The role of MHC class I heterodimer expression in mouse ankylosing enthesopathy

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Abstract Ankylosing enthesopathy (ANKENT) is a spontaneous mouse joint disease with strikingly similar pathology to human HLA-B27-associated enthesopathies such as ankylosing spondylitis. In C57Bl/10 mice, transgenic HLA-B*2702 as well as H2 genes have been shown to be relative risk factors for ANKENT. To investigate the role of major histocompatibility complex (MHC) class I expression in disease pathogenesis, ANKENT occurrence was compared among β_2-microglobulin (β_2m) knockout littermates with or without transgenes for HLA-B*2702 and human β_2m. In the knockout phenotype lacking β_2m, ANKENT occurrence is significantly reduced ($P = 0.016$). In the absence of β_2m, B*2702 is not detected on the cell membrane, nor does it increase the risk for ANKENT. This means that the previous finding that HLA-B*2702 increases susceptibility to ANKENT in C57Bl/10 mice cannot be ascribed to a transgene insertion effect. Rather, in order to increase disease susceptibility, B*2702 must be coexpressed with mouse β_2m (mo-β_2m). In contrast, when HLA-B*2702 is expressed with β_2m of human origin, disease susceptibility is not affected. Thus, both $H2^b$-derived class I heterodimers and HLA-B*2702/mo-β_2m heterodimers contribute to ANKENT susceptibility.

Foreword

A major contribution by George D. Snell to the field of immunogenetics was his early recognition that H2 is the mouse major histocompatibility complex (MHC), and that there is an analogous and homologous MHC in every species. It was a great privilege to have witnessed his pioneering presentation of this topic (Snell 1968), when he visited the Prague MHC immunogenetics group in 1967. At that time Snell received favorably my recommendation to accept as co-worker Peter Demant, who was already well trained in animal and human MHC work. In 1968 I drove Jean and Rosita Dausset through spectacular scenery from Halifax, Nova Scotia to Bar Harbor, to their first meeting with George and Rhoda Snell. There, the similarity between H2 and HLA was discussed. Today it is difficult to appreciate how important it was at the beginning of our MHC era to visualize one homologous system in every species. Thus it is a privilege for us to dedicate a paper to that most ideal MHC subject – the HLA transgenic mouse – in the George Snell Memorial Issue of *Immunogenetics*.

Pavol Ivanyi

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Introduction

Although the association of the seronegative spondylarthropathies with HLA-B27 has been extensively documented, there is still no mechanistic explanation of how this *HLA* class I gene or its product contributes to disease. The leading current theory proposes that B27 exerts a pathogenic effect by presenting microbially derived (or induced) peptides to cytotoxic T cells (Benjamin and Parham 1990). However, other processes are likely to be involved as well, since HLA-B27 is not found in all patients with seronegative spondylarthropathies.

A helpful tool in questioning the pathogenic effect of the HLA-B27 molecule are transgenic and knockout (ko) animals. Our group has previously described a model for B27-associated joint disease called mouse ankylosing enthesopathy [(ANKENT) (Weinreich et al. 1995a)]. ANKENT is a naturally occurring joint disease characterized by progressive ankylosis of the entheses in the ankle and tarsal joints of the hind paw. The prevalence, pathology, and genetic risk factors for this disease have been described (Weinreich et al. 1995a), as well as the existence of environmental risk factors (Weinreich et al. 1995b, 1996). The pathology of ANKENT has remarkable similarities to that of human ankylosing spondylitis (Weinreich et al.

Table 1 Total numbers of mice examined

	Genotype			
mo-β_2m	–	–	–	–
mo HC	+	+	+	+
hu-β_2m	+	+	–	–
HLA-B27	+	–	+	–
	Phenotype			
mo-β_2m	–	–	–	–
mo HC	+	+	–	–
hu-β_2m	+	+	–	–
HLA-B27	+	–	–	–
Total	54	75	39	68

1995a). Also, it has been clearly established that the major histocompatibility (MHC) haplotype is a relative risk factor for ANKENT. In H2 congenic C57Bl/10 mice, ANKENT is more common in mice with the $H2^k$ haplotype than the $H2^b$ haplotype (Weinreich et al.1995a; Capkova and Ivanyi 1992). To help elucidate whether H2 class I gene expression is involved in ANKENT susceptibility, this study addresses the question of whether the disease occurs in β_2m knockout mice which do not express class I heterodimers at all.

It was previously shown that a transgene for HLA-B*2702 is a relative risk factor for ANKENT, reaching the highest significance in $H2^b$ mice (relative risk 9.4, $P < 0.025$, (Weinreich et al. 1995a). Doubly transgenic mice with hu-β_2m in addition to HLA-B*2702 have higher cell-membrane expression of HLA-B2702, but no increased risk for ANKENT as compared with HLA-B*2702 singly trangenic littermates (Weinreich et al. 1995a; Chopin et al. 1996). This suggests that HLA-B*2702/mo-β_2m heterodimers might be a relative risk factor, while HLA-B*2702/hu-β_2m heterodimers might be irrelevant in disease susceptibility. However, another interpretation could be that the B*2702 transgene is pathogenic through an insertion effect, and that the coexpression of β_2m is irrelevant to disease development.

In this study we sought to determine which of these explanations is correct. By introducing transgenes for HLA-B*2702 and/or hu-β_2m in β_2m knockout mice, we were able to answer the following questions: Does the B27 transgene have to be expressed as a heterodimer with β_2m in order to act as a relative risk factor for ANKENT, and if so, does the species origin of the β_2m make a difference?

Materials and methods

The establishment of β_2m knockouts by homologous recombination (Zijlstra et al. 1989) and doubly transgenic HLA-B*2702, hu-β_2m mice has been described previously (Krimpenfort et al. 1987). Mice were housed under conventional conditions. Males used as breeders were excluded from this study, since caging with females decreases the risk for ANKENT (Weinreich et al. 1996). The work reported here was approved by an animal experimentation ethics committee as required by Dutch law.

Cell surface expression of MHC class I and human β_2m was determined by FACS analysis of peripheral blood (or lymph node) cells using the following mouse antibodies: monoclonal anti-HLA-B27

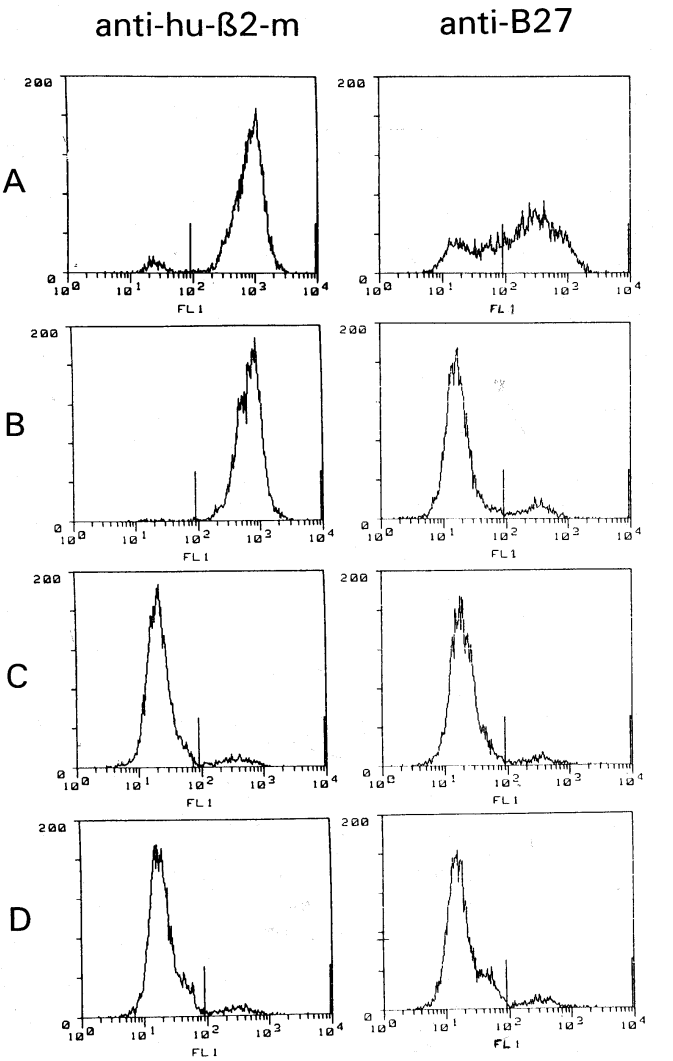

Fig. 1A–D Serological typing of B27 and hu-β_2m transgenic ko mice. FACS analysis with antibodies against hu-β_2m or HLA-B27, on PBL from β_2m ko mice transgenic for **A** B27 and hu-β_2m, **B** hu-β_2m, **C** B27, and **D** no transgenes

(TG1) and polyclonal anti-hu-β_2m (both produced in our laboratory), monoclonal anti-K^b [(Y3) (Hämmerling et al. 1982)], monoclonal anti-D^b (27.11.13, kindly provided by D. H. Sachs; Sachs et al. 1981), and monoclonal rat antibody reactive with mouse class I heterodimers [(M1/42) (Springer 1980)]. The secondary antibody was FITC-conjugated rabbit-anti-mouse F(ab′)$_2$ fragments (Dako, Glostrup, Denmark). To determine T-cell phenotype, cells were stained with CD3PE or a mixture of CD4PE and CD8FITC (Pharmingen, San Diego, CA).

*Genotyping for HLA-B*2702*

DNA was isolated from peripheral blood lymphocytes (PBL). The polymerase chain reaction (PCR)-sequence-specific primer (SSP) method was carried out with HLA-B*27-specific primers on a Corbett thermocytometer, resulting in a product of 222 base pairs (bp). Human controls positive or negative for HLA-B27 were included. PCR product was detected by electrophoresis on a 1.5% agarose gel (Olerup and Zetterquist 1991).

Clinical scoring for ANKENT was performed once per month, as described previously, up to the age of 12 months (Weinreich et al. 1995a). Briefly, paws were gently stretched and assigned a score of

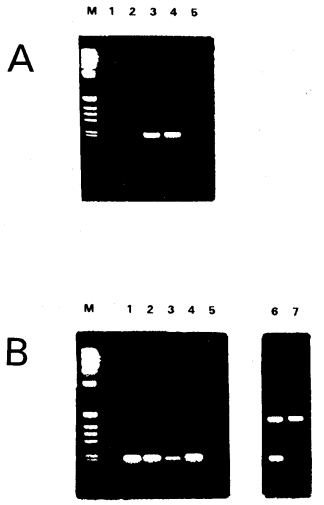

Fig. 2A, B PCR-SSP genotyping of HLAB*2702 in mice without expression of β_2m. Illustrative samples from a single experiment are shown. M = molecular weight markers. Lanes 1–5 on each gel represent different mice. **A** Lanes 4 and 5 respectively show the same mice as in Figure 1C and 1D. **B** Lanes 6 and 7 are human controls previously typed as $B27^+$ and $B27^-$, respectively

0–3 on the basis of stiffness. Scores of 1 or higher were considered positive. The person performing clinical screening was unaware of the segregating transgene genotype.

Statistical analysis

Cumulative incidence of ANKENT among the various groups of mice was compared using Lee Desu analysis (Lee and Desu 1972).

Results

Typing for transgenes in KO mice

Appropriate breeding crosses were performed to introduce transgenes for HLA-B*2702 and hu-β_2m into a β_2m ko strain. C57Bl/10 β_2m ko HLA-B*2702, hu-β_2m transgenic - × C57Bl/10 β_2m ko) F1 littermates were produced, with the genotypes as shown in Table 1.

Each animal was tested for cell surface expression of HLA-B2702 and hu-β_2m. Representative profiles are shown in Figure 1. HLA-B2702 was not detected serologically in mice without β_2m (Fig. 1, C and D).

In order to identify B*2702-positive mice in the absence of β_2m, all hu-β_2m-negative mice were tested for B*2702 by PCR. Figure 2 illustrates electrophoresis of PCR products, showing that B*2702-positive individuals could be identified. Figure 2A, lanes 4 (positive) and 5 (negative) represent the two serologically negative mice shown in Figure 1 C and D. The combined results of cell-membrane analysis and genotyping are shown in Table 1.

ANKENT incidence in the absence of mo-β_2m

Figure 3 shows the cumulative incidence of ANKENT in the four groups of mice studied. ANKENT occurred at low

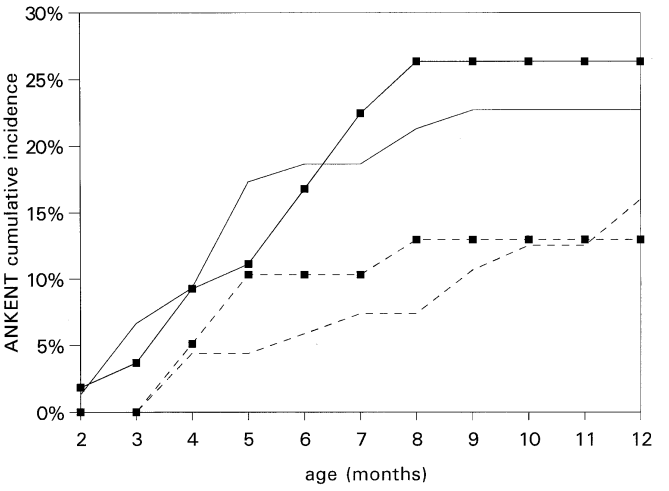

Fig. 3 Cumulative incidence of ANKENT in β_2m ko mice. Hu-β_2m is present (*solid line*) or absent (*dashed line*), and the presence of HLA-B27 is indicated by *square* symbols. Numbers of mice are shown in Table 1

frequency in mice devoid of class I heterodimers. Transgenic HLA-B*2702 did not increase the relative risk for ANKENT in these mice. Reconstitution of ko mice with hu-β_2m was found to increase disease occurrence significantly (P = 0.016, Lee Desu), but the *HLA-B*2702* transgene did not add to this effect.

Reconstitution of knockouts with hu-β_2m: consequences for class I expression and T-cell phenotype

The leading current hypothesis about the pathogenesis of HLA-B27-associated disease implicates the presentation of peptides by class I heterodimers to CD8-positive T cells. Therefore, further experiments were performed to characterize transspecies class I heterodimers and CD8 cells of hu-β_2m reconstituted ko mice. Figure 4 (A–C) shows that antibody binding to K^b and D^b were restored. As expected, the M1/42 epitope was not restored in the absence of mo-β_2m (Fig. 4 D).

We also questioned whether class I expression of mouse heavy chain with hu-β_2m permits thymic selection of CD8-positive cells, since it has previously been shown that β_2m ko mice have virtually no CD8-positive lymphocytes in peripheral blood (reviewed in Lehmann-Grube 1994). Figure 5 shows that CD8 expression in PBL is indeed normalized in hu-β_2m reconstituted mice. Moreover, the proportion of $CD4^+/CD3^+$ PBL is reduced to the wild-type level.

Discussion

Mouse class I heavy chain in ANKENT

Previously it was shown that in C57Bl/10 mice, both the H2 haplotype and transgenic HLA-B27 are relative risk factors for ANKENT (Weinreich et al. 1995a). This study demon-

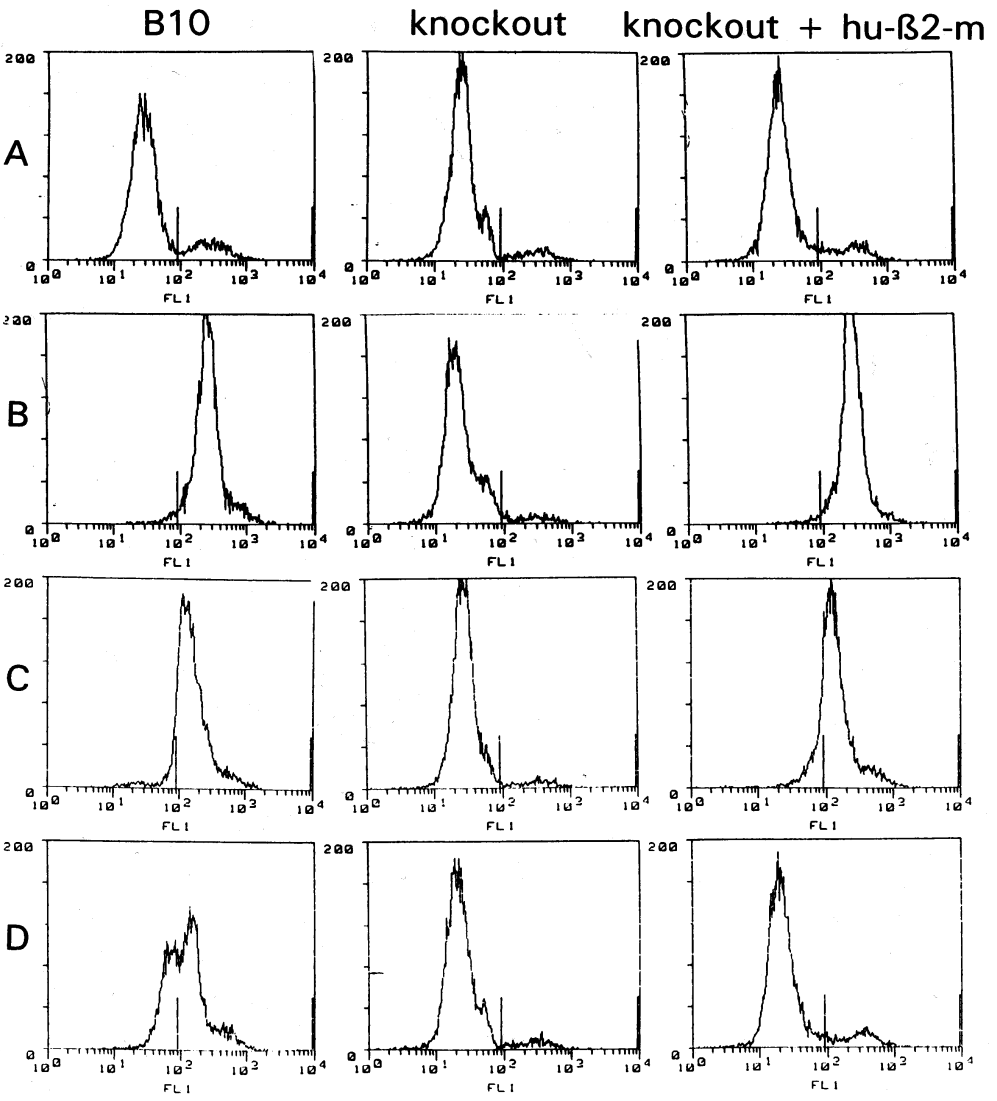

Fig. 4A–D Expression of H-2 epitopes in hu-β_2m reconstituted ko mice. FACS analysis on B10, ko, or hu-β_2m transgenic ko mice with the following antibodies: **A** negative control, **B** anti-K^b (Y3), **C** anti-D^b, and **D** anti-mouse class I heterodimers (M1/42)

strates that mice devoid of class I heterodimers still develop ANKENT, though at low frequency. Thus, class I heterodimers are not an absolute requirement for this disease. Several not mutually exclusive explanations are possible for this finding. First of all, other genes (including H2 class II genes) may be involved in ANKENT pathogenesis. Second, it has been shown that β_2m ko mice do express low levels of certain free class I heavy chains (Bix and Raulet 1992) and that they sometimes are functional in presenting peptides to cytotoxic T cells (Lehmann-Grube 1994; Zugel et al. 1994; Cook et al. 1995). Thus, it is possible that free H2 class I heavy chains are sufficient to effectuate disease. Finally, ko mice may be able to develop ANKENT due to the presence of residual CD8 cells, which have been demonstrated by others (reviewed in (Lehmann-Grube 1994).

Reconstitution of ko mice with hu-β_2m was found to increase disease occurrence significantly (Fig. 3, $P = 0.016$, Lee Desu). This contrasts with previous studies in normal, mo-β_2m expressing mice, where the hu-β_2m transgene is not a relative risk factor for ANKENT (Weinreich et al. 1995a; Chopin et al. 1996). Thus, hu-β_2m increased disease susceptibility only when it was rescuing class I heterodimer expression.

H2 class I epitopes in hu-β_2m-reconstituted ko mice

It was shown that hu-β_2m restored binding of allospecific antibodies to K^b and D^b. However, rat-anti-mouse Mab M1/42 binding was not restored. This antibody has previously been shown to recognize several K and D allotypes, though not separated heavy chain or mo-β_2m (Stallup et al. 1981). Our finding that M1/42 fails to recognize heterodimers on the basis of mouse heavy chain and hu-β_2m confirms a recent report by Bjerager and co-workers (1996). Differential reactivity of antibodies to MHC class I heterodimers, dependent on the species origin of β_2m, has been described before for the mouse anti-human, anti-monomorphic HLA class I Mab W6/32 (reviewed in Luscher et al. 1994), and is now confirmed for M1/42.

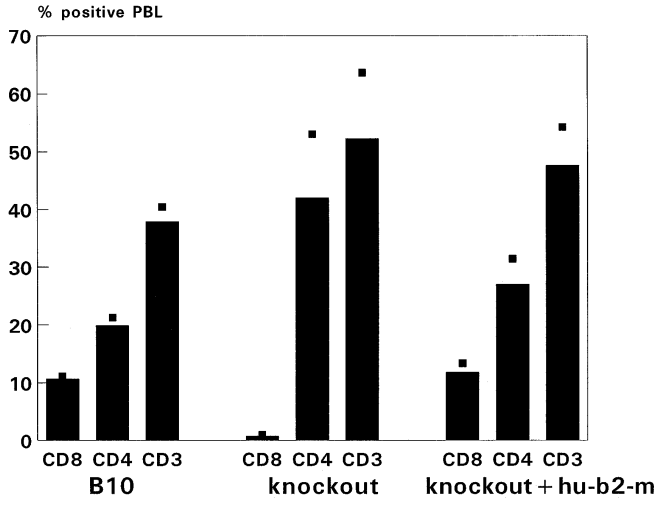

Fig. 5 T-cell phenotype of reconstituted ko mice. The Figure shows a representative experiment with 5 B10, 7 ko, and 5 hu-β_2m reconstituted ko mice. Bars represent average, and points show standard deviation

*B*2702 transgene*

It is shown in Figure 3 that the HLA-B*2702 transgene is not a relative risk factor for ANKENT in the absence of β_2m expression. This means that the B*2702 transgene does not act via an insertion effect, and/or as a free heavy chain, since it would then be expected to be active even in the β_2m knockout phenotype.

Figure 3 also shows that transgenic HLA-B*2702 is not an additional relative risk factor in β_2m ko mice reconstituted with hu-β_2m. In previous studies of wild-type (mo-β_2m-expressing) B10 mice, HLA-B*2702 clearly increased disease susceptibility (relative risk 9.4, $P < 0.025$) (Weinreich et al. 1995a). Taken together, the data suggest that B2702/hu-β_2m heterodimers do not contribute to ANKENT susceptibility, whereas B2702/μ-β_2m heterodimers do. B2702/hu-β_2m heterodimers may lack some functional property involved in ANKENT pathogenesis in the mouse. Human heavy chain and hu-β_2m have each been shown to be functional in mice. First, in singly transgenic mice with B*2702, the transgene is functional in antibody binding, antibody induction, recognition by CTL, CTL induction, skin-graft rejection, and viral antigen presentation (Kievits et al. 1989). Second, CD8 numbers are restored when mouse heavy chain expression is rescued by hu-β_2m (this study). However, it remains possible that B*2702/hu-β_2m heterodimers bind a different repertoire of peptides than do B2702/μ-β_2m. This could influence T-cell recognition.

Khare and co-workers have recently described a spontaneous inflammatory arthritis (SIA), which, in marked contrast to ANKENT, is found only in HLA-B27 transgenic, β_2m ko mice with no class I heterodimer expression (Khare et al. 1995). The ANKENT and SIA models have been described using different B27 transgenes (B*2702 and B*2705, respectively (Krimpenfort et al. 1987; Nickerson et al. 1990), as well as independently produced ko founders (Zijlstra et al. 1989; Koller and Smithies 1989, respectively). Moreover, ANKENT and SIA have distinct pathological characteristics: nail changes, synovial inflammation, and phalangeal joint involvement are present in SIA (Khare et al. 1995) but absent in ANKENT (Weinreich et al. 1995a). SIA occurred only when mice were moved from a specific-pathogen-free to a conventional unit, suggesting that an environmental trigger, in addition to abnormal transport or expression of B27 heavy chain, is involved (Khare et al. 1995). Once this environmental trigger is defined, its effect in the ANKENT model can be examined. In ANKENT an environmental trigger is also strongly suspected, since solitary caging protects animals from disease (Weinreich et al. 1996); however, this protective mechanism has not yet been elucidated. In contrast to SIA, abnormal cellular metabolism of B27 heavy chains is unlikely to play a role in ANKENT, because the B*2702 transgene increases ANKENT susceptibility only when it is coexpressed with mouse β_2m.

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