Recently, autoreactivity of such small numbers of CD8$^+$ T cells from β_2m-deficient mice has been demonstrated against self-MHC (15). Similarly, a low number of CD8$^+$ T cells seen in B27$^+\beta_2m^{-/-}$ mice could be the result of an escape from negative selection and may be autoreactive to the HLA-B27 molecule. Expansion of CD8$^+$ T cells was not observed in the peripheral blood of arthritic mice.

Aberrant processing of B27 HCs could also lead to development of spontaneous arthritis in B27$^+\beta_2m^{-/-}$ mice or in rats with a high copy number of B27 transgenes (16). In the absence of β_2m or overexpression of the B27 gene, the class I molecules may be retained in the endoplasmic reticulum or cytoplasm. Continuous generation of such protein in the cytoplasm would lead to abnormal accumulation. The unassembled HC of HLA-B27 may present extracellu-

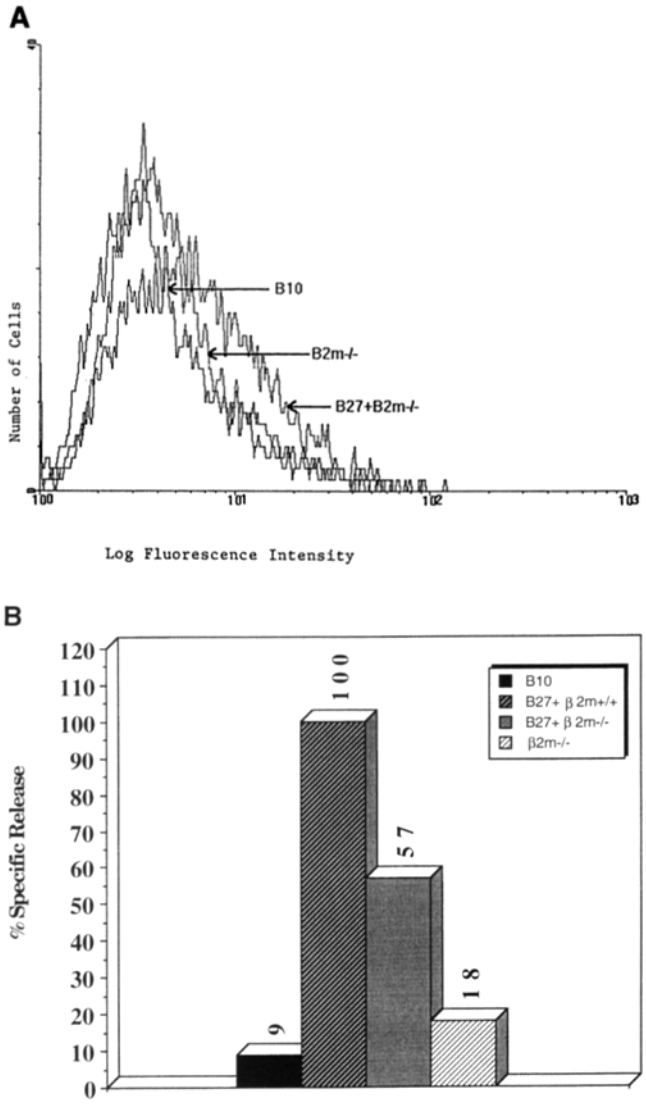

Figure 6. Cell-surface expression of HLA-B27 HC on PBLs after 48 h of stimulation with Con A. (*A*) A shift of 50–70 fluorescence channels was noticed on B27$^+\beta_2m^{-/-}$ PBLs in comparison with nontransgenic B10 or B27-$\beta_2m^{-/-}$ controls by using an HC-specific HC-10 antibody. Other anti–HLA-B27 mAbs did not show such increases of cell-surface expression. The presence of HLA-B27 on the cell surface was also confirmed by using anti-B27 CTL in a cytotoxicity assay. (*B*) Relative cytotoxicity of anti-B27 CTLs against B10, B27$^+\beta_2m^{+/+}$, B27$^+\beta_2m^{-/-}$, and $\beta_2m^{-/-}$ targets at 10:1 E/T ratio.

lar peptides (17) or undergo normal protein degradation. Degraded products of HLA-B27 proteins may behave as an autoantigen and could be presented by the class II molecule to a $CD4^+$ T cell. Exposure of these mice to environmental antigens with molecular mimicry could break self tolerance and may be the cause of disease development (18).

Even though normal expression of B27 is observed in patients, a defect in expression could have occurred during ontogeny or early childhood, causing positive selection of self-reactive T cells. Mutation in β_2m (19) and polymorphism in peptide transporter (20, 21) or proteosome (22) genes could also result in selective peptide binding, inefficient processing and reduced transport of B27 molecules, and perturbation of T cell repertoire. Thus, abnormal processing, transport, and expression of HLA-B27 could predispose certain $B27^+$ individuals to spondyloarthropathies.

To determine whether the HLA-B27 molecule may reach the cell surface in $B27^+\beta_2m^{-/-}$ mice, splenocytes were stimulated in vitro, and expression of free HCs of B27 was determined. Low-level expression of the HC of HLA-B27 was detected on the cell surface of Con A–stimulated splenocytes (Fig. 6 *A*). The presence of HLA-B27 HCs on Con A–stimulated splenocytes was further confirmed when >50% lysis of $B27^+\beta_2m^{-/-}$ targets was seen with anti–HLA-B27 CTLs in a ^{51}Cr release assay (Fig. 6 *B*). The presence of HCs on the cell surface after stimulation suggests several scenarios: (*a*) Environmental antigens in the conventional colony may stimulate cells to express free HCs on the cell surface, (*b*) the presentation of extracellular peptide to residual $CD8^+$ T cells, and (*c*) expansion of self-reactive T cells that could be autoreactive to self-MHC as shown in β_2m-deficient mice (15). Studies are currently underway to determine the mechanism involved in the progression of disease in these mice.

In conclusion, our results clearly demonstrate a specific role for the B27 molecule in disease. It is obvious, though, that additional genetic factors and environmental triggers are required for the onset of autoimmunity. The occurrence of the disease in $B27^+\beta_2m^{-/-}$ mice suggests a possible role for aberrant expression of HLA-B27 in predisposing the mice to self reactivity. This mouse model opens several new avenues to further dissect the role of HLA-B27 in the disease process, since proteosome and transporter knockout animals are now available. Specificity of HLA-B27 association with this disease can be further tested by using other unrelated HLA class I transgenic mice (HLA-A3, HLA-B7) in the absence of β_2m. We can also generate new transgenic mice with a non–disease-associated B*2703 or mutated B27 genes. In addition, a systematic analysis of the environmental agents involved in the disease trigger could pave the way for identification of the exogenous antigen and generation of vaccines and immunotherapeutic agents. We believe that rapid progress can be made in the understanding of the disease mechanism in HLA-B27–linked spondyloarthropathies using this mouse model.

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