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Immunology

Molecular basis of genetic polymorphism in major histocompatibility complex-linked proteasome gene (*Lmp-2*)

(class I molecules/processing/peptides)

PAUL ZHOU, HONG CAO, MICHELE SMART, AND CHELLA DAVID*

Department of Immunology, Mayo Graduate School of Medicine, Rochester, MN 55905

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ABSTRACT Four genes, closely linked to major histocompatibility complex (MHC) class II genes, have been identified in humans, mice, and rats and are thought to be involved in the generation and transport of endogenous immunogenic peptides for the MHC class I antigen-processing pathway. The *Tap-1* and *Tap-2* genes presumably encode a heterodimeric protein complex responsible for transporting endogenous immunogenic peptides to the lumen of the endoplasmic reticulum. The *Lmp-2* and *Lmp-7* gene products are two subunits of the large cytosolic proteasome complex possibly involved in generation of endogenous peptides. To study the genetic polymorphism of the *Lmp-2* gene, we used a published cDNA sequence as a consensus sequence and PCR-amplified, cloned, and sequenced the *Lmp-2* gene from 12 inbred mouse strains. We found three amino acid variants, LMP-2^d, LMP-2^b, and LMP-2^q, which partially correlated with restriction fragment length polymorphism variants identified with Southern blots. Allelic polymorphism of the *Lmp-2* gene may be involved in peptide selection, leading to autoimmune disease susceptibility.

The major histocompatibility complex (MHC) encodes class I and class II glycoproteins that present peptides to T lymphocytes for immunorecognition. The class I glycoproteins present endogenous peptides to cytotoxic T lymphocytes, while the class II glycoproteins mainly present exogenous peptides to helper T cells (1). Until recently, the process of generating endogenous immunogenic peptides for class I was poorly understood (2). Recently, in the human, rat, and mouse systems, several genes clustered within the class II region have been discovered that are thought to be involved in generation and transport of endogenous immunogenic peptides for class I molecules (3–10). In mice, two putative transporter genes *Tap-1* (*Ham-1*) and *Tap-2* (*Ham-2*), encode a heterodimeric protein complex on the membrane of the endoplasmic reticulum and may be responsible for transport of endogenous peptides from the cytosol to the lumen of the endoplasmic reticulum. Two other genes, *Lmp-2* and *Lmp-7*, which are components of the large cytosolic proteasome complex, are believed to be involved in generation of endogenous immunogenic peptides by proteolysis. Although the detailed mechanism of how these genes are involved in these processes is still unknown, their close linkage to the MHC genes and their functional association in the class I antigen-processing pathway suggest that they may have coevolved with MHC genes for optimal functional interaction. Genetic polymorphism of these transporter genes has been found in human and rat systems (6, 11, 12). Furthermore, in the rat system, it has been shown that allelic polymorphism of the *Tap-2* (*mtp-2*) gene correlates with the functional polymorphism in terms of selectively transporting particular peptides for class I (12, 13). Allelic polymorphism of LMP2 and LMP7 has been demonstrated by serological and two-dimensional electrophoresis studies (14–16) as well as by molecular analysis (10). However, the extent of polymorphism at the genomic and cDNA sequence level and whether this polymorphism actually determines the functional polymorphism in terms of selection of the peptide repertoire to be presented by class I molecules is not known.

In both humans and mice, MHC genes have been found to be associated with autoimmune diseases (17). For example, in the mouse collagen II-induced arthritis model, H-$2A^q$ and H-$2A^r$ molecules have been found to be a major genetic predisposing factor for disease susceptibility (18). Similarly, the H-$2A^{g7}$ molecules in NOD mice are involved in their susceptibility to diabetes (19). Perhaps the polymorphic region of the peptide binding pocket of the class II molecules determines selection of certain autoimmunogenic peptide(s) to be presented to T lymphocytes. Alternatively, unidentified closely linked gene(s) may also contribute to the disease process. Thus, both the linkage of *Tap-1*, *Tap-2*, *Lmp-2*, and *Lmp-7* genes to the MHC and the genetic polymorphism of these genes could also be implicated in immunopathogenesis with regard to selection of particular autoimmunogenic peptide(s) for class I to activate self-reactive cytotoxic T lymphocytes.

In this study, the genetic polymorphism of the *Lmp-2* gene was studied by Southern blot analysis and cDNA sequencing in 12 inbred mouse strains. Among these inbred strains, three amino acid allelic variants and at least four genomic variants were identified. The implication of the allelic polymorphism of the *Lmp-2* gene in association with autoimmune diseases is discussed.

MATERIALS AND METHODS

Mice. Strains B6, B10, B10.RIII, RIII, B10.Q, SWR, DBA/1, BUB, B10.AQR, B10.BR, C3H.RKK, CBA/J, BALB/c, B10.D2, and DBA/2 were maintained in our breeding colony at Mayo Clinic (Rochester, MN). NOD mice were purchased from The Jackson Laboratory and maintained in our colony.

RNA Preparation and the First Strand of cDNA Synthesis. For isolation of total RNA, spleens of each mouse strain were removed, immediately frozen in liquid nitrogen, pulverized with mortar and pestle, and extracted by the guanidium/cesium chloride method (20). The first strand of cDNA was made by reverse transcription of 5 μg of total RNA, using Moloney murine leukemia virus reverse transcriptase and oligo(dT) in a final vol of 10 μl (21). After incubation at 37°C for 2 hr, 90 μl of RNase-free water was added to the reaction mixture. The samples were heated at 95°C for 10 min and

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Abbreviations: MHC, major histocompatibility complex; RFLP, restriction fragment length polymorphism.
*To whom reprint requests should be addressed.

chilled on ice for another 5 min before being used as templates for PCR.

PCR Amplifying and Cloning *Lmp-2* Gene. To amplify full-length coding sequences of *Lmp-2*, based on the published *Lmp-2* cDNA sequence (8), a pair of oligonucleotides with the *Sph* I site introduced at their 5′ ends (LMP2-I, 5′-CAC**GCATGC**CGAGCCCCGCTCTGCTGAGA-3′; LMP2-II, 5′-CTAT**GCATGC**GGGGAGGACGCTTCCCTCC-3′) were synthesized using a model 380A DNA synthesizer (Applied Biosystems) and purified through a Sephadex G-50 column. The concentrations of the oligonucleotides were measured by spectrometry (Spectronic 1001, Bausch & Lomb). Twenty-five microliters of each cDNA sample was mixed with each pair of oligonucleotides at 100 pM, 16 μl of dNTP mixture (125 μM each deoxynucleotide), 50 μl of reaction buffer (134 mM Tris·HCl, pH 7.7), 32 mM $(NH_4)_2SO_4$, 20 mM 2-mercaptoethanol, 20% dimethyl sulfoxide, 4 mM $MgCl_2$ (22), and 2 units of *Taq* polymerase (Perkin–Elmer) in a final vol of 100 μl. The reaction was carried out for 30 cycles (1 min at 94°C, 2 min at 55°C, and 1 min and 20 sec at 72°C). The PCR products were digested with *Sph* I and checked by electrophoresis on a 1.4% agarose gel (FMC). The gel-purified PCR products were then ligated into the *Sph* I site of the pGEM7 vector (Promega) and transformed into the bacterial cell line DH5a (GIBCO) by standard combined methods (20). The plasmid DNA from the positive clones was purified through a CsCl gradient.

DNA Sequencing. The purified double-stranded DNA templates described above were sequenced by dideoxynucleotide chain termination with four primers [T7, 5′-d(TAATACGACTCACTATAGGG)-3′; Sp6, 5′-d(GATTTAGGTGACACTATAG)-3′; LMP (III), 5′-d(CATCTTCTGTGCCCTCT)-3′; LMP2 (IV), 5′-d(GATGGTGACCAGGTA)-3′] (23). To minimize the artifacts introduced by PCR amplification, we have sequenced two to four cDNA clones with two different PCR amplifications.

Southern Blot Analysis. Restriction endonuclease-digested liver DNA (15 μg) was size-fractionated on a 0.8% agarose gel in Tris acetate buffer and transferred to a nylon membrane (Hybond N; Amersham). Blots were prehybridized at 60°C in a mixture containing 6× standard saline citrate (SSC), 5× Denhardt's solution, 0.1% SDS, and heat-denatured salmon sperm DNA (100 μg/ml) for 18 hr. Radiolabeled *Lmp-2*

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WEHI-3 (H-2d)          1 CCG-AGC-CCC-GCT-CTG-CTG-AGA-ATG-CTG-CGG-GCA-GGA-GCA-CCT-ACC-GCC-GGC-TCG-TTC-CGG
DBA/1 (H-2q)             --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
B10 (H-2b)               --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
RIII (H-2r)                                    - --- --- --- --- --- --- --- --- --- --- --- --- --- ---

WEHI-3                61 ACG-GAA-GAA-GTC-CAC-ACC-GGG-ACA-ACC-ATC-ATG-GCA-GTG-GAG-TTT-GAC-GGG-GGT-GTC-GTG
DBA/1                    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
B10                      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
RIII                     --- --- --- --- --- --- --- --- --- --- --- --G --- --- --- --- --- --- --- ---

WEHI-3               121 GTG-GGC-TCT-GAT-TCC-CGG-GTG-TCA-GCA-GGA-ACA-GCA-GTG-GTG-AAC-CGC-GTG-TTC-GAC-AAG
DBA/1                    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
B10                      --- --- --- --- --- --- --- --- --G --- --- --- --- --- --- --- --- --- --- ---
RIII                     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

WEHI-3               181 CTC-TCC-CCT-CTG-CAC-CAG-CGC-ATC-TTC-TGT-GCC-CTC-TCG-GGT-TCC-GCT-GCT-GAT-GCC-CAA
DBA/1                    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
B10                      --- --- --- --- --- --- -A- --- --- --- --- --- --A --- --- --- --- --- --- ---
RIII                     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

WEHI-3               241 GCC-ATA-GCT-GAC-ATG-GCC-GCC-TAC-CAG-CTG-GAG-CTA-CAC-GGG-TTG-GAG-CTG-GAG-GAG-CCA
DBA/1                    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --G
B10                      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --G
RIII                     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --G

WEHI-3               301 CCC-CTC-GTT-CTG-GCT-GCT-GCA-AAC-GTG-GTG-AAG-AAC-ATC-TCC-TAC-AAG-TAC-CGT-GAG-GAC
DBA/1                    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
B10                      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
RIII                     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

WEHI-3               361 TTG-TTA-GCG-CAT-CTC-ATA-GTA-GCT-GGC-TGG-GAC-CAA-CGT-GAG-GGG-GGA-CAG-GTG-TAT-GGA
DBA/1                    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --A --- ---
B10                      --- --- --- --- --- --- --- --- --- --- --- --- T-- --- --- --- --- --A --- ---
RIII                     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --A --- ---
                                                                                       -BspEI-
WEHI-3               421 ACC-ATG-GGA-GGG-ATG-CTA-ATT-CGA-CAG-CCC-TTT-ACC-ATC-GGC-GGT-TCT-GGA-AGC-TCC-TAC
DBA/1                    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --C --- --- --- ---
B10                      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --C --- --- --- ---
RIII                     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --C --- --- --- ---

WEHI-3               481 ATT-TAC-GGT-TAT-GTG-GAC-GCA-GCT-TAT-AAG-CCA-GGC-ATG-ACC-CCT-GAG-GAG-TGC-CGG-CGT
DBA/1                    --- --T --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
B10                      --- --T --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
RIII                     --- --T --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

WEHI-3               541 TTC-ACC-ACA-AAT-GCC-ATC-ACT-CTG-GCC-ATG-AAC-CGA-GAT-GGC-TCT-AGT-GGG-GGT-GTC-ATC
DBA/1                    --- --- --- G-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
B10                      --- --- --- G-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
RIII                     --- --- --- G-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

WEHI-3               601 TAC-CTG-GTC-ACC-ATC-ACA-GCT-GCT-GGT-GTG-GAC-CAT-CGA-GTC-ATC-CTG-GGA-GAT-GAG-CTG
DBA/1                    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
B10                      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
RIII                     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

WEHI-3               661 CCA-AAA-TTC-TAC-GAT-GAG-TGA-CTG-ATC-CCC-AGA-AGT-CCC-TTC-CTT-GTT-TGT-AAT-AAA-CTT
DBA/1                    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
B10                      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
RIII                     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

WEHI-3               721 TCT-GGA-ACC-AGA-AGG-CTG-GTG-CGA-TGG-GCA-AAG-GTG-AAA-TAT-GTG-TAT-CAG-AGA-GAC-ACG
DBA/1                    --- --- --- --- --- -C- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
B10                      --- --- --- --- --- -C- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
RIII                     --- --- --- --- --- -C- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

WEHI-3               781 GTG-TGT-GGA-GGG-AAG-CGT-CCT-CCC
DBA/1                    --- --- ---
B10                      --- --- --- --- --- --- --- ---
RIII                     --- --- --- --- --- --- --- ---
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FIG. 1. *Lmp-2* nucleotide sequence polymorphism in four representative H-2 haplotypes. *Lmp-2* sequences from DBA/1 (H-2^q), B10 (H-2^b), and RIII/J (H-2^r) are compared with the published *Lmp-2* sequence derived from the cell line WEHI-3 (H-2^d) (8). *Lmp-2* sequences from DBA/2 (H-2^d), BALB/c (H-2^d), CBA/J (H-2^k), and C3H.RKK are identical to that of WEHI-3. Sequences from SWR (H-2^q), B10.Q (H-2^q), B10.AQR, and NOD (H-2^{g7}) are the same as that of DBA/1. Sequence of B6 (H-2^b) is identical to that of B10. Nucleotide substitution from T to C at position 469 has introduced an additional *BspEI* site.

cDNA probe (the PCR product described above for sequencing) was added and hybridization was carried out at 60°C for another 18 hr. Blots were washed twice with 0.1× SSC/0.1% SDS for 15 min at 60°C.

RESULTS

Nucleotide Sequence and Protein Sequence of *Lmp-2*. To study the genetic coevolution of the *Lmp-2* gene with MHC genes, we have PCR-amplified, cloned, and sequenced *Lmp-2* from inbred strains of H-2^{d} haplotype (DBA/2 and BALB/c), H-2^{k} haplotype (C3H.RKK and CBA/J), H-2^{b} haplotype (B6 and B10), H-2^{q} haplotype (DBA/1, SWR, B10.Q), as well as RIII/J (H-2^{r}) and NOD (H-2^{g7}) mice. The *Lmp-2* gene in the H-2 recombinant B10.AQR (K^{q}I^{k}) maps centromeric to I^{k} and should be of H-2^{q} origin (24). Fig. 1 shows the nucleotide sequence alignment of the *Lmp-2* genes from these inbred strains by using the published sequence of *Lmp-2* derived from the WEHI-3 cell line (H-2^{d}) as a consensus (8). It is clear that the nucleotide sequences of *Lmp-2* from 12 different inbred mouse strains follow the pattern of H-2 haplotypes. Different inbred strains with the same H-2 haplotype have identical *Lmp-2* sequences. Furthermore, the nucleotide sequences of H-2^{d} and H-2^{k} haplotypes and of H-2^{q} and H-2^{g7} are identical. Among the 806 nucleotides of the *Lmp-2* gene, there are 11 overall nucleotide substitutions in these inbred mouse strains. Comparison of H-2^{d} and H-2^{q} haplotypes showed 6 nucleotide substitutions, while H-2^{d} and H-2^{b} haplotypes showed 10 nucleotide substitutions. The *Lmp-2* gene of H-2^{r} has the same nucleotide sequence as that of H-2^{q} except for one position (G to A, nt 96). Between H-2^{b} and H-2^{q}, four nucleotide substitutions were identified. One of these substitutions (T to C, nt 469) introduces a *Bsp*EI site into the cDNA of H-2^{q}, H-2^{r}, H-2^{g7}, and H-2^{b} strains, which has been confirmed by *Bsp*EI digestion of these cDNA clones (data not shown).

As shown in Table 1, there are three amino acid changes (positions 60, 126, and 177) in the protein sequence of LMP2 between H-2^{d} and H-2^{b} haplotypes. All these changes involved charged amino acid residues (Arg to His, Arg to Cys, and Asn to Asp). H-2^{d} and H-2^{q} haplotypes showed only one amino acid change at position 177 (Asn to Asp). The amino acid sequence of LMP2 from H-2^{r} is identical to that of the H-2^{q} haplotype. Thus, at the protein sequence level, three amino acid variants LMP2^{d}, LMP2^{b}, and LMP2^{q} were found among these inbred strains.

Southern Analysis of Genomic DNA of LMP2. To further investigate coevolution of the *Lmp-2* gene with the MHC gene family, we performed restriction fragment length polymorphism (RFLP) analysis of 15 inbred mouse strains. Southern blots were screened with the cDNA *Lmp-2* probe described above. No RFLP was found when the genomic DNAs from these strains were digested with *Eco*RI, *Bam*HI, and *Bgl* II. However, digestion with *Msp* I yielded a RFLP pattern along the H-2 haplotypes (Fig. 2). Different strains with the same H-2 haplotypes have identical RFLPs. In addition, RFLPs between H-2^{d} and H-2^{k} haplotypes and between H-2^{q}, H-2^{b}, and H-2^{g7} haplotypes are identical. The H-2^{r} strain has a unique RFLP. Thus, RFLP variants identified by Southern blot with the *Msp* I digestion partially correlated with allelic polymorphism shown by cDNA sequencing.

DISCUSSION

The LMP complex, originally discovered in the early 1980s by Monaco and McDevitt (14), was recently demonstrated to be closely related to the cytosolic proteasome by their similarity in size, shape, and subunit composition (25). Because of its cytosolic location, its proteolytic activity, and the mapping of two of its genes, *Lmp-2* and *Lmp-7*, to the MHC region, this complex was assumed to catalyze endogenous immunogenic peptides for the MHC class I pathway. The close linkage of *Lmp-2*, *Lmp-7*, *Tap-1*, and *Tap-2* to the MHC and their putative functional association with class I molecules lead to the speculation that these genes may have coevolved with MHC genes to obtain optimal interaction. In the present studies, genetic polymorphism of the *Lmp-2* gene at the genomic and cDNA levels was found in 12 inbred mouse strains. Clearly, this polymorphism follows the H-2 pattern; i.e., different strains with the same H-2 haplotypes have identical *Lmp-2* sequences and RFLPs.

Based on amino acid sequences, three allelic variants were found. The LMP2^{d} allele includes LMP2 from H-2^{d} and H-2^{k}, H-2^{b} bears the LMP2^{b} allele, and LMP2 from H-2^{q,r,g7} is the LMP2^{q} allele. Between LMP2^{d} and LMP2^{b}, 10 nucleotide substitutions were found, which resulted in three amino acid changes at positions 60, 126, and 177. All these changes

Table 1. Amino acid allelic variants of LMP2

	LMP2 allele/H-2			
Position	d/d and k	b/b	q/g7 and q	r
25	gca	gca	gca	gcg
	A	A	A	A
42	gca	gcg	gca	gca
	A	A	A	A
60	cgc	cac	cgc	cgc
	R	H	R	R
66	tcg	tca	tcg	tcg
	S	S	S	S
93	cca	ccg	ccg	ccg
	P	P	P	P
126	cgt	tgt	cgt	cgt
	R	C	R	R
131	gtg	gta	gta	gta
	V	V	V	V
149	tct	tcc	tcc	tcc
	S	S	S	S
155	tac	tat	tat	tat
	Y	Y	Y	Y
177	aat	gat	gat	gat
	N	D	D	D

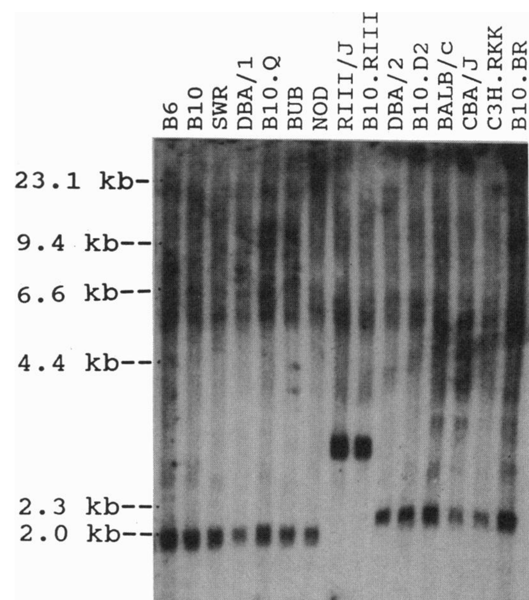

FIG. 2. Allelic polymorphism of *Lmp-2* gene by Southern blot analysis.

involved charged amino acid residues (Arg to His, Arg to Cys, and Asn to Asp). Between $LMP2^d$ and $LMP2^q$, there are six nucleotide substitutions but only one amino acid change at position 177 (Asn to Asp). These sequence results should explain the serological observations made several years ago by Monaco *et al.* (15). Anti-H-2 antisera [BALB.B anti-BALB/c and (BALB.B × B10.AQR)F_1 anti-B10.MBR] precipitated not only LMP antigens from the $H\text{-}2^d$ haplotype but also other distinct haplotypes. However, the antisera failed to precipitate LMP antigens from the $H\text{-}2^{b,q,p,r}$. Likewise, anti-$H\text{-}2^k$ antiserum (C.SW anti-C3H) recognized LMP antigens from $H\text{-}2^k$ and $H\text{-}2^d$ but not from $H\text{-}2^{q,r,b}$. Furthermore, cell lysates from $H\text{-}2^{b,q,r,p}$ failed to absorb anti-$H\text{-}2^d$ derived from BALB.B anti-BALB/c, but those from $H\text{-}2^{a,k,s,v,u,f,z}$ did. In the present study, there are three amino acid changes at positions 60 (Arg to His), 126 (Arg to Cys), and 177 (Asn to Asp) in the whole protein sequence between $LMP2^d$ and $LMP2^b$. However, $LMP2^b$ and $LMP2^q$ only share the Asp residue at position 177. Thus, it is very likely that the Asn residue at position 177 of the $LMP2^d$ allele must involve the antigenic epitope recognized by the anti-$H\text{-}2^d$ and anti-$H\text{-}2^k$ antisera mentioned above.

Demonstration of allelic variants in the *Lmp-2* gene among inbred strains raises the question as to whether this genetic polymorphism gives rise to functional polymorphism of the proteasome in terms of generating the endogenous peptide repertoire for MHC class I. The biochemical mechanism of how subunits of the proteasome complex are involved in generating endogenous immunogenic peptides is still unknown. Thus, it is difficult to speculate how allelic polymorphism of LMP2 could influence generation of the immunogenic peptide repertoire. Nevertheless, recent studies by Powis *et al.* (12) demonstrated that in the rat system, allelic polymorphism of the transporter gene *Tap-2* determines the peptides assembled in class I molecules. Furthermore, from the serological studies mentioned above and the present sequencing analysis, we can conclude that at least the polymorphic residue at position 177 is present on the surface of LMP2. Thus, it is possible that the different LMP2 allelic variants alone or in combination with the LMP7 allelic variants (16) may influence the endogenous immunogenic peptide repertoire for class I molecules.

Interestingly, the inbred strains $H\text{-}2^r$, $H\text{-}2^q$, and $H\text{-}2^{g7}$, which share the same $LMP2^q$ allele, are known to be prone to develop autoimmune diseases. $H\text{-}2^q$ and $H\text{-}2^r$ are the only haplotypes susceptible to collagen-induced arthritis, and NOD mice ($H\text{-}2^{g7}$) develop spontaneous insulin-dependent diabetes mellitus. In both these autoimmune diseases, H-2A molecules were previously indicated to be disease associated. However, recent studies in NOD mice, as well as in insulin-dependent diabetes mellitus patients implicated reduced surface expression of class I molecules in disease susceptibility (26). Currently, it is not clear whether disease susceptibility is due to the reduced class I expression or to sequence polymorphism of class II molecules. Studies are necessary to test whether the $LMP2^q$ allele contributes to alteration in the level of class I expression in these strains.

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