The Specificity of Peptides Bound to Human Histocompatibility Leukocyte Antigen (HLA)-B27 Influences the Prevalence of Arthritis in HLA-B27 Transgenic Rats

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Summary

Human histocompatibility leukocyte antigen B27 is highly associated with the rheumatic diseases termed spondyloarthropathies, but the mechanism is not known. B27 transgenic rats develop a spontaneous disease resembling the human spondyloarthropathies that includes arthritis and colitis. To investigate whether this disease requires the binding of specific peptides to B27, we made a minigene construct in which a peptide from influenza nucleoprotein, NP383-391 (SRYWAIRTR), which binds B27 with high affinity, is targeted directly to the ER by the signal peptide of the adenovirus E3/gp19 protein. Rats transgenic for this minigene, NP1, were made and bred with B27 rats. The production of the NP383-391 peptide in $B27^+NP1^+$ rats was confirmed immunologically and by mass spectrometry. The NP1 product displaced ~90% of the ^{3}H-Arg-labeled endogenous peptide fraction in $B27^+NP1^+$ spleen cells. Male $B27^+NP1^+$ rats had a significantly reduced prevalence of arthritis, compared with $B27^+NP^-$ males or $B27^+$ males with a control construct, NP2, whereas colitis was not significantly affected by the NP1 transgene. These findings support the hypothesis that B27-related arthritis requires binding of a specific peptide or set of peptides to B27, and they demonstrate a method for efficient transgenic targeting of peptides to the ER.

Key words: human histocompatibility leukocyte antigen B27 • transgenic rat • antigenic peptide • arthritis • mass spectrometry

HLA-B27 refers to a group of closely related alleles of the HLA-B locus that shows strong genetic predisposition to the rheumatic diseases termed spondyloarthropathies (1, 2). The association of B27 with these disorders has been known for 25 years, but the molecular mechanism remains to be identified. The known physiologic function of class I MHC molecules such as B27 is to present peptide antigens, predominantly of intracellular origin, to the immune system (3, 4), and it has been hypothesized that the role of B27 in disease pathogenesis involves presentation of one or more "arthritogenic" peptides (5). It has been difficult, however, to find strong experimental support for this hypothesis. Most of the T cells isolated from patients with B27-related disease that are reactive with implicated pathogens have been of the MHC class II–restricted CD4 variety (6), although occasional $CD8^+$ B27-restricted clones have been described (7). Results from studies of B27 transgenic rodents that develop spontaneous arthritis have also not established a definite role for $CD8^+$ B27-restricted T cells (8–11).

In the studies described here, we have specifically addressed the role of HLA-B27 in transgenic rats expressing this molecule. These rats develop a spontaneous multisystem disease that shares several pathologic features with the human spondyloarthropathies, including arthritis and colitis (8–10, 12–15). We provide evidence that the specificity of

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the peptide population bound to HLA-B27 in vivo has a significant effect on at least one important aspect of the disease course in these rats, peripheral arthritis.

Materials and Methods

Rats. The transgenic lines on a Lewis (LEW)[1] background, 21-4H and 21-4L, each expressing HLA-B*2705 and human β2m, have been previously described (13, 15). The 21-4H line (150 B27 gene copies, 90 hβ2m gene copies) develops a spontaneous multisystem disease, whereas the 21-4L line (6 gene copies each of B27 and hβ2m), which has a lower transgene copy and level of expression, remains completely healthy. The DA.33-3 line, expressing the B*2705/hβ2m transgene locus of the 33-3 line (55 copies of B27, 66 copies of hβ2m; references 13, 15) on a DA background, has likewise been described (16, 17). The LEW.33-3 line, not previously described, was produced by backcrossing the 33-3 transgene locus >10 generations onto the LEW background. This line shows a phenotype similar to 21-4H, but without neurologic manifestations (15).

Sprague Dawley (SD) founder rats transgenic for the NP1 and NP2 peptide expression constructs, described below, were produced by pronuclear microinjection, as previously described (15). All nontransgenic LEW and SD breeding stock was originally obtained from Charles River, Inc. (Wilmington, MA).

Production of the NP1 and NP2 Minigene Constructs. A modification of the method described by Anderson et al. (18) was used to make two minigenes as outlined in Fig. 1 *A*. Overlapping oligonucleotides were synthesized to encode a 27mer peptide containing the 18mer signal sequence, MRYMILGLLALAAVCSAA, of the adenovirus protein E3/gp19K (19) at the NH_2 terminus, and the 9mer influenza A nucleoprotein, NP383-391 (SRYWAIRTR) (20), at the COOH terminus. 40 ng each of the oligonucleotides were annealed, made fully double stranded with Klenow enzyme and dNTPs, and then amplified by PCR with oligonucleotide primers containing SalI and BamHI restriction sites at the 5′ and 3′ ends, respectively. These sites were used to clone the minigene into the expression vector pHSE3′ (21) under the control of the H-2K^b promoter, as shown in Fig. 1 *B*. This construct is hereafter referred to as the NP1 minigene. A control minigene construct, hereafter called the NP2 minigene, was similarly produced, in which the codon for Arg at P2 of the NP383-391 peptide was replaced by Leu to encode the peptide SLYWAIRTR, which does not bind to B27 (22). The DNA sequences of the minigene inserts were confirmed to be correct by dideoxy sequencing. Preliminary transfection experiments in B27$^+$ human and mouse cells lines confirmed expression of mRNA from both constructs and immunologically authentic SRYWAIRTR from NP1 (data not shown). For microinjection into fertilized rat eggs, the NP1 and NP2 inserts were excised from the vector at the flanking XhoI sites.

Northern Analysis. Northern blot hybridization of whole cellular RNA was carried out on cell lines and rat tissues as previously described (13). Hybridization specific for the NP1 and NP2 constructs was carried out with a ^{32}P-labeled 1.6-kb BamHI-PstI fragment from the human β-globin gene 3′-untranslated region contained within the pHSE3′ vector (21). Quantitation of mRNA by normalization to 18S RNA was carried out as previously described (13).

Flow Cytometry. Surface B27 expression on lymphoid cells was detected by flow cytometry with the anti–HLA-B,C mAb B1.23.2, as previously described (13, 15).

CTL Generation and Assays. The expression of antigenic NP1 peptide was assayed in B*2705/hβ2m-bearing transfected cell lines or Con A–stimulated lymph node (LN) lymphoblasts by using these cells as labeled targets in a 4-h ^{51}Cr-release assay with effectors from the B27-restricted, NP peptide-specific human CTL line Q124 (23; the gift of Dr. W.E. Biddison, NIH, Bethesda MD). CTL effectors were generated by stimulating Q124 cells with irradiated B27$^+$ human peripheral blood mononuclear cells pulsed with NP peptide for 6 d in vitro, as previously described (23).

Recognition of the B27-presented HY minor histocompatibility antigen by polyclonal rat CTL was assayed as previously described (16, 17). The same protocol was modified to generate (*a*) polyclonal CTL allospecific for HLA-B27 by priming nontransgenic DA rats with lymphoid cells from sex-matched line 21-4L rats on a LEW background congenic for RT1^{av1} and (*b*) B27-restricted NP peptide-specific CTL by priming 21-4L (B27$^+$NP$^-$) recipients with B27$^+$NP1$^+$ transgenic lymphoid cells.

CTL assay of targets sensitized with HPLC-fractionated peptides was carried out as previously described (17, 24). Cold target competition experiments were carried out as previously described (16, 17, 24). All incubations with synthetic NP383-391 peptide were at 2–4 μM.

Peptide Analysis. Metabolic labeling, peptide isolation, fractionation by reverse phase HPLC, and sequence analysis by quadrupole time-of-flight mass spectrometry (QTOF/MS) were carried out as previously described (17, 24).

IL-1β Assay. The IL-1β content of proximal colon was assayed by ELISA with polyclonal goat anti–rat IL-1β antibodies, as previously described (25).

Clinical Assessment. Rats were scored twice a week, usually without knowledge of their genotypes, for arthritis, diarrhea, and other clinical manifestations of the B27/hβ2m transgenic rats, as previously described (8–10, 13, 15).

Histopathology. Formalin-fixed, paraffin-embedded, hematoxylin- and eosin-stained sections of proximal colon and acid-decalcified ankle joints were prepared as previously described (8). Histologic colitis was graded on a semiquantitative scale of 0–4, as previously described (25). All histologic assessment was done without knowledge of the genotypes of the specimen donors.

Statistical Analysis. Comparisons among groups of rats were made by a χ^2 test, *t* test, ANOVA, or Wilcoxon rank sum test.

Results

Production of the NP1 and NP2 Transgenic Rat Lines. The NP1 and NP2 minigene constructs were used to produce transgenic SD by pronuclear microinjection. Genomic integration of the NP1 and NP2 constructs was observed in nine and six founders, respectively. Two NP1 lines, 293-5 and 300-5, and one NP2 line, 338-2, were established. The relative transgene mRNA levels in spleen in these three lines were 1.0, 1.3, and 3.9, respectively. In an mRNA tissue survey in the 300-5 line, the highest mRNA levels were found in thymus and spleen, with weaker expression in jejunum and liver (data not shown).

Immunologic Identification of the NP1 Transgene Product. To test for the presence of the NP383-391 peptide in the

[1]*Abbreviations used in this paper:* LEW, Lewis; LN, lymph node; SD, Sprague Dawley.

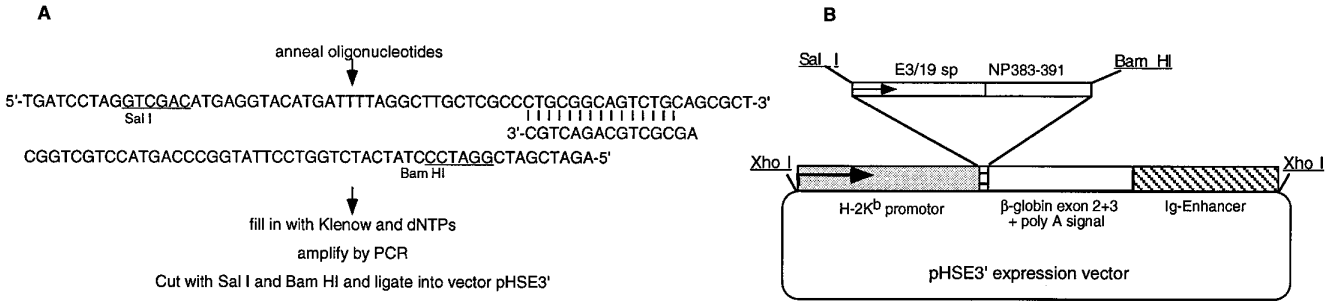

Figure 1. Construction of the minigenes encoding the adenovirus E3/19K signal peptide and influenza NP383-391 peptide. (*A*) Procedure for generation of the double-stranded DNA insert. The indicated overlapping oligonucleotides were synthesized and annealed. Double stranded synthesis was completed with Klenow enzyme, and the resulting construct was cleaved with SalI and BamHI and ligated into the expression vector pHSE3′ (21). For the NP2 minigene, the residue at P2 in the B27-binding epitope was changed from Arg to Leu by replacing the codon AGG with the codon TTG. This substitution abrogates binding of the peptide to B27 (22). (*B*) Map of the complete construct for NP1 and NP2.

NP1 rats, the NP1 and NP2 transgene loci were backcrossed to the LEW background and crossed with the B27/hβ2m transgenic lines 21-4H, LEW.33-3, and 21-4L. B27 surface expression as detected by flow cytometry was not significantly affected by the simultaneous expression of either the NP1 or the NP2 transgene (data not shown). Con A blast LN targets from the single and double transgenic offspring were tested for lysis by the human CTL line, Q124, which is specific for B27 and the NP383-391 peptide. As shown in Fig. 2 *A*, the $B27^+NP1^+$ targets were lysed well by the specific CTL, whereas the $B27^+NP^-$ and $B27^+NP2^+$ targets were not lysed. Moreover, lysis of the $B27^+NP1^+$ targets was inhibited by $B27^+NP^-$ cold targets pulsed with synthetic NP383-391 peptide (see Fig. 2 *B*), consistent with the predicted specificity of the transgene product. In a separate experiment, LN and spleen cells from a $B27^+NP1^+$ rat were used to prime an RT1-matched $B27^+NP^-$ recipient, and subsequently to restimulate the primed LN cells in vitro, which were then tested for lysis of the mouse lymphoma EL-4 cells transfected with HLA-B27 and hβ2m in the presence or absence of added NP383-391 peptide. As shown in Fig. 2 *C*, CTL effectors were generated that were specific for B27 and the added peptide, indicating that the NP1 transgene product is presented by B27 in vivo.

HPLC Isolation and MS Sequencing of the NP1 Peptide. To identify the NP1 product biochemically, B27 molecules were immunoprecipitated from detergent lysates of $B27^+NP1^+$, $B27^+NP^-$, and $B27^+NP2^+$ spleen cells, and the bound peptides were dissociated in acid and fractionated by reverse phase HPLC. When aliquots of the HPLC fractions were tested for sensitization of $B27^+$ human C1R targets, all of the immunologic activity was found in fraction 83 from the $B27^+NP1^+$ peptides (Fig. 3 *A*), whereas no activity was found in any of the $B27^+NP^-$ (Fig. 3 *B*) or $B27^+NP2^+$ (data not shown) peptide fractions.

Electrospray mass spectrometric analysis of the active fraction from $NP1^+$ spleen indicated five peptides with molecular masses 1207.7, 1303.6, 1313.8, 1379.8, and 1473.7 (Fig. 4, *top*). The most abundant ion, of m/z 403.6, was selected for tandem MS analysis and shown to correspond to the $[M+3H]^{3+}$ ion of the NP383-391 peptide, SRYWAIRTR (Fig. 4, *bottom*). Analysis of the other four peptides indicated that they were modifications of the SRYWAIRTR sequence by adducts of 96, 106, 172, and 266 Da. Full characterization of these adducts is in progress. It also remains to be determined whether these modifications to the NP1 transgene product occur in vivo or as a result of the purification procedure.

Estimates of Endogenous B27 Peptide Displacement by NP1. To estimate the extent to which surface B27 molecules were engaged by NP1-encoded peptides, spleen cells from $B27^+NP1^+$, $B27^+NP2^+$, and $B27^+NP^-$ rats were metabolically labeled with ^{3}H-Arg, and the peptides bound to B27 were isolated and separated by RP-HPLC, as described above. As shown in Fig. 5, *B* and *C*, the peptides extracted from both $B27^+NP^-$ and $B27^+NP2^+$ B27 molecules eluted in a broad peak between fractions 30 and 70, containing ~98% of the total eluted ^{3}H. This result was similar to the pattern that we have previously reported for endogenous B27-bound peptides in transgenic mouse spleen eluted under the same conditions (24). In contrast, as shown in Fig. 5 *A*, the B27-bound peptides from $B27^+NP1^+$ spleen eluted in two peaks. The larger peak was centered at the same position as the NP1-encoded peptide product identified immunologically and confirmed by mass spectrometry (Figs. 3 and 4). This peak represented ~87% of the total eluted radioactivity. The smaller peak, containing ~9% of the eluted counts, appeared to be an attenuated version of the peak of endogenous peptides found in the $B27^+NP^-$ and $B27^+NP2^+$ eluates. These results suggest that a ~10-fold reduction in the usual B27-bound endogenous peptide population occurs in the $B27^+NP1^+$ rats through displacement by NP1-encoded peptide.

To assess the functional consequences of NP1 expression, Con A blast targets were compared as targets for lysis by B27-restricted anti-HY CTL and by anti-B27 allogeneic CTL. As shown in Fig. 6 *A*, male $B27^+NP1^+$ targets were lysed by B27-restricted anti-HY CTL to only ~50% the level of male $B27^+NP1^-$ targets. Similar results were seen with anti-B27 allospecific CTL (Fig. 6 *B*). These find-

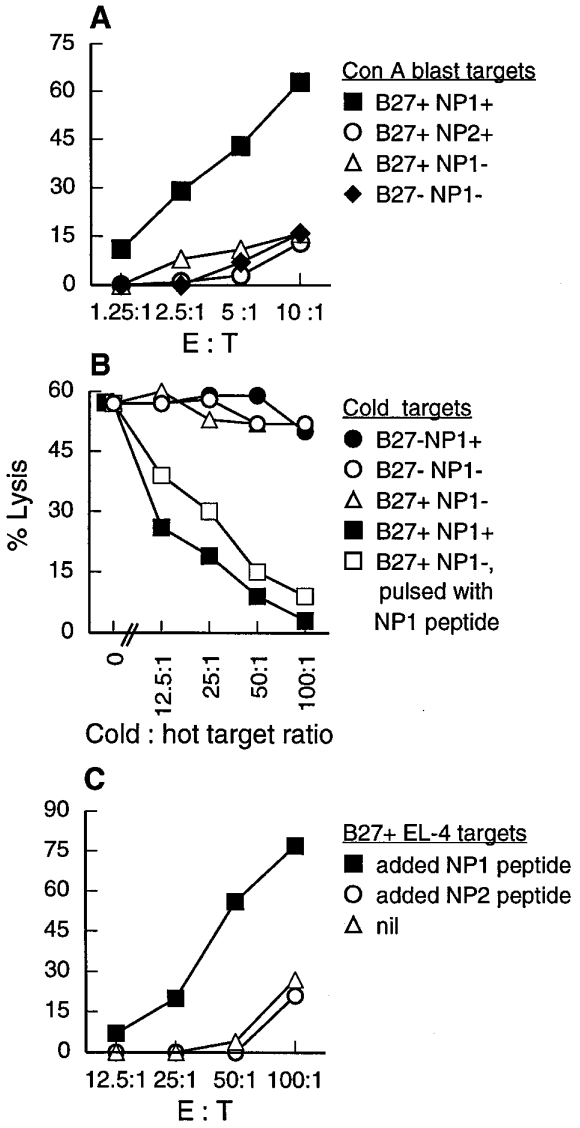

Figure 2. The NP1 transgene construct directs expression of antigenically authentic NP1 peptide. (*A*) Con A–stimulated LN lymphoblast targets from the indicated transgenic rats were tested for lysis by the human CTL line Q124, which is specific for HLA-B27 and the NP383-391 peptide. Only cells from rats carrying both the B27/hβ2m and NP1 transgene loci were lysed significantly. (*B*) Con A blast targets from a $B27^+$ $NP1^-$ rat were pulsed with synthetic NP peptide (10 nM), then washed and tested for lysis by the Q124 CTL line at an E:T of 5:1, in the presence of varying concentrations of the indicated unlabeled (cold) targets. Lysis was efficiently inhibited by double transgenic $B27^+NP1^+$ targets and by $B27^+$ targets pulsed with synthetic NP peptide. (*C*) $B27^+NP1^-$ rats were immunized in vivo with cells from RT1-matched $B27^+NP1^+$ rats, and the immune LN cells were restimulated in an MLR. The resulting CTL effectors lysed B27/hβ2m-transfected mouse EL-4 tumor cells to which authentic NP1 peptide, but not NP2 peptide, had been added.

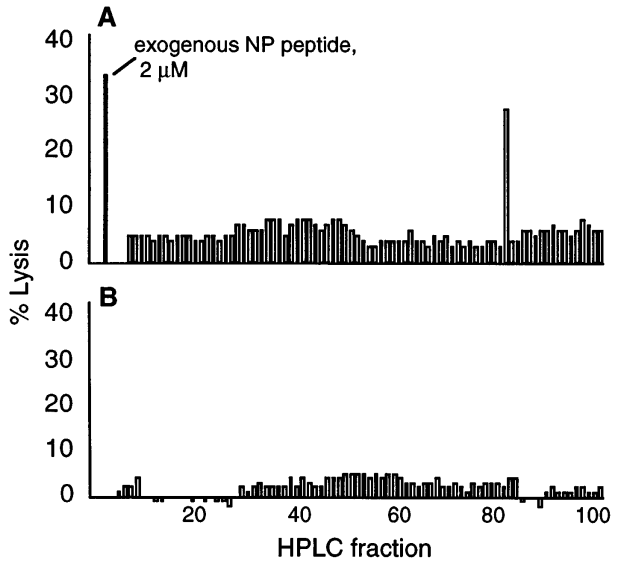

Figure 3. Isolation and immunologic identification of NP peptide from transgenic rats. B27 molecules were immunoprecipitated from 10^8 $B27^+NP1^+$ (*A*) and $B27^+NP^-$ (*B*) Con A–stimulated spleen cells and the bound peptides were extracted with 0.1% TFA and separated by RP-HPLC. One-tenth of each fraction was added to 5,000 ^{51}Cr-labeled $B27^+$ C1R cells, which were then tested for lysis by 2.5×10^4 line Q124 CTL effectors. Lysis above background was seen only with the peptides from the $B27^+NP1^+$ cells, with all of the immunologic activity in fraction 83.

ings indicate at least a twofold reduction of the endogenous peptides antigenic in these respective systems as a result of expression of the NP1 transgene locus.

Effects of the NP1 and NP2 Transgenes on Inflammatory Disease in B27 Rats. To test the effect of the NP1 and NP2 transgenic products on spontaneous clinical disease in rats, the 293-5 and 300-5 NP1 lines and 338-2 NP2 line were backcrossed to the disease-prone LEW lines 21-4H and LEW.33-3, and the double transgenic $B27^+NP^+$ and single transgenic $B27^+NP^-$ offspring were observed for the disease manifestations characteristic of the 21-4H line (13, 15). The clinical data from all of the rats observed to age 6 mo are shown in Table 1. The rats were of backcross generation N2-N6 to the LEW background (median generation N4 for all three genotypes, $NP1^+$, $NP2^+$, and NP^-; mean ± SD 3.8 ± 1.2, 3.7 ± 1.0, and 3.5 ± 0.5, respectively).

Almost all of the rats developed diarrhea, and there was no significant difference among the groups regarding the age of onset of the diarrhea or its maximum severity (data not shown). Similarly, there was no difference among the groups in which the IL-1β content of proximal colon was measured at sacrifice. Histologic examination of proximal colon was carried out on a subset of the males. There was a trend toward more severe disease in the groups lacking NP1, but this was not statistically significant (data not shown). WBC measurements at 3 mo of age showed no difference among the groups (Table 1), and there was similarly no difference in the pattern of WBC elevation upon serial measurements (data not shown).

The one parameter showing a significant difference among the groups was the prevalence of arthritis, with only 6 of 25 $NP1^+$ male rats developing arthritis during observation to age 6 mo, compared with 15 of 22 NP^- and 4 of 7 $NP2^+$ controls ($P = 0.005$ for $NP1^+$ versus $NP1^-$). Among the female $NP1^+$ and NP^- rats, the prevalence of arthritis was very low (1 of 20 and 3 of 22, respectively),

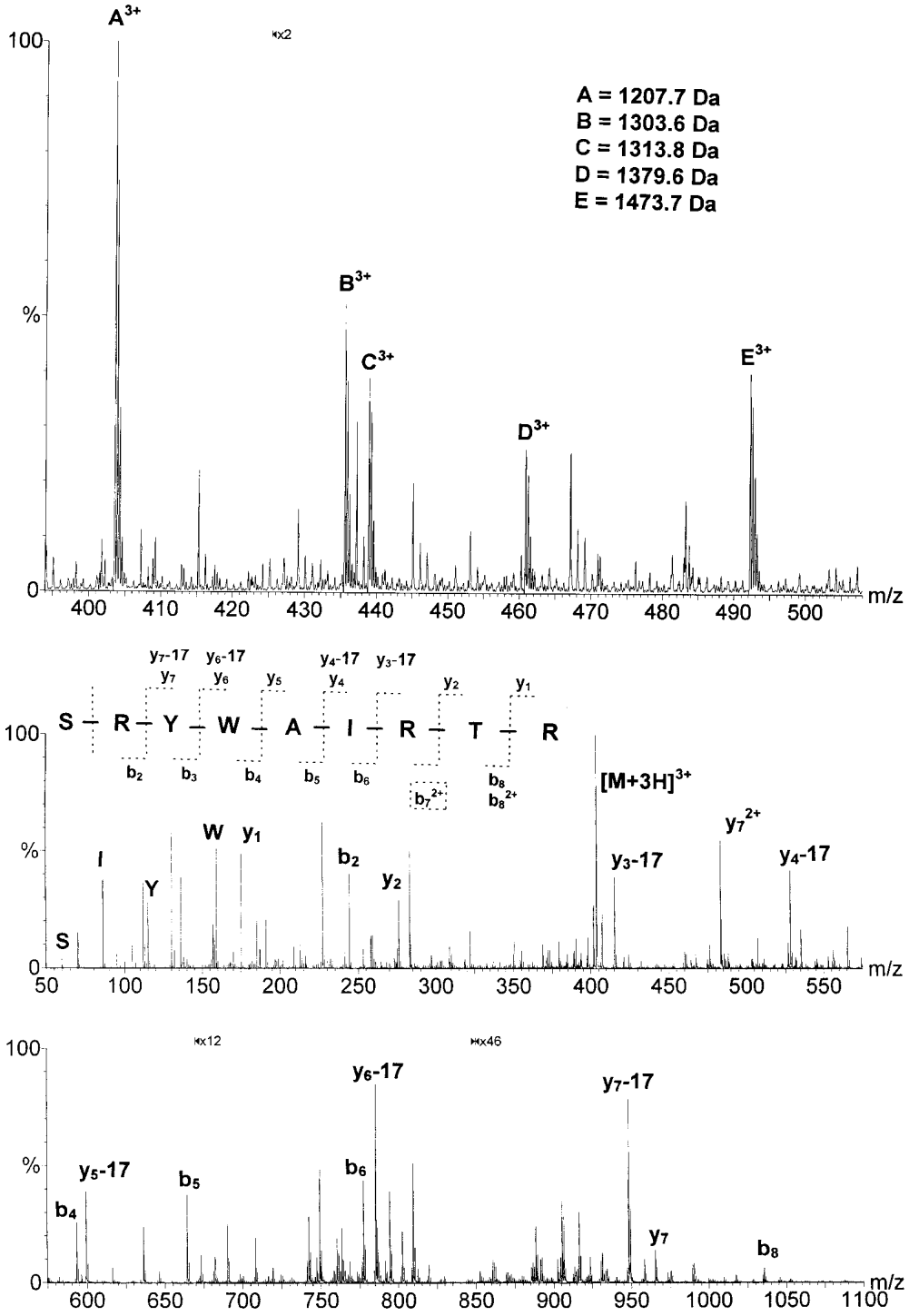

Figure 4. Mass spectrometric analysis of the immunologically active NP fraction, as shown in Fig. 3. (*Top*) Electrospray MS spectrum. The $[M+3H]^{3+}$ ions of components *A–E* are indicated, with the charge state evident from the m/z ratio separation of the isotopic components. Corresponding $[M+2H]^{2+}$ and $[M+4H]^{4+}$ ions were also observed outside the m/z range shown. (*Bottom*) Product ion spectrum recorded during tandem MS analysis. The selected precursor ion was m/z 403.6 ($[M+3H]^{3+}$). Fragment ions are designated using the nomenclature of Biemann (41). Confirmatory evidence was obtained from the product ion spectrum of m/z 604.8 ($[M+2H]^{2+}$); b_7^{2+} was observed only in the latter spectrum.

whereas 2 of 6 $NP2^+$ females developed arthritis. Among rats developing arthritis, there was no difference in the age of onset or severity of the arthritis among the six groups. Among the $NP1^+$ males, arthritis was observed in 4 of 17 rats backcrossed to 21-4H and 2 of 8 backcrossed to LEW.33-3. Among the NP^- males, arthritis was observed in 10 of 13 backcrossed to 21-4H and 5 of 9 backcrossed to LEW.33-3. Among $NP2^+$ males, the corresponding numbers were 2 of 3 and 2 of 4. Among the joints assessed histologically, 26 were from rats that never showed clinically evident arthritis, and these are listed in Table 1. Only three of these sections showed any microscopic lesions, two from NP^- females and one from an $NP2^+$ male.

A subsequent cohort of 300-5 (NP1) × 21-4H and 338-2 (NP2) × 21-4H rats, of backcross generations N5-N7 to LEW, was observed to age 4 to 6 mo for arthritis, which

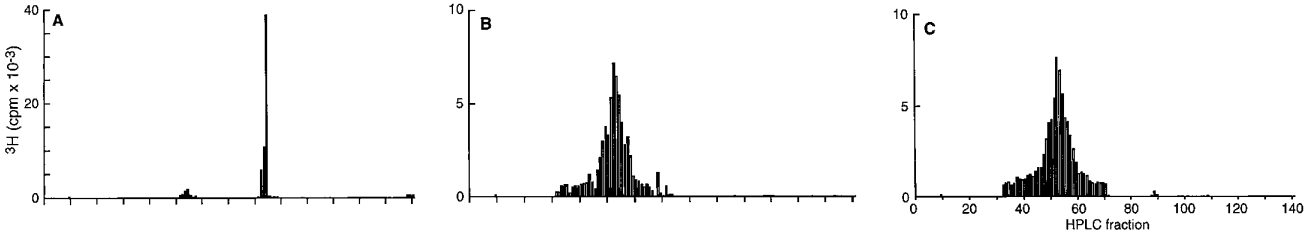

Figure 5. The NP1 transgene construct dramatically alters the profile of peptides bound to B27. Spleen cells from NP1$^+$ (*A*), NP$^-$ (*B*), and NP2$^+$ (*C*) LEW.33-3 rats were metabolically labeled with ^{3}H-Arg, and the B27 molecules were immunoprecipitated from detergent lysates of the labeled cells, as previously described (24). The bound peptides were eluted and separated by RP-HLPC, as in Fig. 3, *A* and *B*, and the resulting fractions counted for ^{3}H. Total ^{3}H cpm in the B27 immunoprecipitates were ($\times 10^{-7}$): NP1$^+$ 2.75, NP2$^+$ 3.00, NP$^-$ 2.75. Total ^{3}H cpm in the peptide pools eluted from B27 were ($\times 10^{-4}$): NP1$^+$ 6.5, NP2$^+$ 8.4, NP$^-$ 6.8.

again was seen almost exclusively in males. Of the B27$^+$ males of this group, arthritis was observed in 1 of 4 NP1$^+$, 3 of 4 NP2$^+$, and 6 of 8 NP$^-$ rats. Upon adding these results to the tally shown in Table 1, the prevalence of arthritis was found to be 7 of 29 in NP1$^+$ rats, and 28 of 41 in NP1$^-$ rats (χ^2 with Yates' correction = 11.54, $P < 0.0006$).

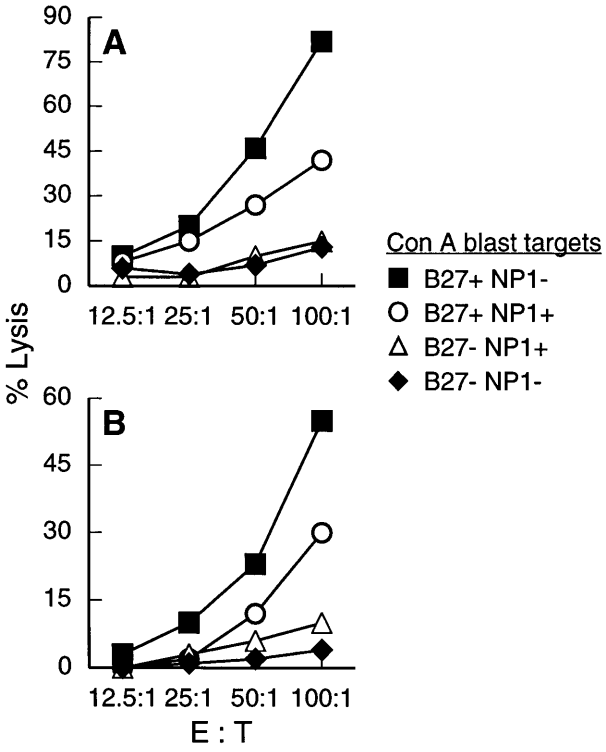

Figure 6. Expression of the NP1 transgene construct reduces recognition of other B27-presented peptides. (*A*) CTL against the male-specific HY minor histocompatibility antigen presented by HLA-B27 were generated in LEW transgenic rats as previously described (10, 16, 17, 24). These were tested for lysis of Con A lymphoblasts from male rats carrying the indicated transgenes. The rats were from a backcross of the SD 300-5 line to the DA.33-3 line, and therefore did not share any RT1 alleles with the effectors. (*B*) Allospecific CTL were generated by in vivo immunization of nontransgenic DA rats with cells from sex-matched B27/hβ2m LEW 21-4L rats congenic for *RT1*^{av1}, and the immune LN cells were similarly restimulated in an MLR. The CTL were tested for lysis of the Con A blast target cells described in *A*.

Discussion

The prevalence of arthritis was significantly reduced in male B27/hβ2m transgenic rats also carrying a transgene targeting the influenza A NP383-391 peptide to the ER, compared with B27/hβ2m rats lacking the NP1 transgene. In the B27/hβ2m female littermates, the prevalence of arthritis was very low in both the NP1$^+$ and NP$^-$ groups. Arthritis is typically more common in male B27/hβ2m rats than in the females (13, 15). The sex difference in these studies was more pronounced than usual, and the low prevalence of arthritis in the NP$^-$ females made it difficult to draw any conclusions from a comparison of the groups of female rats. Nonetheless, considering both sexes, of the rats shown in Table 1, 6 out of 13 rats transgenic for the control NP2 construct developed arthritis, providing evidence that the suppression of arthritis in the NP1$^+$ rats was not a nonspecific artifact of the NP1 transgene construct itself. This was further supported by a subsequent cohort, in which 3 of 4 NP2$^+$ males developed arthritis, and overall, there was a significant difference in arthritis prevalence between NP1$^+$ and NP2$^+$ males (7/29 vs. 7/11). The data from the males thus suggest that the specificity of the peptides bound to B27 in vivo is a key factor in the pathogenesis of spontaneous arthritis in B27/hβ2m transgenic rats.

Although the B27 transgenic lines were produced in inbred rats (15), the NP constructs were introduced into SD eggs because of the far greater efficiency of the procedure in this outbred strain compared with inbred rat strains, particularly LEW. It is of some potential concern that the rats in this study were not completely inbred. However, the correlation of NP1 with reduced prevalence of arthritis is statistically quite significant and there was no significant effect of either the backcross generation or the origin of the B27/hβ2m transgene locus (21-4H or 33-3) on the prevalence of arthritis in any of the three NP genotypes. The finding of a lower prevalence of arthritis in NP1$^+$ rats compared with NP2$^+$ and NP$^-$ rats in a subsequent, more extensively backcrossed cohort further supports this concept. Overall, the data support the conclusion of a peptide-specific suppression of arthritis.

Sequence analysis of the peptides in the one immunologically active HPLC fraction of the peptides eluted from B27 molecules of B27$^+$NP1$^+$ rats confirmed that the pep-

Table 1. *Clinical Manifestations in NP1$^+$, NP2$^+$, and NP$^-$ Rats Carrying a Disease-prone B27/hβ2m Transgene Locus*

Sex	Genotype	Total	WBC at 3 mo $\times 10^{-3}$	Joint swelling		Microscopic joint abnormality*	Diarrhea		Proximal colon IL-1β
				n affected	Age onset		*n* affected	Age onset	
		n			*d*			*d*	*pg/mg*
Male	NP1$^+$	25	27 ± 10‡ (21)§	6	128 ± 25	0/7	24	76 ± 25	19 ± 16 (8)
	NP2$^+$	7	28 ± 12 (5)	4	114 ± 29	1/2	7	87 ± 21	ND
	NP$^-$	22	20 ± 9 (13)	15	128 ± 22 (11)	0/3	21	86 ± 24 (19)	35 ± 35 (7)
Female	NP1$^+$	20	31 ± 12 (19)	1	145	0/4	20	69 ± 15	22 ± 8 (2)
	NP2$^+$	6	23 ± 5 (5)	2	146 ± 6	0/3	6	76 ± 9	ND
	NP$^-$	22	25 ± 6 (17)	3	142 ± 26	2/7	22	79 ± 16	24 ± 4 (3)

Arthritis was found in 6 of 25 NP1$^+$ males and 19 of 29 NP1$^-$ males (χ^2 with Yates' correction = 7.71, $P = 0.005$).
*Number of rats with no clinically evident arthritis that showed microscopic joint abnormalities (mild periarthritis and peritendonitis in one NP2$^+$ male and mild synovitis in two NP$^-$ females), out of the 26 examined.
‡Mean ± SD (WBC of healthy 21-4L rats of this age average 9,500 ± 1,500).
§Number of rats for which a determination was made.

tide species in this fraction all derived from the transgene, both the expected NP383-391 peptide and an unexpected series of chemical derivatives of this peptide. Determination of the origin and nature of these derivatives is in progress. However, for the main purpose of this study, the most significant characteristic of these derivatives is that all were bound to B27.

The pattern of ^{3}H-labeled peptides eluted from B27 molecules differed dramatically between the NP1$^+$ and NP$^-$ or NP2$^+$ rats, and the findings suggested that ~90% of the endogenous peptides normally binding to B27 were displaced by the NP1-encoded peptide. This was supported by the substantial inhibition of CTL recognition of endogenous B27-presented peptides in B27$^+$NP1$^+$ targets. The reduction of endogenous antigenic peptide seen in these experiments was probably greater than twofold, because of the sigmoidal nature of peptide/CTL lysis dose response curves (17, 26). These results indicate that the strategy to displace endogenous B27-bound peptides through expression of the transgene construct was successful. To our knowledge, this is the first report of the targeting of a highly expressed MHC class I–presented peptide antigen to the ER via a transgene.

Although arthritis was suppressed in the NP1$^+$ rats, there was no pronounced effect of this transgene construct on gut inflammation. This finding is consistent with the possibility that peptide specificity is of less overall relevance in the development of colitis than of arthritis. However, the finding does not necessarily preclude the need for peptide specificity in the pathogenesis of colitis in the B27 transgenic rats. We have previously shown that germ-free B27 transgenic rats do not develop either gut inflammation or arthritis (14, 25). Since luminal bacteria are abundant in the gastrointestinal tract, it may be that in the gut but not in the joint the generation of a putative B27-presented, disease-related antigenic peptide from an intracellular bacteria is quantitatively sufficient to overcome the blockade imposed by the NP1 transgene. Alternatively, class I molecules are capable of acquiring exogenous antigen (27), and if this were the mechanism operating in the gut to induce B27-related colitis, then in this case the nature of the peptides acquired by B27 molecules in the ER, whether endogenous or arising from the NP1 transgene, would presumably be relatively unimportant. Although the spondyloarthropathies in humans are strongly associated both with HLA-B27 and with gut inflammation (which can range from subclinical histologic changes to classic ulcerative colitis or Crohn's disease), there is no particular association between HLA-B27 and classic inflammatory bowel disease in the absence of arthritis (reviewed in reference 28). This is quite different from the case of the disease-prone lines of the B27 transgenic rats, in which marked colitis has virtually a 100% prevalence and usually develops before any arthritis is seen (13). In this respect, the disease in rats most resembles human reactive arthritis that arises after intestinal infection. Based on these observations, as well as on the findings that both the gut disease and arthritis in the B27 rats are abrograted by the germ-free state (14) and by the DA genetic background (29 and Taurog, J.D., S.D. Maika, N. Satumtira, M.L. Dorris, I.L. McLean, W.A. Simmons, A.T.

Le, A. Sayad, J.B. Splawski, J.A. Richardson, and R.E. Hammer, manuscript in preparation), we favor a model in which arthritis in the rats is dependent upon gut inflammation. This model would not require the B27 transgene product to play the same role in the pathogenesis of the gut and joint disease, and the evidence from the current study at least suggests the possibility that it in fact does not. Consistent with this interpretation is the finding that rats transgenic for a B*2705 gene with a mutation in the B pocket (67Cys→Ser) develop severe gut disease but very little arthritis (Taurog, J.D., S.D. Maika, N. Satumtira, M.L. Dorris, I.L. McLean, W.A. Simmons, A.T. Le, A. Sayad, J.B. Splawski, J.A. Richardson, and R.E. Hammer, manuscript in preparation).

Because this disease has only been observed in rats with high gene copy number and supraphysiologic expression of the B27 and hβ2m transgenes (13, 15), it has been of some concern whether the role of B27 in the rat disease is similar to that in the human spondyloarthropathies. There is no evidence that the disease is simply an artifact of high HLA class I expression, since control rats with equally high expression of HLA-B7 or HLA-Cw6 do not develop this disease and the overwhelming majority of these rats remain healthy (30 and Taurog, J.D., S.D. Maika, N. Satumtira, M.L. Dorris, I.L. McLean, W.A. Simmons, A.T. Le, A. Sayad, J.B. Splawski, J.A. Richardson, and R.E. Hammer, manuscript in preparation). Moreover, as already noted, the rat disease resembles the human spondyloarthropathies in its relationship to the gut flora, modifying background genes, and a requirement for T cells, as well as in phenotype. Previous investigation of the cellular pathogenesis has suggested that both $CD4^+$ and $CD8^+$ can separately transfer disease to athymic B27 transgenic rats (9). However, the recipients of these transferred cells predominantly exhibited gut and skin disease, with very little arthritis. Thus, it is not yet clear which effector cells mediate arthritis in these rats. Moreover, even if CD4 cells were found to be effectors of arthritis, this would not preclude the requirement for the antecedent participation of B27-restricted, peptide specific, CD8 T cells, for example through a mechanism involving epitope spreading (31). Part of the rationale for the present study was the observation that high B27 expression was required for disease expression. This suggested that an arthritogenic peptide may be presented above a critical threshold level in the high transgene copy rats. If so, it would be predicted that disease would be prevented or suppressed by reducing the level of presentation of this putative peptide. As noted above, the present data suggest that distinct cellular processes and molecular recognition events may be operating in the pathogenesis of the arthritis and gut disease in the B27 rats, and they provide the best evidence to date that B27 presents a specific peptide at some stage in the development of arthritis in these animals.

Further work will be needed to gain insight into the mechanism by which arthritis is suppressed. The effect may be occurring in the peripheral immune system and/or at the level of thymic selection, and these possibilities can be better investigated once the NP lines are sufficiently inbred to carry out thymus graft and cell transfer experiments. Finally, it remains formally possible that the critical influence of the NP1 transgene product on disease pathogenesis is not exerted through displacement of a peptide or set of peptides that is recognized by conventional peptide-specific T cells, but rather on some aspect of the metabolism of the B27 molecules in a critical intracellular compartment. This might make a difference, for example, if the critical role of B27 in arthritis were to provide a B27-derived peptide presented by MHC class II, as has been hypothesized (32). However, the similar levels of surface-expressed B27 and of ^{3}H-Arg incorporated into immunoprecipitated B27 in the $NP1^+$, $NP2^+$, and NP^- rats tend to weigh against this possibility.

There are 12 known subtypes of HLA-B27. Epidemiologic studies of disease association have been carried out for seven of them, B*2702, -03, -04, -05, -06, -07, and -09; the others are too rare and/or too recently discovered to have been studied or to have yielded susceptibility data (1, 2, 33). Of these seven subtypes, two, HLA-B*2706 and -B*2709, have shown little or no association with the spondyloarthropathies in recent epidemiologic studies (33–36). These two subtypes differ from the disease-associated subtypes at position 116, in the floor of the F-pocket of the peptide binding groove, and B*2706 also differs at an adjacent floor position, 114. The major effect of these differences would be expected to be exerted on the spectrum of peptides accommodated by the binding groove, particularly at the peptide COOH terminus, and indeed significant differences in this regard have been identified (37, 38). The data from these recent studies of the B27 subtypes and from the experiments in B27 rats reported here thus both support the "arthritogenic peptide" hypothesis. Similar structural correlations with genetic epidemiology have also implicated peptide binding in the pathogenetic role of other disease-associated HLA alleles (reviewed in 39).

Studies of the B27 subtypes have also indicated that the peptides eluted from the disease-prone subtypes B*2702, -04, and -07, unlike those from B*2705 (and also -01, -03, and -10), do not include peptides with positively charged COOH termini. These data suggest that if there is a common arthritogenic peptide bound by all of the disease-prone subtypes, it most likely carries an aliphatic or aromatic COOH terminus (reviewed in references 1, 2). This may explain our previous observation that polymorphism of the MHC-linked peptide transporters in the rat had no significant effect on disease in B*2705 transgenic rats, despite an appreciable influence on peptide presentation by B27 (10), since this polymorphism would not necessarily affect transport of peptides with noncharged COOH termini (24, 40).

In summary, this study in transgenic rats provides evidence consistent with recent findings in humans that the peptide specificity of B27 is critical to its role in enhancing susceptibility to the spondyloarthropathies. Further work in the B27 transgenic rat system may help to identify the relevant peptides and the mechanism by which they induce arthritis. This study also suggests the potential feasibility of gene therapy specifically targeting peptides to particular MHC alleles.

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