Proteasome Immunosubunits Protect against the Development of CD8 T Cell-Mediated Autoimmune Diseases

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*J Immunol* 2011; 187:2302-2309; Prepublished online 29 July 2011;
doi: 10.4049/jimmunol.1101003
http://www.jimmunol.org/content/187/5/2302

References

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Proteasome Immunosubunits Protect against the Development of CD8 T Cell-Mediated Autoimmune Diseases

Dietmar M. W. Zaiss,* Cornelis P. J. Bekker,* Andrea Gröne,† Benedicte A. Lie,‡ and Alice J. A. M. Sijts*

Exposure of cells to inflammatory cytokines induces the expression of three proteasome immunosubunits, two of which are encoded in the MHC class II region. The induced subunits replace their constitutive homologs in newly formed “so-called” immunoproteasomes. Immunosubunit incorporation enhances the proteasome’s proteolytic activity and modifies the proteasome’s cleavage-site preferences, which improves the generation of many MHC class I-presented peptides and shapes the fine specificity of pathogen-specific CD8 T cell responses. In this article, we report on a second effect of immunoproteasome formation on CD8 T cell responses. We show that mice deficient for the immunosubunits β5i/low molecular mass polypeptide (LMP7) and β2i/multicatalytic endopeptidase complex-like–1 develop early-stage multiorgan autoimmunity following irradiation and bone marrow transplantation. Disease symptoms are caused by CD8 T cells and are transferable into immunoproteasome-deficient, RAG1-deficient mice. Moreover, using the human Type 1 Diabetes Genetics Consortium MHC dataset, we identified two single nucleotide polymorphisms within the ββ5i/LMP7-encoding gene sequences, which were in strong linkage disequilibrium, as independent genetic risk factors for type 1 diabetes development in humans. Strikingly, these single nucleotide polymorphisms significantly enhanced the risk conferred by HLA haplotypes that were previously shown to predispose for type 1 diabetes. These data suggested that inflammation-induced immunosubunit expression in peripheral tissues constitutes a mechanism that prevents the development of CD8 T cell-mediated autoimmune diseases.

CD8 T cells play an important role in immune protection against intracellular pathogens and tumor growth; however, they also can be mediators of autoimmune disease. CD8 T cells recognize short peptide epitopes that are presented on the cell surface by MHC class I (MHC-I) molecules. These peptides usually are generated upon degradation of intracellular proteins by an abundant protease complex, the proteasome (1). The catalytic activity of proteasomes is exerted by three subunits, β1, β2, and β5, which are constitutively expressed in all cells. In hematopoietic cells and cells exposed to inflammatory cytokines, such as TNF-α and IFN-γ, these subunits are exchanged for three cytokine-inducible homologs, β1i/low molecular mass polypeptide (LMP2), β2i/multicatalytic endopeptidase complex-like–1 (MECL-1), and β5i/LMP7 (2, 3), resulting in the formation of immunoproteasomes.

The three immunosubunits have coevolved with the adaptive immune system, and two of these, β1i/LMP2 and β5i/LMP7, are encoded in the MHC class II (MHC-II) region, suggesting an important role in immune responses. Indeed, the cleavage site preferences of immunoproteasomes differ from those of constitutive proteasomes; consequently, the repertoire of peptides produced by immunoproteasomes differs from that produced by constitutive proteasome complexes (4, 5). These qualitative differences in peptide generation are reflected in the immunodominance hierarchy of pathogen-specific CD8 T cell responses, thus several epitopes that are dominant targets of the pathogen-specific CD8 T cell response in infected wild type (wt) mice are not targeted by CD8 T cells in immunoproteasome-deficient mice due to inefficient generation (6, 7). However, CD8 T cell responses to immunoproteasome-generated epitopes are not essential for clearance of most pathogens (5, 8). Recent studies indicated that, under conditions of IFN-induced oxidative stress, immunoproteasomes could play a more general role in the maintenance of protein homeostasis (9). Whereas IFN-exposed immunoproteasome-deficient cells were found to accumulate oxidant-damaged, polyubiquinated unfolded nascent proteins in aggresome-like induced structures, formation of such structures was only transient in cells with immunoproteasomes, as a result of the enhanced proteolysis.

Recently, it has become more appreciated that, in addition to CD4 T cells, the CD8 T cell subset plays an important role in tissue destruction during the progression of autoimmune disease (10–13). In this article, we show that mice, gene-deficient for β2i/MECL-1 and β5i/LMP7, develop CD8 T cell-mediated, early-stage, multi-tissue autoimmune syndrome after irradiation and bone marrow (BM) reconstitution. These data indicate that one important function of the immunosubunits, as well as their inducible
expression in inflamed tissues, is the prevention of CD8 T cell-mediated autoimmune reactions.

Materials and Methods

**Mice**

C57BL/6 (B6), B6 RAG1-, and β5i/LMP7 and β2i/MECL-1 gene-deficient mice (14), backcrossed >10 times onto the B6 background, were maintained by in-house breeding under specific pathogen-free conditions. RAG1+/− mice were crossed with β5i/LMP7 and β2i/MECL-1 gene-deficient mice to generate β5i/LMP7 and RAG1 gene-deficient mice. PSMB8 deficiency was determined by PCR, using the oligonucleotides 5′-CCGACCGGACAGCTTTAC-TACGTAGATG-3′ on PSMB8 or 5′-CCGACCGGACAGCTTTAC-TACGTAGATG-3′ as reverse primer. RAG1 deficiency was verified by flow cytometry, using anti-TCRβ and anti-CD19 Abs. To generate BM chimeric mice, BM cells flushed from the femurs of donor mice were depleted of mature T lymphocytes by incubation with anti-CD4 (clone GK1.5 and RL7A.2) and anti-CD8 mAb (clone 3–155) and guinea pig complement. Depletion efficiency was determined by TCRβ staining.Recipient mice were irradiated with 10 Gy as a single dose from an x-ray irradiator and were injected i.v. reverse primer. RAG1 deficiency was verified by flow cytometry, using b  

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**Glucose challenge**

After measurement of blood glucose levels, mice were deprived of food for ≥7 h. Fasting blood glucose levels were determined, and mice were injected i.p. with 1.5 mg/g glucose (Sigma) per gram body weight. Blood glucose levels were monitored.

**Vasopressin treatment and measurement of urine osmolality**

Mice were weighed and placed in metabolic cages with free access to water for 2 h. Access to water was removed: 1 h later, the mice were injected s.c. with 1 ng/g body weight vasopressin (DDAVP [Sigma]) dissolved in 200 μl PBS or were left untreated. Urine was collected at regular time intervals, and osmolality was determined by freeze-point reduction.

**T cell depletion and transfer of splenocytes**

Mice were injected twice i.p. with 200 μg purified CD4-depleting (GK1.5) or CD8-depleting (3–155) Abs. Efficiency of T cell depletion was checked in the spleen of one mouse, 2 d after the last treatment, by TCRβ staining and flow cytometry. For splenocyte transfers, β5i/LMP7 and β2i/MECL-1 gene-deficient mice were either irradiated and reconstituted with BM or left untreated. Six weeks after irradiation, when water consumption had increased, mice were sacrificed. Splenocytes were isolated, depleted of CD4 T cells using anti-CD4 mAbs and complement, and transferred into RAG1+/− or β5i/LMP7+/− and RAG1−/− mice at a ratio of 1:1 donor recipient.

**Conditional type 1 diabetes risk analysis**

Sixteen single nucleotide polymorphisms (SNPs) (rs4713598, rs3763365, rs3763349, rs9357155, rs9276810, rs2071543, rs2071541, rs3198005, rs1057373, rs4711312, rs4713600, rs991760, rs241419, rs17587, rs2071476, rs241417) covering the PSMB8 and PSMB9 genes (including 2 kb up-stream and downstream) were extracted for the Type-1 Diabetes Genetics Consortium (T1DGC) MHC fine-mapping dataset (consisting mostly of type 1 diabetes [T1D] families of white origin) and analyzed in combination with T1D-susceptibility determinants identified in previous fine-mapping studies (15), (i.e., HLA-B, HLA-C, HLA-DPB1, rs4122198, rs1619379, rs1611133, rs2394186, rs6926530, rs3130695, rs4713468, rs2246626, rs3132959, rs2076522, rs2187818, rs660895, rs456717, rs3116999, rs9560757, rs421446, rs399121, as well as HLA-DRB1-DQA1-DQB1). Linkage disequilibrium (LD) was calculated by HaploView. Preparation of the T1DGC MHC dataset, including re-coding of DRB1, DQA1, and DQB1 haplotypes, was described previously (15).

**IFN-γ-ELISPOT**

Ninety-six-well ELISPOT plates (Milenpore, Billerica, MA) were coated with 2 μg/ml AN18 (anti-mouse IFN-γ) in 100 μl PBS for ≥2 h at room temperature. Wells were then washed and blocked twice with RPMI 1640 medium. Splenocyte dilutions, starting with 1 × 10^6 cells/well, were incubated for ≥4 h in the presence or absence of 2 μg/ml synthetic peptide epitopes in 100 μl IMDM medium. Thereafter, the plates were washed with PBS plus 0.01% Tween 20, and IFN-γ was detected by incubation with 2 μg/ml biotinylated XMG1.2, followed by 1 μg/ml alkaline phosphatase-conjugated streptavidin (Jackson Immunobiology, Bar Harbor, ME) in PBS plus 0.01% Tween 20 supplemented with 2% BSA. The assay was developed with the Vector AP substrate kit (Vector Laboratories). The plates were then washed, dried, and analyzed using the A.EL. VIS ELIspot Reader (A.ELVIS, Hannover, Germany).

**MHC-I stability**

RMA-S cells, lacking the TAP transporter, were incubated with or without 60 μM synthetic peptide overnight (o/n), at 37°C, in protein-free hybridoma medium (Invitrogen, Carlsbad, CA). Cells were then harvested, washed three times with PBS, and chased at 37°C in the absence of peptide. Samples were taken after 0, 1, 2, and 4 h; washed with ice-cold PBS with 1% BSA and 0.02% NaN3 (PBS buffer); and stained for H2-Kb class I epitopes in 100 μl PBS containing with 2 μg/ml AN18 (anti-mouse IFN-γ) and biotinylated PE-conjugated streptavidin (BD Biosciences, San Diego, CA). Cells were analyzed on a FACS流入arib, using CellQuest software.

**Results**

**Immunosubunit-deficient mice develop signs of autoimmune disease following irradiation and BM reconstitution**

C57BL/6 (B6) β5i/LMP7 and β2i/MECL-1 double gene-deficient mice have a normal, healthy appearance (7, 14). However, following irradiation and BM transplantation, we found that β5i/LMP7 and β2i/MECL-1–deficient recipients developed different signs of potential autoimmune disease. The most striking symptoms were inflammation of the skin (dermatitis) (Fig. 1A) and dramatically increased water consumption (Fig. 1C), both of which were not observed in nonirradiated controls or wt recipients. Both β5i/LMP7 and β2i/MECL-1–deficient recipients of wt and of β5i/LMP7 and β2i/MECL-1–deficient BM consumed an enhanced amount of water (Fig. 1B), and water consumption progressively increased over time, until the mice drank ~10-fold more water per day than did untreated mice, which constituted about twice their body weight (Fig. 1C). Such an increase in water consumption far exceeds the regular variation between individual mice or increases over the lifespan of a mouse. Taken together, these findings suggested that β5i/LMP7 and β2i/MECL-1–deficient BM recipients had developed different autoimmune diseases.

**Development of central diabetes insipidus contributes to increased water consumption by immunosubunit-deficient BM-recipient mice**

Dramatic increases in water consumption often are caused either by diabetes insipidus, which results from a lack of function of anti-diuretic hormone (vasopressin) and often has an autoimmune etiology, or by insulin-dependent diabetes mellitus (IDDM), during which autoreactive T cells destroy the insulin-producing cells of the islets of Langerhans in the pancreas. To test the potential development of diabetes insipidus, irradiated BM-transplanted mice were deprived access to water, and urine production was measured. Although urine excretion by wt recipients rapidly decreased, β5i/LMP7 and β2i/MECL-1–deficient recipients continued to excrete urine (Fig. 2A), and the experiments had to be terminated when the mice had lost >20% of their body weight. Osmolality of the urine produced by β5i/LMP7 and β2i/MECL-1–deficient recipient mice did not significantly increase during water...
with two to four mice per group per cage. BM transplantation (stained with H&E (original magnification water (recipients developed dermatitis (donor mice (C), A (arrows). Urine production (vasopressin s.c. injected s.c. with physiological amounts of synthetic vasopressin mone (vasopressin) or from vasopressin hyposensitivity, mice were absence of water intake resulted from a lack of antidiuretic hor-

To determine whether the continuing urine production in the BM-recipient mice (ko) recipients of ko BM were deprived of water for the in-

CD8 T cell responses to islet β cell-associated Ags in immunosubunit-deficient BM recipients

Because the immunosubunits are components of the MHC-I Ag-processing pathway and determine which peptides are displayed on the cell surface, we determined whether CD8 T cells play a role in the autoimmune disorders observed in BM-recipient β5i/LMP7 and β2i/MECL-1–deficient mice. The Ags targeted during IDDM have been well studied, and several epitopes targeted by CD8 T cells during experimental diabetes in H-2b mouse models have been described (16). Therefore, we quantified CD8 T cell responses to these epitopes, as well as an IGRP-derived peptide selected for the presence of an H-2Kb–binding motif (see later discussion), in the spleens of the BM-recipient mice ex vivo, using an IFN-γ-ELISPOT assay (Fig. 4A, 4B). Significant responses were detected in the spleens of β5i/LMP7 and β2i/MECL-1–deficient mice but not in wt recipient mice or untreated β5i/LMP7 and β2i/MECL-1–deficient mice (data not shown) against each of the epitopes, including the newly selected IGRP-derived H-2Kb binder (Fig. 4B), although there was some variation in responses between individual mice. Remarkably, this epitope (IGRP 5 untranslated region [UTR] aa 7–14: RWISFIGV; Fig. 4C) is encoded by a 32-aa-long polypeptide that is translated from the first ATG codon of the IGRP-encoding mRNA sequence. The full-length IGRP protein is translated from the fifth ATG codon only. Restimulation with IGRP 5 UTR7–14 also expanded CD8 T cells in primary spleen cell cultures of immunosubunit-deficient, but

Immunosubunit-deficient BM recipients develop a latent form of IDDM

IDDM is well known to lead to enhanced water consumption and is further characterized by defective glucose uptake from the blood. To test for IDDM development, we assessed the blood glucose levels in irradiated BM-recipient mice. Individual β5i/LMP7 and β2i/MECL-1–deficient BM recipient mice displayed highly variable glucose levels; however, on average, they were not significantly increased over those in wt recipient mice (Fig. 3A). In contrast, following o/n fasting, the blood glucose levels in irradiated β5i/LMP7 and β2i/MECL-1–deficient recipient mice were significantly higher than those in irradiated wt recipients (Fig. 3B). In addition, glucose clearance from the blood after challenge was significantly delayed in irradiated β5i/LMP7 and β2i/MECL-1–deficient recipient mice (Fig. 3C). Analysis of the pancreas showed substantially enlarged pancreatic lymph nodes in β5i/LMP7 and β2i/MECL-1–deficient recipient mice, a loss of Langerhans islet fine structure, and infiltration of mononucleated cells (Fig. 3D), of which some were CD3+ (data not shown). Overall, no significant differences were observed in the size or frequency of islets of Langerhans in pancreata of β5i/LMP7 and β2i/MECL-1–deficient and wt recipients. Further histological analysis of β5i/LMP7 and β2i/MECL-1–deficient BM recipients revealed enlarged salivary glands, with substantially enlarged draining lymph nodes (data not shown). Taken together, we inferred that β5i/LMP7 and β2i/MECL-1–deficient recipients suffer from multiorgan disease.

CD8 T cell responses to islet β cell-associated Ags in immunosubunit-deficient BM recipients

Because the immunosubunits are components of the MHC-I Ag-processing pathway and determine which peptides are displayed on the cell surface, we determined whether CD8 T cells play a role in the autoimmune disorders observed in BM-recipient β5i/LMP7 and β2i/MECL-1–deficient mice. The Ags targeted during IDDM have been well studied, and several epitopes targeted by CD8 T cells during experimental diabetes in H-2b mouse models have been described (16). Therefore, we quantified CD8 T cell responses to these epitopes, as well as an IGRP-derived peptide selected for the presence of an H-2Kb–binding motif (see later discussion), in the spleens of the BM-recipient mice ex vivo, using an IFN-γ-ELISPOT assay (Fig. 4A, 4B). Significant responses were detected in the spleens of β5i/LMP7 and β2i/MECL-1–deficient mice but not in wt recipient mice or untreated β5i/LMP7 and β2i/MECL-1–deficient mice (data not shown) against each of the epitopes, including the newly selected IGRP-derived H-2Kb binder (Fig. 4B), although there was some variation in responses between individual mice. Remarkably, this epitope (IGRP 5 untranslated region [UTR] aa 7–14: RWISFIGV; Fig. 4C) is encoded by a 32-aa-long polypeptide that is translated from the first ATG codon of the IGRP-encoding mRNA sequence. The full-length IGRP protein is translated from the fifth ATG codon only. Restimulation with IGRP 5 UTR7–14 also expanded CD8 T cells in primary spleen cell cultures of immunosubunit-deficient, but
not wt, recipient mice, and analysis of the congeneric markers CD45.1 (present on cells derived from the BM donor mice) and CD45.2 (present on cells of the immunosubunit-deficient recipient mice) revealed that the IGRP 5’UTR7–14-reactive CD8 T cells derived from the BM donors (data not shown).

Taken together, we showed the presence of islet Ag-reactive CD8 T cell responses and identified a novel IGRP-derived epitope that is targeted in diseased BM-transplanted β5i/LMP7 and β2i/MECL-1–deficient B6 mice. The presence of islet Ag-specific CD8 T cells in the spleens of immunosubunit-deficient, but not of wt, recipients further supports our conclusion that the former mice develop a CD8 T cell-mediated latent form of IDDM following irradiation and BM reconstitution.

**The newly identified IGRP 5’UTR7–14 epitope binds MHC-I H2-K^b molecules with low affinity**

Autoreactive CD8 T cells often recognize self-peptides that bind their presenting MHC-I molecule with relatively low affinity (16). To determine the relative affinity of IGRP 5’UTR7–14 for MHC-I, we cultured the T cell line RMA-S, which lacks the TAP and, therefore, expresses “empty” MHC-I molecules on its cell surface, o/n with synthetic IGRP 5’UTR7–14 or with the control peptides OVA257–264 or TRP2180–188 (a self-peptide derived from a melanoma-associated Ag). As shown in Fig. 4D, IGRP 5’UTR7–14 stabilized the H2-K^b molecules on RMA-S, but to a lower extent than did TRP2180–188 or OVA257–264. During a subsequent chase in the absence of peptide, H2-K^b/IGRP 5’UTR7–14 complexes were lost from the RMA-S cell surface with a t_1/2 of ~30 min (Fig. 4E). In comparison, H2-K^b/OVA257–264 and H2-K^b/TRP2180–188 disappeared with a t_1/2 of 4 and 2 h, respectively. These data indicated that IGRP 5’UTR7–14, similar to most diabetogenic CD8 T cell epitopes identified in B6 models, binds its presenting MHC-I molecule (H2-K^b) with low affinity.

**Development of disease in immunosubunit-deficient BM recipient mice is CD8 T cell dependent and transferable into RAG1-deficient mice that lack immunosubunit expression**

Because the immunosubunits play a central role in MHC-I Ag processing, and autoreactive CD8 T cells were detected in diseased β5i/LMP7 and β2i/MECL-1–deficient mice, but not in wt recipient mice, we assumed that CD8 T cells played a causative role in the development of disease in BM-transplanted β5i/LMP7 and β2i/MECL-1–deficient mice. To test this hypothesis, immunosubunit-deficient BM recipients were injected with CD8 or CD4 T cell-depleting Abs 3 wk after irradiation, prior to the onset of enhanced water intake. Whereas CD8 T cell depletion prevented increases in water consumption, the depletion of CD4 T cells had no effect (Fig. 5A). Moreover, CD8 T cell depletion also abrogated the delay in glucose clearance (Fig. 5B) found in nondepleted β5i/LMP7 and β2i/MECL-1–deficient recipient mice following challenge (Figs. 2C, 5B). Thus, CD8 T cells play a causative role in the disorders observed in β5i/LMP7 and β2i/MECL-1–deficient recipient mice.

The notion that only the β5i/LMP7 and β2i/MECL-1–deficient recipient mice, reconstituted with either wt or β5i/LMP7 and β2i/MECL-1–deficient BM, developed autoimmune disease (Fig. 1A), in contrast to the wt recipients, led us to infer that not the T cell repertoire, but the absence of immunosubunit expression in the targeted tissues was a causative factor in the observed disorders. To test whether the absence of β5i/LMP7 and β2i/MECL-1 in the peripheral tissues of BM chimeras supports the development of autoimmune disease, RAG-1–deficient mice were backcrossed into β5i/LMP7-deficient background. CD4 T cell-depleted spleen cells of diabetic β5i/LMP7 and β2i/MECL-1–deficient BM recipients were adoptively transferred into these or control RAG1–deficient mice. At day 28 after transfer (Fig. 5C), water consumption by recipient β5i/LMP7- and RAG-1–deficient mice had significantly increased, up to ~10 ml/mouse/d, compared with untreated, age-matched mice that consumed ~5 ml/mouse/d. Water consumption by recipient control RAG-1–deficient mice was only slightly elevated, to ~6 ml/mouse/d, and transfer of CD4 T cell-depleted spleen cells of untreated β5i/LMP7 and β2i/MECL-1–deficient controls did not enhance the water consumption of β5i/LMP7 and RAG1–deficient mice (Fig. 5C).

Taken together, these data indicated that CD8 T cells are the main mediators of disease in the β5i/LMP7 and β2i/MECL-1–deficient BM recipients and that a lack of immunosubunit expression in the target tissue plays a causative role in the development of autoimmune disease.

**Haplotype-dependent association between β5i/LMP7 allelic SNPs and T1D development in a human population**

The data obtained so far indicated that immunosubunit expression provides protection against the development of autoimmune disease in mice. In humans, the T1DGC dataset, which contains data on 2957 SNPs across the MHC region for 2321 T1D families, has allowed the identification of multiple new genetic risk factors for T1D development, including several MHC-I alleles (15). Thus, to test whether immunosubunits play a role in the development of
autoimmune disease in a human population, we decided to analyze this dataset for associations between T1D and SNPs within the PSMB8 and PSMB9 genes, which encode the immunosubunits β5i/LMP7 and β2i/MECL-1, respectively. Both of these genes are located in the MHC-II region.

Disease-association analyses conditional on the main genetic HLA determinant DRB1*03–DQA1*0501–DQB1*0201 haplotype, the PSMB8 SNPs rs3763365 and rs9276810 increased the disease associations conferred by HLA-B8 to odds ratios (ORs) of 3.02 (95% confidence interval [CI], 1.9–4.7) and 3.1 (95% CI 2.1–4.8), respectively, and by HLA-B18 to ORs of 3.6 (95% CI, 1.9–6.9) and 4.2 (95% CI 2.2–8.1), respectively. For rs9276810, the predisposing, minor allele was carried by 41% (133/323) of DR3-B8+ subjects with T1D and 18% (37/207) of same-haplotype subjects without T1D. For rs3763365, the predisposing allele was carried by 35% (146/418) of DR3-B18+ subjects with T1D and 46% (23/50) of same-haplotype subjects without T1D. For rs3763365, the predisposing allele was carried by 35% (146/418) of DR3-B18+ subjects with T1D and 46% (23/50) of same-haplotype subjects without T1D. For rs9276810, the predisposing allele was carried by 35% (146/418) of DR3-B18+ subjects with T1D and 46% (23/50) of same-haplotype subjects without T1D. For the two SNPs, the frequency in the human population. In contrast to other HLA haplotypes, we found these associations to be particularly pronounced in combinations with the HLA-B8 and -B18 alleles (Table I). Thus, on the DRB1*03–DQA1*0501–DQB1*0201 haplotype, the PSMB8 SNPs rs3763365 and rs9276810 increased the disease associations conferred by HLA-B8 to odds ratios (ORs) of 3.02 (95% confidence interval [CI], 1.9–4.7) and 3.1 (95% CI 2.1–4.8), respectively, and by HLA-B18 to ORs of 3.6 (95% CI, 1.9–6.9) and 4.2 (95% CI 2.2–8.1), respectively. For rs9276810, the predisposing, minor allele was carried by 41% (133/323) of DR3-B8+ subjects with T1D and 18% (37/207) of same-haplotype subjects without T1D, as well as by 78% (146/186) of DR3-B18+ subjects with T1D and 46% (23/50) of same-haplotype subjects without T1D. For rs3763365, the predisposing allele was carried by 35% (146/418) of DR3-B18+ subjects with T1D and 46% (23/50) of same-haplotype subjects without T1D. Thus, the two SNPs occurred significantly more frequently in persons with T1D than in healthy persons carrying either the HLA-DRB1*03–DQA1*0501–DQB1*0201-B8 or -B18 haplotypes. The effects of HLA-B39 on disease association could not be determined because of the low frequencies of this class I allele among the T1DGC families.

In conclusion, our analyses identified two PSMB8 SNPs, rs3763365, located 1048 bp downstream, and rs9276810, in intron 3, with minor allele frequencies of 41.6% and 44.9% in the mainly white population selected for the T1DGC MHC fine-mapping data set, as genetic risk factors for T1D development.

Discussion

The immunosubunits, first discovered during efforts to obtain a complete sequence and gene map of the MHC region, are well known for being expressed in inflamed tissue, as well as for their dominant role in shaping the fine specificity of pathogen-specific CD8 T cell responses. To our knowledge, in this article, we demonstrate for the first time that immunosubunits also may protect the inflamed tissue against autoimmune CD8 T cell responses. Thus, our data showed that proteasome immunosubunit-deficient mice developed CD8 T cell-mediated multiorgan immunity, including a latent form of IDDM, following irradiation and BM transplantation. These findings, as well as the identification of two linked PSMB8–β5i/LMP7 allelic SNPs as haplotype-dependent risk factors for development of human T1D, indicated a direct link between immunoproteasome-mediated proteolysis or Ag processing and autoimmune disease in mice, as well as in humans. Apparently, these two linked SNPs mark a specific β5i/LMP7 allele, which occurs with a minor, but significant, frequency in the human population. In contrast to other β5i/LMP7 alleles, this β5i/LMP7 allelic form fails to confer disease protection, at least when expressed in the context of a predisposing HLA haplotype. The SNPs in PSMB8 that were found to be associated with T1D in this study did not fall in any of the regions mapped to harbor T1D-associated variants in previous studies (10, 15). This probably results from the fact that the association is most evident in combination with specific HLA-B alleles; thus, the conferred predisposition went unnoticed in the overall association analysis.
Most autoimmune diseases progress through different stages that are accompanied by complex immunological cascades, including activation of the innate immune system, chronic inflammation, epitope spreading of MHC-I- and MHC-II-presented epitopes, and recruitment of B cells, with each stage controlled by different susceptibility loci (17). We found that IDDM in immunosubunit-deficient recipient mice did not proceed further than a latent form, which was characterized by elevated blood glucose levels following o/n fasting and glucose intolerance; this led us to infer that immunoproteasomes may play a critical role, most likely in a very early stage of development of autoimmune disease. This conclusion would be in good agreement with previous studies in the NOD mouse model (18), showing that CD8 T cells mainly play a role in the early stages of disease.

It is not entirely clear how CD8 T cells, recognizing tissue-specific Ags, are activated in our mouse model. Different studies indicated that immunosubunit expression in lymphoid cells has diverse effects and can influence immune responses at different levels (e.g., by regulating cytokine production) (19–22). Because both immunosubunit-deficient mice reconstituted with wt or with immunosubunit-deficient BM developed disease (Fig. 1A), whereas disease was absent in wt recipients, we concluded that immunosubunit expression in the lymphoid compartments and T cell repertoire play a minor role at most. Thus, these findings suggest a direct regulatory role for the increased expression of immunosubunits in the development of autoimmune disease.

Table I. Association analyses of SNPs in the PSMB8 and PSMB9 gene regions conditional on the main genetic HLA determinants in T1D

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
<th>MAF</th>
<th>p Value*</th>
<th>HLA-B</th>
<th>B18</th>
<th>B8</th>
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<td>rs4713598</td>
<td>PSMB8</td>
<td>0.465</td>
<td>&lt;0.0006</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>&lt;0.009</td>
<td>1.2 × 10⁻⁴</td>
<td>7.7 × 10⁻⁷</td>
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<tr>
<td>rs3763349</td>
<td>PSMB8</td>
<td>0.398</td>
<td>&lt;0.03</td>
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<td>–</td>
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</tr>
<tr>
<td>rs9357155</td>
<td>PSMB8</td>
<td>0.186</td>
<td>–</td>
<td>–</td>
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<td>–</td>
</tr>
<tr>
<td>rs9276810</td>
<td>PSMB8</td>
<td>0.449</td>
<td>&lt;0.0009</td>
<td>&lt;0.02</td>
<td>1.2 × 10⁻⁵</td>
<td>5.7 × 10⁻⁸</td>
</tr>
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*The p values given are the least significant p value of either the main effect test or the homozygous parent transmission disequilibrium test.

-, not significant; MAF, minor allele frequency.
role of immunoproteasomes in inflamed tissue in deviating CD8 T cells from tissue-associated Ags.

We propose that the autoimmune phenotype observed in our model develops as a consequence of a wave of apoptotic cell death (23, 24), induced by irradiation of the recipient mice. Most likely, these T cells then expand under the lymphopenic conditions following irradiation, a particularly permissive environment for the initiation of autoimmunity (25, 26). A potential explanation for the particular susceptibility of immunosubunit-deficient mice to whole body irradiation-induced autoimmune CD8 T cell responses was provided by a recent study by Seifert et al. (9), who showed that immunoproteasome formation in cells exposed to IFN was essential for efficient removal of damaged defective ribosomal products that accumulated as a consequence of IFN-induced oxidative stress. Immunoproteasome deficiency increased the sensitivity of IFN-exposed cells to apoptosis and enhanced clinical scores during experimental autoimmune encephalomyelitis-induced inflammation in mice. A potential role for CD8 T cells was not addressed.

However, altered Ag processing in immunosubunit-deficient tissues, compared with immunoproteasome-sufficient tissues, may provide an alternative or complementary explanation for the development of CD8 T cell-mediated autoimmune reactions in recipients lacking immunosubunits. An enhanced sensitivity of immunosubunit-deficient tissue to autoreactive CD8 T cells is suggested by Fig. 5, showing that lymphocytes of diseased mice transfer disease symptoms to β2i/LMP7 and RAG1-deficient mice but barely to RAG1-deficient mice. The effects of immunosubunits on Ag processing are well documented (5). In particular, β2i/ LMP7-deficient cells display less MHC-I on their cell surface than wt cells do (14, 21, 27, 28), with levels restored to those on wt cells when cells are incubated with synthetic MHC-I ligands (data not shown). In other words, in the absence of immunoproteasomes, a relatively high percentage of MHC-I molecules associated with low-affinity binders traffic to the cell surface and disintegrate there. Such peptides, mainly exposed in immunosubunit-deficient tissues, may be the targets of autoreactive CD8 T cell responses.

Indeed, different studies suggested that the early autoimmune response in T1D-prone individuals targets peptides bound to MHC molecules with low affinity (16). Also, in mouse models of experimental diabetes, except for those in which foreign Ags are expressed from the rat insulin promoter, most described CD8 T cell targets are peptides that bind MHC-I molecules with low affinity (29, 30). The low affinity of the diabetogenic epitope IGRP 5i/UTR5–14, targeted in our model (Fig. 4), further supports this notion. In a recent study (31), diabetes in RIP-B7 transgenic mice (32), induced by immunization with an endoplasmic reticulum-targeted preproinsulin cDNA construct that contained a low-affinity H2-KK1–presented diabetogenic CD8 T cell epitope, was prevented by coimmunization with a second cDNA construct encoding a peptide that bound this MHC-I molecule with high affinity. These data demonstrated that outcompetition of diabetes-associated epitopes by higher-affinity MHC-I binders can prevent autoimmune disease. Conversely, we propose that when the Ag-processing pathway is disrupted at the level of efficient processing of high-affinity MHC-I binders, potential targets of autoimmune T cells will not be replaced upon inflammation, and a window of opportunity for autoreactive CD8 T cells will be created that ultimately may initiate autoimmune diseases.

It is tempting to speculate that a similar situation as we described in this article for β2i/LMP7 and β2i/MECL-1 gene-deficient mice after BM transplantation could occur in patients undergoing BM transplantation. These patients have to take immunosuppressive medication for the rest of their lives. So far, it has been assumed that such treatments may function mainly to prevent possible graft-versus-host side effects. However, our data suggest that, particularly in predisposed patient groups, the suppression of autoimmune diseases may also be an important aspect encouraging the use of such medication. Such a hypothesis is supported by a number of cases of de novo development of autoimmune disorders in patients following BM transplantations (33, 34).

In conclusion, we propose that immunoproteasome formation in inflamed tissues forms a protective mechanism that can prevent the onset or progression of autoimmune diseases.

Acknowledgements

We thank J. Jooles for helpful advice regarding measurements of urine excretion and osmolality and the use of vasopressin, J. Monaco for providing B6 β2i/LMP7 and β2i/MECL-1 gene-deficient mice, and M. Eike for preparation of the T1DGC MHC dataset.

Disclosures

The authors have no financial conflicts of interest.

References


