The Journal *of*
Immunology

Spontaneous Inflammatory Disease in HLA-B27 Transgenic Mice Is Independent of MHC Class II Molecules: A Direct Role for B27 Heavy Chains and Not B27-Derived Peptides

Sanjay D. Khare, Michael J. Bull, Julie Hanson, Harvinder S. Luthra and Chella S. David

---

References This article **cites 36 articles**, 13 of which you can access for free at:
http://www.jimmunol.org/content/160/1/101.full#ref-list-1

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days*** from submission to initial decision

- **No Triage!** Every submission reviewed by practicing scientists

- **Fast Publication!** 4 weeks from acceptance to publication

*average

Subscription Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

Spontaneous Inflammatory Disease in HLA-B27 Transgenic Mice Is Independent of MHC Class II Molecules: A Direct Role for B27 Heavy Chains and Not B27-Derived Peptides[1]

Sanjay D. Khare,* Michael J. Bull,* Julie Hanson,* Harvinder S. Luthra,[†] and Chella S. David[2]*

Although association of HLA-B27 with human spondyloarthropathies has been known for several years, its role in disease pathogenesis is not understood. Recently, a few investigators have proposed that presentation of B27-derived peptides by MHC class II molecules may be the underlying mechanism. HLA-B27 transgenic rat and mouse models have provided a new tool for understanding the exact role of B27 in disease pathogenesis. HLA-B27 mice lacking endogenous β_2-microglobulin (B27$^+\beta_2$m^o) develop disease after they are transferred from the barrier facility to the conventional colony. This model was utilized to test the hypothesis that B27-derived peptide presented by MHC class II molecules is the cause of the disease. The MHC class II knockout gene, Aβ^o, was bred into our B27$^+\beta_2$m^o mice, and disease manifestation was monitored. These mice develop spontaneous disease, demonstrating that MHC class II molecules do not play a major role in B27-related disease. Thus, the disease is not manifested by presentation of B27-derived peptides by class II molecules, since these mice are devoid of H2-A and H2-E molecules. Furthermore, in vivo treatment with mAb against the heavy chain of B27 reduced the incidence of disease in B27$^+\beta_2$m^o mice. Our results clearly demonstrate that B27 heavy chains are directly involved in the disease process. *The Journal of Immunology,* **1998, 160: 101–106.**

The MHC class I gene, HLA-B27, is strongly implicated in a group of human diseases called spondyloarthropathies (1, 2). Unlike other autoimmune arthritidis, males are more affected than females with spondyloarthropathies (2). We and others have shown spontaneous disease symptoms in experimental animals with human HLA-B27 transgenes (3–5). In addition to HLA-B27, the role of unknown environmental Ags has also been implicated in these transgenic HLA-B27 animals (4–6). How HLA-B27 interacts with an environmental Ag to trigger the disease is not yet clear. In a subgroup of B27-linked diseases, such as reactive arthritis, the disease is seen after an enterobacterial infection of the small or large intestine or genitourinary tract (7).

Several hypotheses have been proposed to explain the role of enterobacteria in HLA-B27-associated diseases including: 1) molecular mimicry of HLA-B27 with enterobacteria, 2) presentation of arthritogenic peptide from environmental Ag by HLA-B27 to CD8$^+$ T cells, and 3) presentation of exogenous Ag by empty HLA-B27 (8, 9). More recently, a few investigators have proposed a role for B27-derived peptides in the disease. Sequence analysis of hypervariable regions of HLA-B27 with disease-implicated enterobacteria with B27-binding motif showed that a number of peptides fulfill this criteria and could be important in the initiation of autoimmune response (10). Binding of such peptides with HLA-

B27 has been demonstrated in an in vitro assembly assay (11). On the other hand, HLA-B27-derived peptides may also be presented by MHC class II molecules (12, 13).

The role of CD8$^+$ T cells vs CD4$^+$ T cells in B27-linked diseases has been a controversial issue. Even though the HLA-B27 molecule as a class I Ag would be expected to present Ags to CD8$^+$ T cells, most of the reports describe CD4$^+$ T cells in the diseased joint or an MHC class II-restricted immune response against bacteria implicated in spondyloarthropathies (14–16). Only a few reports describe isolation of bacteria-specific B27-restricted CD8$^+$ T cells from the involved joints of patients with B27-associated reactive arthritis and ankylosing spondylitis (17, 18). This further suggests the involvement of a CD4$^+$ T cell recognizing a B27-derived peptide presented by class II molecules (13, 19).

We have reported an animal model of B27-associated arthropathy in which β_2m^3-deficient HLA-B27 mice develop disease after they are transferred from a barrier facility to the conventional colony (4). These mice have low cell surface expression of B27 heavy chains (HC) and normal expression of endogenous class II molecules. In this mouse model of human disease, two possibilities may exist for the role of B27 in the disease process: 1) presentation of exogenous environmental peptide by β_2m-free HLA-B27 HC; 2) presentation of B27-derived peptides by endogenous MHC class II molecules. If the presentation of B27-derived self peptide by mouse H2-A molecule is related to disease development, MHC class II-deficient B27$^+\beta_2$m^{o}Aβ^o mice should not develop spontaneous disease. On the contrary, B27$^+\beta_2$m^{o}Aβ^o mice developed spontaneous disease similar to the previously described disease in B27$^+\beta_2$m^o mice, suggesting that class II molecules are not required for disease development. Furthermore, in vivo treatment

Departments of *Immunology and [†]Rheumatology, Mayo Clinic and Medical School, Rochester, MN 55905

[2] Address correspondence and reprint requests to Dr. Chella S. David, Department of Immunology, Mayo Clinic and Medical School, Rochester, MN 55905. E-mail address: david.chella@mayo.edu

[3] Abbreviations used in this paper: β_2m, β_2-microglobulin; HC, heavy chain; HV3, third hypervariable region.

with HC-specific HC10 Ab decreased the incidence of spontaneous disease in B27$^+$$\beta_2$m^o mice. Thus, blocking of B27 HC may prevent disease in experimental animals. Our data suggest a direct involvement of HLA-B27 HC as an Ag-presenting molecule, and not as an autoantigen in B27-associated disease in transgenic mice. Likewise, B27-linked human spondyloarthropathies may also be the result of direct function by B27 HC.

Materials and Methods
Animals

Generation of B27$^+$$\beta_2$m^o mice has been described before (4). β_2m^{o}Aβ^o mice and B27$^+$Aβ^o mice were produced by mating β_2m^o mice and B27$^+$ mice with Aβ^o mice, respectively (20) (kindly provided by Drs. C. Benoist and D. Mathis, INSERM, Strasbaurg, France). To generate MHC class II-deficient B27$^+$$\beta_2$m^o mice (B27$^+$$\beta_2$m^{o}A$\beta^o$), β_2m^{o}Aβ^o mice were mated with B27$^+$Aβ^o mice, and in the F$_2$ population, B27$^+$$\beta_2$m^{o}A$\beta^o$ mice were identified and intercrossed to generate the B27$^+$ and B27$^-$ mice in the context of β_2m^o and Aβ^o mutant genes. A flow chart describing the generation of B27$^+$$\beta_2$m^{o}A$\beta^o$ mice is shown in Figure 2. All breedings were conducted in the barrier facility of the Immunogenetics mouse colony of Mayo Clinic (Rochester, MN).

Screening of mice

In the absence of β_2m, MHC class I molecules are rarely expressed on cell surface. Therefore, the presence of MHC class I transgenes in β_2m^o mice was analyzed by PCR. DNA was extracted from the peripheral blood according to manufacturer's instructions using the Isoquick nucleic acid extraction kit. Four microliters of DNA were added to 0.2 mM dNTPs, 1 μM each 3′ and 5′ primers in the PCR buffer in a total volume of 25 μl. A quantity amounting to 0.625 U of *Taq* polymerase was added to this mixture and amplified in 30 cycles under the following conditions: 3 min at 94°C (94°C for 1 min, annealing temperature 62°C for 1 min, and 72°C for 1 min), × 30, and 7 min at 72°C. PCR products were analyzed by electrophoresis, and their m.w. was compared with a standard m.w. marker.

Presence of HLA-B27 transgene was identified by PCR using the following oligonucleotides: 3′-(CTC TGC CTT GGC CTT GCA GA) and 5′-(CCA CTC CAT GAG GTA TTT CCA).

The presence of homozygous mutation in mouse β_2m was identified by PCR (5). We used three primers to identify the presence of wild-type and mutated mouse β_2m gene. Mβ_2m-1 and Mβ_2m-2 oligonucleotide sequences amplify wild-type β_2m. Neomycin and Mβ_2m-1 primer sequences amplify mutated β_2m. Presence of both of the PCR products indicates heterozygocity for the knockout gene. Mβ_2m-l: 3′-(GAA AAC CCC TCA AAT TCA AGT ATA CTC A). Mβ_2m-2: 5′-(GAC GGT CTT GGG CTC GGC CAT ACT). Neomycin: 5′-(TCG AAT TCG CCA ATG ACA AGA CGC T).

The presence of wild-type gene for Aβ^b was analyzed by using 3′-(TGA GTT GTG TCT GCA CAC GG) and 5′-(AGT TCA TGG GCG AGT GCT AC). Presence of neomycin gene for mutated Aβ^b was analyzed by using 3′-(GGA TCG GCC ATT GAA CAA G) and 5′-(CAC CAT GAT ATT CGC CAA GC) oligonucleotide sequences. Presence of both genes indicates the mouse is heterogyzous for the mutated gene.

Immunofluorescence and flow cytometry

Expression of HC of B27 and endogenous H2-Aβ^b molecules on the cell surface was conducted by immunofluorescence using HC10 (21) (kindly provided by Dr. Hidde Plough, MIT, Cambridge, MA) and 3F12 or AF6.120 (HB163) mAbs (American Type Culture Collection, Rockville, MD), respectively. Briefly, mononuclear cells from peripheral blood were incubated with Abs for 30 min at 4°C. After washing with FACS buffer (PBS containing 1% BSA and 0.05% sodium azide), cells were incubated with a fluorescence-labeled secondary Ab (IgG goat anti-mouse F(ab′)$_2$; Accurate Chemical & Scientific Corporation, Westbury, NY). Expression of cell surface molecules was analyzed on 10,000 gated lymphocytes on forward and side scatter by flow cytometry by using CellQuest software.

Peptide synthesis

Three overlapping synthetic peptides covering the third hypervariable region (HV3) of B*2705 molecule were chemically synthesized by solid-phase procedure using Applied Biosystems (Foster City, CA) at Protein Core Facility, Mayo Clinic (Rochester, MN). The amino acid sequences of these overlapping peptides are as follows:B27#1 (57–76): PEYWDRET QICKAKAQTDRE.B27#2 (66–85): ICKAKAQTDREDLRTLLRYY.B27 #3 (77–90): DLRTLLRYYNQSEA.

Sequence integrity and homogeneity of these peptides were confirmed by reverse-phase HPLC.

Lymphoproliferative response

A 2-day-long culture in the presence or absence of specific peptides was conducted to analyze lymphocyte proliferation. Briefly, mice were immunized with 200 μg of peptide emulsified with CFA intradermally at the base of the tail (100 μg) and rear footpads (50 μg each). After 7 to 10 days, draining lymph nodes were removed and a single cell suspension was prepared. RBC were lysed using hypotonic ACK (ammonium chloride and potassium chloride) solution, and after washing, mononuclear cells were resuspended in complete media (RPMI 1640 + 10% heat-inactivated FBS + penicillin and streptomycin). Now, 10^6 cells/well were plated in 96-well flat-bottom tissue culture plate in the presence or absence of indicated amount of peptide (Fig. 1*A*). In some experiments, mAbs (10 μg/ml purified Ab or 20 μl culture supernatant) were added in addition to the peptide to block specific immune response (Fig. 1*B*). On the next day, 1 μCi of [^{3}H]TdR (Amersham, Arlington Heights, IL) was added to each well, and 24 h later, cells were harvested onto glass fiber paper. Scintillation fluid was added, and β emissions were counted and calculated in cpm. The data were analyzed by using the following calculations: Δ cpm (δ cpm) = mean cpm in experimental wells − mean cpm in control wells without peptide.

In vivo treatment with mAb

Mice used in the study were between 9 and 12 wk of age and age/sex matched. Experimental groups of mice were i.v. injected with 1 mg of HC10 (human MHC class I HC specific) (21) or 3F12 mAb before transferring them from the barrier facility to the conventional mouse colony. Control groups of mice received either the same amount of an isotype-matched irrelevant Ab or PBS.

Disease monitoring

As described before, mice were monitored for the development of arthritis, nail disease, and other clinical changes (such as skin inflammation and hair loss) twice per week for a period of 12 wk in the conventional area.

Results
B27$^+$$\beta_2$m^o mice are not tolerant to HLA-B27-derived peptides

B27$^+$$\beta_2$m^o mice have very low level expression of MHC class I molecules and few CD8$^+$ T cells. These mice have intact mouse class II molecule H2-A^b and a normal population of CD4$^+$ T lymphocytes. To investigate whether the H2-A molecule could be presenting HLA-B27-derived peptides to CD4$^+$ T cells to initiate disease in these mice, we first examined the immune response to peptides derived from HV3 of HLA-B27 molecule. As shown in Figure 1*A*, B27$^+$$\beta_2$m^o mice showed vigorous lymphoproliferative response to B27 peptide 57–76 (Δ cpm > 20,000). These mice also responded to B27 peptide 77–90, but to a lesser extent. B27$^+$$\beta_2$m^o mice did not show lymphoproliferative response to B27 peptide 66–85. To determine the specificity of the immune response, we added mAbs against CD4, CD8, and MHC molecules 1 h before addition of B27 peptide 57–76 to the culture. mAbs against the CD4 and H2-A molecules (Fig. 1*B*) completely inhibited the lymphoproliferative response. Addition of anti-CD8, anti-B27, and other anti-mouse MHC class I (H2-K^b and H2-D^b)-specific Abs in lymphocyte cultures with B27 peptide 57–76 did not show such effect on the lymphoproliferative responses (Fig. 1*B*). These data suggest that immune response to B27 peptide 57–76 is mediated by CD4$^+$ T cells and is restricted to the H2-A molecule. These experiments imply that B27$^+$$\beta_2$m^o mice are not tolerized to self B27 unless these peptide responses represent cryptic determinants (22). Since H2-A molecule is capable of presenting at least one of the three B27-derived peptides from HV3 region of the B27 molecule, is it possible that the disease in B27$^+$$\beta_2$m^o mice is due to such presentation of B27-derived peptide by MHC class II molecule. To directly address this question, we bred MHC class II knockout gene (Aβ^o) into B27$^+$$\beta_2$m^o mice.
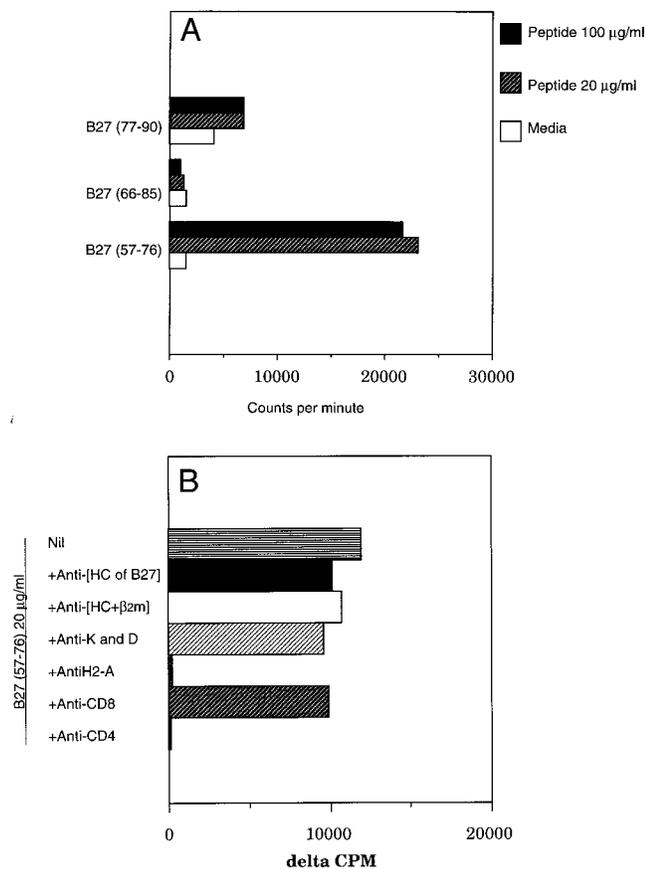
FIGURE 2. Generation of MHC class II-deficient B27$^+\beta_2$m^{o}Aβ^o mice.

FIGURE 1. *A,* Lymphoproliferative response to overlapping peptides (57–76, 66–85, and 77–90) derived from HV3 of HLA-B27 in B27$^+\beta_2$m^o mice. Draining lymph node cells (LNCs) from immunized mice were challenged in vitro with indicated amount of peptides in a 2-day culture. Sixteen hours before harvesting the cells, radioactive [^{3}H]thymidine was added. Results are expressed as mean cpm of the triplicate wells. Representative figure shown here is from one of the four experiments conducted. The SE for the triplicate samples was less than 10% of the mean value. B27$^+\beta_2$m^{o}Aβ^o mice did not respond to B27-derived peptides (data not shown). *B,* H2-A-restricted and CD4$^+$ T cells mediated immune response to B27-derived peptide (57–64). LNCs from B27$^+\beta_2$m^o mice immunized with peptide B27 (57–64) were preincubated with 20 μl of Ab (culture supernatants GK1.5 for CD4; Lyt-2 for CD8 or 10 μg/ml purified Ab 3F12 for H2-A; ME1 for HLA-B27; 28-14-8 for D^b and 34-1-2S for K^b; and HC10 for HC of B27) 1 h before challenge with B27-derived peptide (57–64). The B27-derived peptide (57–64) was used at a concentration of 20 μg/ml. Culture conditions for these experiments were similar to the experiment described in *A.* The SE for the triplicate samples was less than 10% of the mean value.

Generation of B27$^+\beta_2$m^{o}Aβ^o mice and disease susceptibility

To determine any contribution by endogenous class II molecules in the disease, MHC class II-deficient, Aβ^o mice were mated with HLA-B27 transgenic mice, and the F$_1$ population was intercrossed to obtain B27$^+$Aβ^o mice (Fig. 2). B27$^+$Aβ^o mice lack expression of MHC class II molecules (H2-A and H2-E), and thus have a negligible number of CD4$^+$ T cells in the periphery (Fig. 3). Unlike previously described B27$^+\beta_2$m^o mice, B27$^+$Aβ^o mice do not develop spontaneous disease (Table I). The B27$^+$Aβ^o mice were mated with β_2m^{o}Aβ^o mice to obtain B27$^+\beta_2$m^{o}Aβ^o mice. These mice lack normal expression of H2-K^b, H2-D^b, HLA-B27 (data not shown), and H2-A molecules, and therefore have low levels of mature CD4 and CD8$^+$ T cells in the periphery.
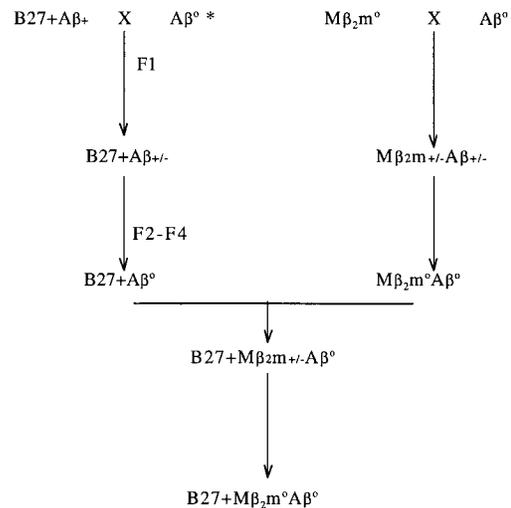
B27$^+\beta_2$m^{o}Aβ^o mice developed spontaneous disease similar to that of B27$^+\beta_2$m^{o}A$\beta^{+/-}$ fullsibs and previously described B27$^+\beta_2$m^o mice (Table I). B27$^+\beta_2$m^{o}Aβ^o mice developed arthritis and nail disease within 2 to 3 wk after transferring them from the specific pathogen-free colony to the conventional area. Similar to B27-associated spondyloarthropathy in humans, male mice were primarily affected (Table I). Since these mice lack functional H2-A and H2-E molecules, it is clear that B27-derived peptides presented by MHC class II molecules are not the cause of disease in B27 transgenic animals. Moreover, lymph node cells from B27$^+\beta_2$m^{o}Aβ^o mice immunized with B27-derived peptides do not respond to B27-derived peptides when challenged in vitro (data not shown). B27$^+$A$\beta^o\beta_2$m$^{+/+}$ mice did not develop arthritis and nail disease. A few B27$^+$A$\beta^o\beta_2$m$^{+/-}$ mice showed lower incidence of disease susceptibility (23% among male animals). The heterozygocity of wild-type β_2m gene results in lower expression of B27 molecules due to competition with endogenous class I molecules. A few B27 molecules may reach the cell surface free/empty. In addition, background genes may also have some influence in disease susceptibility.

In vivo treatment with HC10 Ab decreases incidence of arthritis and nail disease in B27$^+\beta_2$m^o mice

We have hypothesized previously that the development of spontaneous disease in B27$^+\beta_2$m^o mice may be the result of exogenous Ag presentation by the few β_2m-free B27 HC expressed on the cell surface. To confirm a direct role of B27 HC, mice were treated with appropriate mAbs to block presentation of unknown environmental Ags before transferring them from specific pathogen-free barrier facility. At the same time, control groups of mice were treated with isotype-matched Ab (L368) or left untreated. As shown in Figure 4, in vivo treatment with the HC-specific HC10 Ab decreased the incidence of disease in B27$^+\beta_2$m^o mice. No such effect was observed in the group of mice treated with 3F12 mAb. Only 25% of mice treated with the HC10 Ab developed arthritis and/or nail disease compared with 60 and 73% of mice in 3F12 (anti-H2-A^b) or control Ab-treated groups. In vivo treatment with an Ab (ME1) reactive to [HC of B27$^+\beta_2$m] complex was also found ineffective. Since the HC10 Ab recognizes and binds to the $\alpha_1\alpha_2$ peptide binding domains of the HLA-B27 molecule and not the α_3 domain (data not shown), in vivo treatment was thought to block binding and presentation of environmental Ags by HCs.
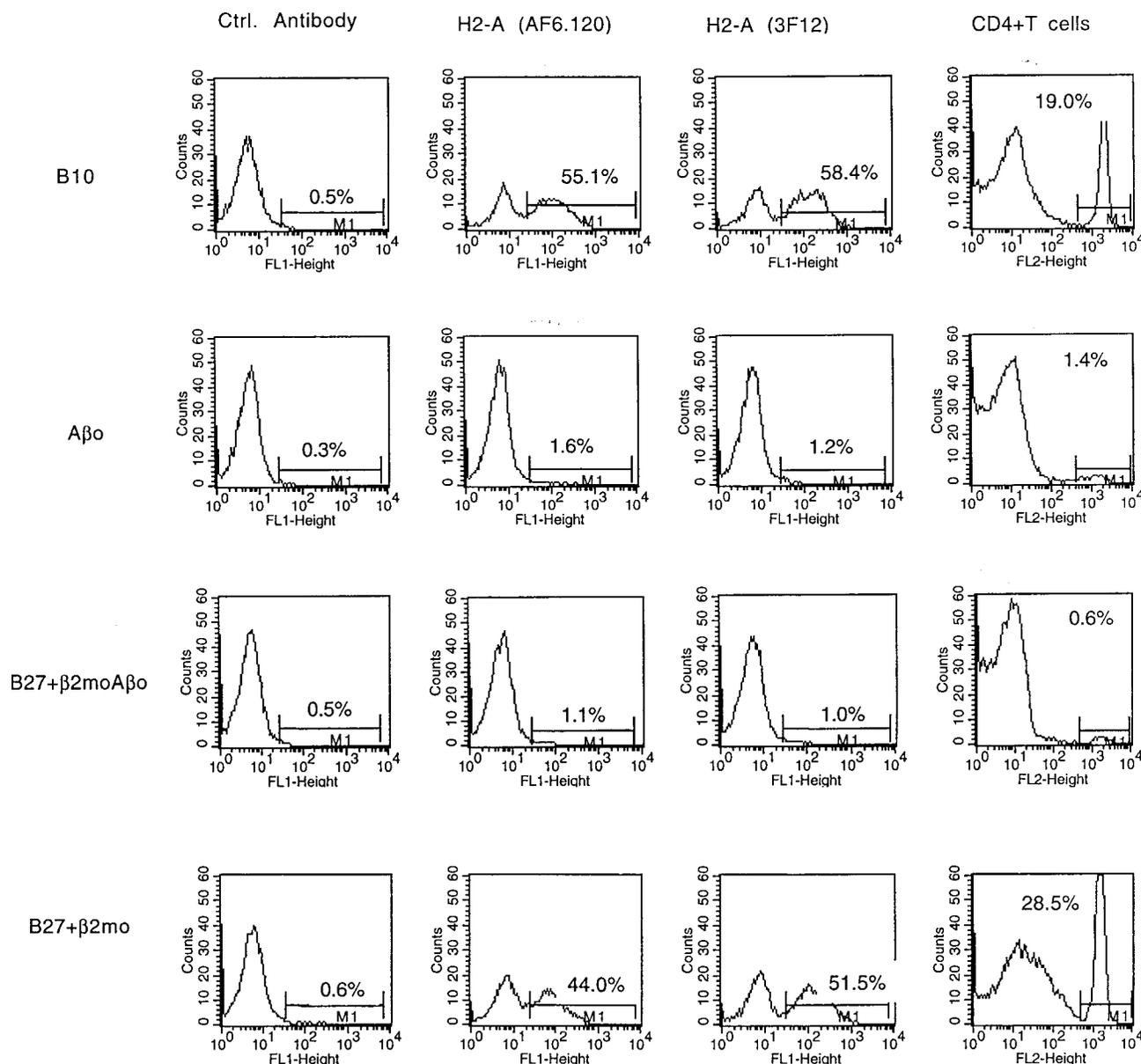
FIGURE 3. Flow-cytometric analysis for the cell surface expression of MHC class II (H2-A) molecule and CD4$^+$ T cells. *First column* shows background staining by using an isotype control and secondary (goat anti-mouse) Abs. *Second* and *third columns* reveal expression of H2-A molecule by using AF6.120 and 3F12. The histograms were compared with negative controls stained with only FITC-conjugated secondary Ab (goat anti-mouse IgG). *Fourth column* shows expression and percentage of positive CD4$^+$ T cells, as analyzed by using anti-CD4 mAb directly conjugated to phycoerythrin (PE).

These results demonstrate that in vivo treatment with the HC10 Ab blocks presentation of unknown environmental Ags by HLA-B27 HC, thereby delaying development of spontaneous disease in B27$^+$$\beta_2$m^o animals. In addition, in vivo treatment with HC10 Ab may also interfere with the generation of B27-derived peptides.

Discussion

In the present study, we analyzed two potential reasons for the involvement of the HLA-B27 molecule in disease pathogenesis in transgenic mice. These possibilities include 1) presentation of exogenous Ag by B27 HC or by a functional H2-A molecule, and 2) presentation of B27-derived peptides by MHC class II molecules.

Table I. *Arthritis and nail disease in MHC class II deficient B27 transgenic mice*

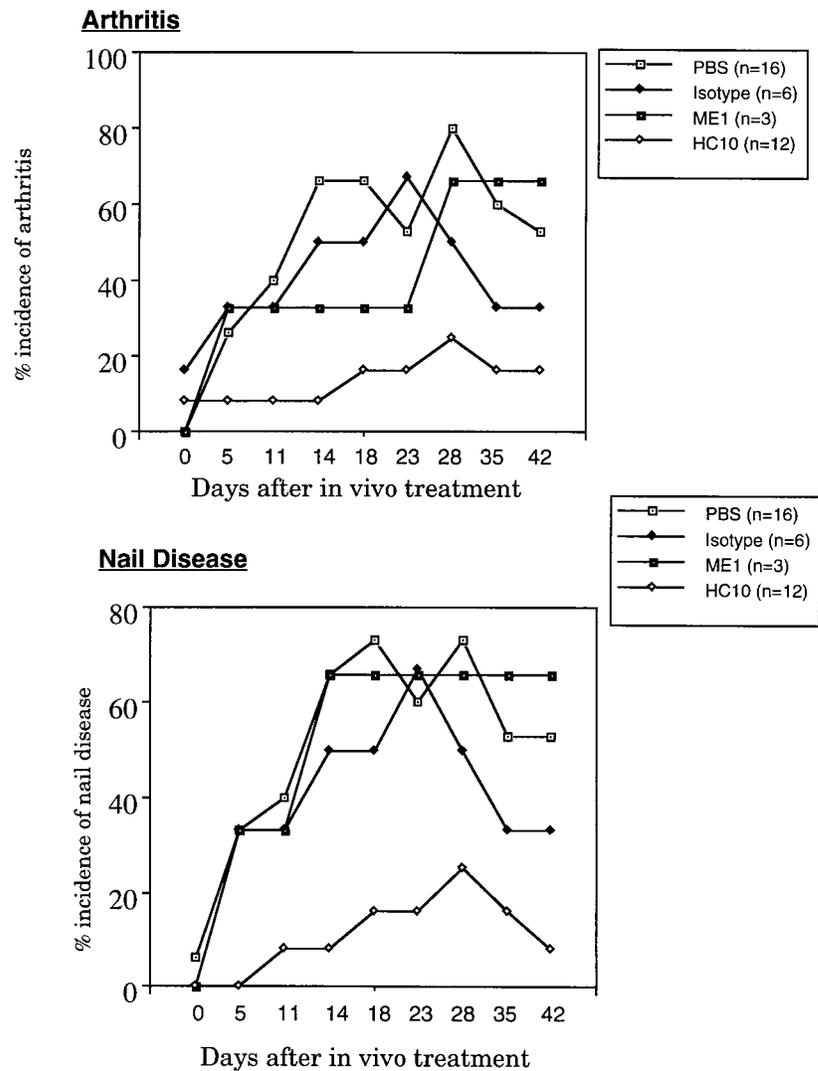| B27	Aβ	Mβ_2m	Sex	Total	Arthritis	Nail Disease
+	+/+	−/−	M	86	59 (68%)	63 (73%)
			F	59	19 (32%)	16 (27%)
+	−/−	−/−	M	33	16 (49%)	18 (55%)
			F	11	1 (9%)	1 (9%)
−	+/+	−/−	M	13	1 (7%)	0
			F	9	0	0
+	−/−	+/+	M	18	1 (5%)	0
			F	19	0	0
+	−/−	+/−	M	13	3 (23%)	3 (23%)
			F	15	0	0

FIGURE 4. Percentage of incidence of arthritis and nail disease in B27$^+$$\beta_2$m^o mice after in vivo treatment with HC10 or other control Abs. Data shown here are from a total of three experiments. Purified Ab or PBS was injected within 30 min after transferring mice from barrier facility to the conventional colony. Mice were monitored for the development of disease twice/week for a period of 6 to 8 wk.

Previously described B27$^+$$\beta_2$m^o mice lack normal expression of class I molecules, but they do express H2-A^b molecule and have normal levels of CD4$^+$ T cells (4). In this model, because of low expression of HC of B27, an intriguing possibility includes presentation of B27-derived peptides by class II molecules (13, 19). Peptide elution from two TAP polymorphic alleles in B27 transgenic rats shows different pattern of eluted peptides, regardless of disease susceptibility (23). These authors suggested that binding of specific peptide to HLA-B27 probably does not play a role in the disease.

To address the question of whether class II molecules in the B27$^+$$\beta_2$m^o mice may be presenting a B27-derived peptide, these mice were treated with the 3F12 (anti-H2-A^b) Ab. This treatment had no effect on the disease, suggesting that class II molecules may not have any role in disease pathogenesis. Since B27$^-$$\beta_2$m^o full-sibs are not susceptible to the disease, we know that class II molecules are not involved in the disease by presenting an exogenous peptide. Since B27$^+$$\beta_2$m^o mice express low levels of HCs of B27 on cell surface, it is possible that these HCs may be presenting an environmental Ag to initiate the disease. Similarly, presentation of peptide by H2-K and H2-D molecules has been shown in β_2m-deficient mice (24, 25). Our findings on the decreased incidence of spontaneous arthritis and nail changes after treatment with the anti-HC of B27-specific HC10 Ab suggest a role for these B27 HC in

the disease process by presenting an unknown environmental peptide.

Since several enterobacteria are known to be part of the normal gut flora in mice, they could be the source of disease-causing peptide in these mice. Although Ags presented in association with MHC-encoded class I molecules are generally derived from intracellular proteins (26), under some circumstances exogenous Ags have been reported to enter class I presentation pathway (27–32). Processing of bacterial Ag by phagocytic route in mast cells and presentation by the class I molecule to T cells have recently been described (33). Moreover, presentation of exogenous Ags by empty HLA-B27 has been described (34). Furthermore, transfer of inflammatory disease in B27 transgenic rats by bone marrow engraftment suggests the presentation of exogenous Ags by HLA-B27. Very recently, presentation of tissue-associated self Ags in the context of class I via an exogenous processing pathway has been described (35). Our data indicate a direct role for B27 molecules in the disease process.

Even though a role for class II molecules in B27-linked spondyloarthropathies has been minimum, certain observations and hypotheses have raised some questions (12, 13, 19). The first observation is that a low level relative risk in some B27-linked diseases has been attributed to the HLA-DR or HLA-DQ genes (36). The next observation is that class II-restricted CD4$^+$ T cells have been

found in affected tissues in certain patients with B27-associated spondyloarthropathies (14–16). Are these purely bystander lymphocytes or are they generated during chronic infection by an epitope-spreading phenomena, or are they actually effector T cells involved in joint injury? A recent hypothesis has proposed that the disease may be initiated by the presentation of HLA-B27-derived peptides by class II molecules (13). Although $B27^+\beta_2m^o$ mice showed immune response to B27-derived peptide (57–76), in vivo treatment with anti-H2-A^b Ab had no effect on the disease. $B27^+\beta_2m^oA\beta^o$ mice lacking endogenous class II molecules developed spontaneous disease similar to that of $B27^+\beta_2m^o$ transgenic mice. Thus, B27-derived peptides presented by class II molecules are not the cause of spontaneous disease in these mice. Prevention of disease with in vivo administration of anti-HC Ab shows that B27 HC play a direct role in the disease pathogenesis. We cannot rule out the possibility that the disease is mediated by the presentation of B27-derived peptides by B27 molecule. Could the slightly reduced incidence in the $B27^+\beta_2m^oA\beta^o$ mice compared with $B27^+\beta_2m^o$ mice be due to reduced levels of CD4$^+$ T cells? We are currently exploring the role of CD4 vs CD8 T cells, the source of peptide and potential immunotherapies.

Acknowledgments

References

1. Brewerton, D. A., F. D. Hart, A. Nicholls, M. Caffrey, D. C. James, and R. D. Sturrock. 1973. Ankylosing spondylitis and HL-A 27. *Lancet i:904.*

2. Khan, M. A. 1992. An overview of clinical spectrum and heterogeneity of spondyloarthropathies. *Rheum. Dis. Clin. North Am. 18:1.*

3. Hammer, R. E., S. D. Maika, J. A. Richardson, J.-P. Tang, and J. D. Taurog. 1990. Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human β_2m: an animal model of HLA-B27 associated human disorders. *Cell 63:1099.*

4. Khare, S. D., H. S. Luthra, and C. S. David. 1995. Spontaneous inflammatory arthritis in HLA-B27 transgenic mice lacking β_2-microglobulin: a model of human spondyloarthropathies. *J. Exp. Med. 182:1153.*

5. Khare, S. D., J. Hansen, H. S. Luthra, and C. S. David. 1996. HLA-B27 heavy chains contribute to spontaneous inflammatory disease in B27/human β_2-microglobulin double transgenic mice with disrupted mouse β_2m. *J. Clin. Invest. 98: 2746.*

6. Taurog, J. D., J. A. Richardson, J. T. Croft, W. A. Simmons, M. Zhou, J. L. Fernandez-Sueiro,. E. Balish, and R. E. Hammer. 1994. The germfree state prevents development of gut and joint inflammatory disease in HLA-B27 transgenic rats. *J. Exp. Med. 180:2359.*

7. Burmester, G. R., A. Daser, T. Kamradt, A. Krause, N. A. Mitchison, J. Sieper, and N. Wolf. 1995. Immunology of reactive arthritides. *Annu. Rev. Immunol. 13:229.*

8. Geczy, A. F., K. Alexander, H. V. Bashir, J. P. Edmonds, L. Upfold, and J. S. Sullivan. 1983. HLA-B27, *Klebsiella* and ankylosing spondylitis: biological and chemical studies. *Immunol. Rev. 70:23.*

9. Benjamin, R., and P. Parham. 1990. Guilt by association: HLA-B27 and ankylosing spondylitis. *Immunol. Today 11:137.*

10. Scofield, R. H., W. L. Warren, G. Koelsch, and J. B. Harley. 1993. A hypothesis for the HLA-B27 dysregulation in spondyloarthropathy: contribution from enteric organisms, B27 structure, peptides bound by B27, and convergent evolution. *Proc. Natl. Acad. Sci. USA 90:9330.*

11. Scofield, R. H., B. Kurien, T. Gross, W. L. Warren, and J. B. Harley. 1995. HLA-B27 binding of peptide from its own sequence and similar peptides from bacteria: implications for spondyloarthropathies. *Lancet 345:1542.*

12. Sieper, J., and G. Kingsley. 1996. Recent advances in the pathogenesis of reactive arthritis. *Immunol. Today 17:160.*

13. Parham, P. 1997. Presentation of HLA class I-derived peptides: potential involvement in allorecognition and HLA-B27-associated arthritis. *Immunol. Rev. 154: 137.*

14. Hermann, E., B. Fleischer, W. J. Mayet, T. Poralla, and K. H. Meyer zum Buschenfelde. 1989. Response of synovial fluid T cell clones to *Yersinia enterocolitica* antigens in patients with reactive *Yersinia* arthritis. *Clin. Exp. Immunol. 75:365.*

15. Viner, N. J., L. C. Bailey, P. F. Life, P. A. Bacon, and J. S. H. Gaston. 1991. Isolation of *Yersinia*-specific T-cell clones from the synovial membrane and synovial fluid of patients with reactive arthritis. *Arthritis Rheum. 34:1151.*

16. Hermann, E., W. J. Mayet, H. Thomssen, J. Sieper, T. Poralla, K. H. Meyer zum Buschenfelde, and B. Fleischer. 1992. HLA-DP restricted *Chlamydia trachomatis* specific synovial fluid T cell clones in *Chlamydia*-induced Reiter's disease. *J. Rheumatol. 19:1243.*

17. Hermann, E., D. T. Yu, K. H. Meyer zum Buschenfelde, and B. Fleischer. 1993. HLA-B27-restricted CD8 T cells derived from synovial fluids of patients with reactive arthritis and ankylosing spondylitis. *Lancet 342:646.*

18. Hermann, E. M., R. Duchmann, E. May, B. Ackermann, and K. H. Meyer zum Buschenfelde. 1996. The T cell receptor (TCR) in HLA-B27-restricted T cell responses: an introduction. *Clin. Rheumatol. 15(Suppl. 1):86.*

19. Lopez-Larrea, C., S. Gonzalez-Roces, and V. Alvarez. 1996. HLA-B27 structure, function and disease association. *Curr. Opin. Rheumatol. 8:296.*

20. Cosgrove, D., D. Gray, A. Dierich, J. Kaufman, M. Lemeur, C. Benoist, and D. Mathis. 1991. Mice lacking MHC class II molecules. *Cell 66:1051.*

21. Stam, N. J., H. Spits, and H. L. Ploegh. 1986. Monoclonal antibodies raised against denatured HLA-B locus heavy chains permit biochemical characterization of certain HLA-C locus products. *J. Immunol. 137:2299.*

22. Sercarz, E. E., P. V. Lehmann, A. Ametani, G. Benichou, A. Miller, and K. Moudgil. 1993. Dominance and crypticity of T cell antigenic determinants. *Annu. Rev. Immunol. 11:729.*

23. Simmons, W. A., L. Y. W. Leong, N. Satumtira, G. W. Butcher, J. C. Howard, J. A. Richardson, C. A. Slaughter, R. E. Hammer, and J. D. Taurog. 1996. Rat MHC-linked peptide transporter alleles strongly influence peptide binding by HLA-B27 but not B27-associated inflammatory disease. *J. Immunol. 156:1661.*

24. Glas, R., C. Ohlen, P. Hoglund, and K. Karre. 1994. The CD8$^+$ T cell repertoire in β_2-microglobulin mice is biased towards reactivity against self-major histocompatibility complex I. *J. Exp. Med. 179:661.*

25. Zugel, U., B. Schoel, and S. H. E. Kaufman. 1994. β_2-microglobulin independent presentation of exogenously added foreign peptide and endogenous self epitope by MHC class I α-chain to a cross reactive CTL clone. *J. Immunol. 153:4070.*

26. Germain, R. N. 1994. MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. *Cell 76:287.*

27. Yewdell, J. W., J. R. Bennink, and Y. Hosaka. 1988. Cells process exogenous proteins for recognition by cytotoxic T lymphocytes. *Science 239:637.*

28. Carbone, F. R., and M. J. Bevan. 1990. Class I-restricted processing and presentation of exogenous cell-associated antigen in vivo. *J. Exp. Med. 171:377.*

29. Rock, K. L., S. Gamble, and L. Rothstein. 1990. Presentation of exogenous antigen with class I major histocompatibility complex molecules. *Science 249:918.*

30. Pfeifer, J. D., M. J. Wick, R. L. Roberts, K. Findlay, S. J. Normark, and C. V. Harding. 1993. Phagocytic processing of bacterial antigens for class I MHC presentation to T cells. *Nature 361:359.*

31. Norbury, C. C., L. J. Hewlett, A. R. Prescott, N. Shastri, and C. Watts. 1995. Class I MHC presentation of exogenous soluble antigen via macropinocytosis in bone marrow macrophages. *Immunity 3:783.*

32. Jondal, M., M. Schirmbeck, and Jörg Reimann. 1996. MHC class I-restricted CTL responses to exogenous antigens. *Immunity 5:295.*

33. Malaviya, R., N. J. Twesten, E. A. Ross, S. N. Abraham, and J. D. Pfeifer. 1996. Mast cells process bacterial antigens through a phagocytic route for class I MHC presentation to T cells. *J. Immunol. 156:1490.*

34. Benjamin, R. J., J. A. Madrigal, and P. Parham. 1991. Peptide binding to empty HLA-B27 molecules of viable human cells. *Nature 351:74.*

35. Kurts, C., W. R. Heath, F. R. Carbone, J. Allison, J. F. A. P. Miller, and H. Kosaka. 1996. Constitutive class I-restricted exogenous presentation of self antigen in vivo. *J. Exp. Med. 184:923.*

36. Yuokko, J., H. Reijonen, J. Ilonen, K. Anttila, S. Kikkari, T. Mottonen, U. Yli-Kerttula, and A. Toivanen. 1997. Increase of HLA-DRB1*0408 and -DQB1*0301 in HLA-B27 positive reactive arthritis. *Ann. Rheum. Dis. 56:37.*