The Structure of H-2K\textsuperscript{b} and K\textsuperscript{bm8} Complexed to a Herpes Simplex Virus Determinant: Evidence for a Conformational Switch That Governs T Cell Repertoire Selection and Viral Resistance

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The Structure of H-2K\textsuperscript{b} and K\textsuperscript{bm8} Compressed to a Herpes Simplex Virus Determinant: Evidence for a Conformational Switch That Governs T Cell Repertoire Selection and Viral Resistance\textsuperscript{1,2}

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Polymorphism within the MHC not only affects peptide specificity but also has a critical influence on the T cell repertoire; for example, the CD8 \textsuperscript{T} cell response toward an immunodominant HSV glycoprotein B peptide is more diverse and of higher avidity in H-2\textsuperscript{bm8} compared with H-2\textsuperscript{b} mice. We have examined the basis for the selection of these distinct antiviral T cell repertoires by comparing the high-resolution structures of K\textsuperscript{b} and K\textsuperscript{bm8}, in complex with cognate peptide Ag. Although K\textsuperscript{b} and K\textsuperscript{bm8} differ by four residues within the Ag-binding cleft, the most striking difference in the two structures was the disparate conformation adopted by the shared residue, Arg\textsuperscript{62}. The altered dynamics of Arg\textsuperscript{62}, coupled with a small rigid-body movement in the \textalpha{} helix encompassing this residue, correlated with biased V\textalpha{} usage in the B6 mice. Moreover, an analysis of all known TCR/MHC complexes reveals that Arg\textsuperscript{62} invariably interacts with the TCR CDR1\textalpha{} loop. Accordingly, Arg\textsuperscript{62} appears to function as a conformational switch that may govern T cell selection and protective immunity. The Journal of Immunology, 2004, 173: 402–409.

The cytotoxic T cell response toward viruses is mediated by class I MHC molecules complexed to viral peptide Ags. These complexes are expressed on the surface of infected cells and are subsequently recognized by clonally distributed TCR on CD8\textsuperscript{T} lymphocytes. Appropriately armed and activated CD8\textsuperscript{T} cells can eliminate infected cells and prevent viral replication. The CD8\textsuperscript{T} cell response toward many viruses is extremely focused with viral eradication occurring through the recognition of only one or two immunodominant epitopes. Polymorphism in class I molecules not only diversifies selection of peptide Ags (1) but also broadens the T cell repertoire that is used to recognize pathogens. Even single amino acid differences in MHC molecules can exert profound effects on T cell repertoire selection and dictate subtle changes in peptide ligand selection (2) and Ag processing (3, 4, 38). The effect of the polymorphism may be to alter the thymic peptide repertoire and influence thymic positive and negative T cell selection, to alter the viral determinants presented during infection, or to present the same viral determinants in an altered conformation, resulting in selection of different T cell clonotypes. We have examined the CD8\textsuperscript{T} cell response to infection with HSV-1 in C57BL/6 (B6) mice to study the influence of polymorphism on antiviral responses at a molecular level. The response to HSV-1 in B6 mice is almost entirely focused on a single immunodominant determinant (glycoprotein B 498–505 (SSIEFARL)), which encompasses up to 90% of the total response. Moreover, in B6 mice, SSIEFARL-specific CD8\textsuperscript{T} T cells exhibit TCR V\textalpha{} usage (such that 70% use V\textalpha{}2 and V\textbeta{} usage bias (60% use V\textbeta{}10, and 20% use V\textbeta{}8S1) (5, 6), T cell clonotypes expressing these V\textalpha{} and V\textbeta{} pairings are selected from a diverse naïve repertoire.

Spontaneous mutations in the H-2K\textsuperscript{b} molecules of B6 mice has been used as a tool to investigate the role of MHC polymorphism in the immune response and were initially identified by their ability to elicit allogeneic T cell responses in wild-type mice (7). Of particular interest in our study is the mutant molecule H-2K\textsuperscript{bm8}, which differs from K\textsuperscript{b} at four amino acids (Y22→F, M23→I, E24→S, and D30→N) that are located within the Ag-binding cleft and are inaccessible to TCRs (8). The ability of H-2K\textsuperscript{bm8} (Bm8) mice to elicit allogeneic T cell responses, therefore, most likely reflects altered peptide repertoire and/or changes in MHC-peptide conformation expressed on the allograft, consistent with recent structures of monoallelic variants and analysis of their ligand repertoires (2, 9–13). The response of Bm8 mice to viral and other Ags has been studied in detail. CD8\textsuperscript{T} cell immunity to OVA is poor in Bm8 mice, yet this response in B6 mice is robust and dominated by the SIINFEKL determinant. This difference in response is due to poor K\textsuperscript{bm8}-restricted presentation of SIINFEKL (14, 15). In contrast,
comparable immune responses and equivalent protective immunity in both strains are seen following challenge with vesicular stomatitis virus (VSV) or Sendai virus (SEV). This observation suggests that both strains produce diverse naïve T cell repertoires capable of clearing pathogens using similar T cell determinants and hierarchies of immunodominance. Analysis of the structures of Kb and Kbm8 complexed to dominant peptide determinants from VSV and SEV revealed subtle conformational changes in the complexes, but these were not associated with major functional differences in the CD8+ T cells generated in B6 and Bm8 mice (8, 16, 17). However, it is remarkable that, although in HSV-1 infection, the SSIEFARL epitope dominates the CD8+ T cell response in both strains of mice, Bm8 mice are five times more resistant to lethal challenge with HSV-1 than B6 mice. This observation correlates with a more diversified repertoire and higher functional avidity of SSIEFARL-specific CD8+ T cells in Bm8 mice (18). The basis of this MHC-linked enhancement in antiviral resistance and T cell selection is not well understood but is thought to be related to differences in T cell selection in the two strains of mice. In this study, we investigate the structural influence of the MHC class I polymorphism between Kb and Kbm8 in the presentation and recognition of the SSIEFARL determinant and examine potential extrathymic mechanisms of clonotypic expansion based on the differences observed in the two structures.

Materials and Methods

Expression, purification, crystallization, and structure determination

Four amino acid substitutions differentiate H-2Kb from Kbm8. Truncated Kbm8 (aa 1–285) was generated by site-directed mutagenesis using a Kb-plasmid template (QuickChange; Stratagene, La Jolla, CA). Recombinant Kb and Kbm8 molecules were expressed as inclusion bodies, refolded, and purified, as previously described (19).

All crystallization trials were conducted using the hanging-drop vapor diffusion technique (20, 21). The crystals were grown under identical conditions for each allele, by mixing equal volumes of 10 mg/ml Kb and Kbm8 peptide complexes with the reservoir buffer (0.1 M sodium cacodylate, 0.2 M calcium acetate, and 14% (v/v) polyethylene glycol 8000 (pH 6.5)) and microseeded from crystals grown in 16% (v/v) polyethylene glycol 8000 at room temperature. Crystals were frozen following a stepwise transfer from 5 to 10% of the cryoprotectant glycerol with 5 min per condition. The crystals were transferred to a solution of 10 mM Tris and 150 mM NaCl (pH 8.0).

CD spectra were measured on a Jasco 810 spectropolarimeter using a thermostatically controlled cuvette at temperatures between 20 and 90°C as described in detail elsewhere (25–27). Far-UV spectra from 195 to 250 nm were collected and averaged over 10 individual scans; θ218 measurements for the thermal melting experiments were made at intervals of 0.1°C at a rate of 1°C/min. The midpoint of thermal denaturation (Tm) for each protein was calculated by taking the first derivative of the ellipticity data and identifying the inflection point. Both complexes were measured at 30 μg/ml in a solution of 10 mM Tris and 150 mM NaCl (pH 8.0).

Results

Greater thermostability of H-2Kb vs H-2Kbm8 complexes

The improved protective immunity against HSV observed in mutant Bm8 mice could reflect a higher affinity for the SSIEFARL determinant by Kbm8 molecules. This might lead to higher determinant density, which might explain the greater diversification of the T cell repertoire in this strain. Therefore, we examined the thermostability of the Kbm8-SSIEFARL complexes by CD. Both complexes gave similar spectra at 20°C, however, the Tm of Kb was found to be ~7°C higher than Kbm8 bound to the SSIEFARL peptide in two independent experiments (Tm of 61 and 54°C, respectively; Fig. 1), suggesting greater stability and longer half-life of Kbm8-SSIEFARL complexes in vivo. This finding is consistent with the lower half-life of surface Kb relative to Kbm8 bound to other viral Ags (8). In contrast, the lower stability of Kbm8 complexes indicates that the enhanced protective immunity to HSV in Bm8 mice is not the result of higher levels of Ag presentation and must therefore reflect altered selection of the T cell repertoire.

Impact of polymorphic residues on the conformation of the MHC-peptide complexes

The structures of Kb and Kbm8 complexed to the SSIEFARL have been determined to 2.0- and 1.8-Å resolution, respectively (Table I). Both complexes crystallize in the same space group under identical conditions with isomorphous unit cell dimensions. In addition, the freezing protocol for the complexes were identical. Accordingly, conformational differences that are observed between the two crystal structures can be attributed to the polymorphic amino acid differences between Kb and Kbm8. Moreover, all of the regions of interest that are discussed below do not participate in crystal contacts. Table II describes all peptide-H chain (hc) interactions in detail.

Within both Ag-binding clefts, SSIEFARL is bound in an extended conformation, with a small centrally located bulge around the relatively mobile Glu residue. Other surface-exposed residues are Ser, Ala, and Arg, which accordingly may play a role in contacting the TCR in both strains (Fig. 2, A and B). Three of the four polymorphic amino acids (positions 22, 23, and 24) between Kb and Kbm8 are clustered on the floor of the Ag-binding cleft, whereas the Asp50Asn substitution is located on a solvent-exposed loop remote from known sites of TCR recognition. Residues at 22, 23, and 24 are solvent inaccessible and located within the β2 strand of the cleft. Residues 22 and 24 project into the cleft, whereas residue 23 points toward the β2-microglobulin domain (Fig. 2, C and D).

In Kb, Glu24, found at the base of the B pocket, is only the polymorphic residue that directly contacts the peptide (Fig. 2C). The carboxylate of Glu24 forms a direct H-bond with P2-SerOγ of the bound peptide, as well as making H-bonds to the h c residues Asn70-Asn76 (located on the α1 helix) and the Tyr22-Oγ and Tyr45-Oγ groups. The aliphatic moiety of Glu24 also packs against the polymorphic Tyr22 residue, as well as ValP and PheE, a residue on the
The aromatic ring of Tyr23 residue packs against the backbone of the α1 helix, and the side chains of Asn70, Glu71, and Tyr74.

In Kbm8, the polymorphic residues do not directly contact the peptide. Glu24 is replaced by the smaller Ser residue. The Ser O\(^{\cdots}\)H-bond to the peptide, and the loss of the H-bonding network that is centered around Glu24 in Kb, and a H-bond to the α1 helix is also lost in the Kbm8 complex. Structural perturbations can be seen to flow on from position 24. In Kbm8, Tyr45 moves toward Ser24, such that the Tyr45-O\(_N\) groups are 1.0 Å apart in the respective structures (Fig. 2E), and the tilt of the aromatic ring of Tyr45 has changed by ~45°. In Kb, Tyr45 lies flat against the base of the α1 helix, whereas in Kbm8, the plane of the aromatic ring is more perpendicular to the axis of the α1 helix, nesting within the groove of the helix as well as participating in a unique water-mediated H-bond to P2-Ser of the peptide. The plane of the aromatic ring of Phe22, a residue that contacts the polymorphic residue at position 22, has also been adjusted by ~30° in the two structures. These polymorphism-mediated structural perturbations result in a local readjustment of the core-packing residues, resulting in a rigid-body shift in the α1 helix spanning from residues 62 to 73. These changes also influence the positioning and the dynamics of Arg62 (Fig. 2, A, B, and E).

![FIGURE 1. Thermostability of polymorphic H-2K molecules bound to SSIEFARL, as revealed by CD spectropolarimetry.](http://www.jimmunol.org/)

### Table I. Data collection and refinement statistics\(^a\)

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<td>1.74</td>
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\(^a\) The values in parentheses are for the highest resolution bin (approximate interval, 0.1 Å).

\(^b\) R\(_factor\) = \(\sum_{n=1}^{N} |F_{o}| - |F_{c}| / \sum_{n=1}^{N} |F_{o}| \) for all data except for 4%, which was used for the \(^d\) R\(_merge\) calculation.

\(^d\) R\(_merge\) = \(\sum_{hkl} \sum_{i=1}^{N} [I_{hi} - \langle I_{hi} \rangle]^2 / \sum_{hkl} \sum_{i=1}^{N} I_{hi} \) for all data except for 4%, which was used for the \(^d\) R\(_merge\) calculation.
In the Kb complex, the Arg62 side chain is disordered, with limited electron density observed beyond the Cβ atom of the Arg side chain. Attempts to model the Arg62 into a discrete conformation, such as that observed in the Kbm8 complex, resulted in significant negative peaks in the $F_o$ - $F_c$ difference maps, and discontinuous electron density for this side chain in the $2F_o$ - $F_c$ difference maps as well as high temperature factors. Moreover, simulated-annealing omit maps (28) confirmed the disordered nature of the Arg62 side chain for both molecules in the asymmetric unit (data not shown). In contrast, within the Kbm8 complex, the discrete conformation of Arg62 side chain (temperature factor of 44 Å²) was clearly evident in the initial electron density maps and later unambiguously confirmed using simulated-annealing omit maps. In the Kbm8 structure, Arg62 clearly forms a salt bridge with Glu63, and van der Waals contacts with Trp167, such that the side chain lies parallel to the α1 helix (Fig. 2, D and E). The slightly broader cleft in the Kb complex and more optimal packing of the B pocket associated with direct interaction of Glu63 with the peptide act in concert to mobilize Arg62.

**Discussion**

We have determined the high-resolution structures of Kb and Kbm8 complexed to the immunodominant HSV determinant gB498–505. These structures have revealed small rigid-body shifts in the α1 helix and changes in the H-bonding network associated with the four polymorphic amino acids, altered water structure, and changes in the position and dynamics of Arg62. The four polymorphic amino acids present between Kb and Kbm8 are not in a position to interact with the TCR directly, consistent with previous structures of related complexes (8). In our structures, the bound conformation of SSIEFARL in the Kb and Kbm8 complexes is very similar, with subtle structural perturbations evident toward the N terminus of the peptide. Subtle changes in the α helices bounding the peptide can impact upon T cell recognition and thymic selection, as highlighted, for example, by our recent studies on the ligand repertoire and conformation of HLA B44 allotypes (2). Moreover, differences in T cell selection by Kb and Kbm8 have previously been documented (15) and suggested that altered peptide repertoire and conformation of MHC-peptide complexes can impact significantly on T cell selection in B6 and Bm8 mice. Consistent with this concept was the ability of certain mAb to differentiate between cells expressing Kb and Kbm8 (17).

The substitution of Glu24 to Ser24 in Kbm8 not only substantially reduces the electrogative potential of the B pocket of these molecules but also results in the loss of a direct H-bond between the peptide ligand and the peptide ligand. A series of conformational adjustments are observed to compensate for the loss of this interaction with changes in the hydrophobic packing of the B pocket and surrounding residues evident. Interestingly, the loss of these interactions probably accounts for the considerable decrease in the thermostability of the complex as measured by CD and increased crystallographic temperature factors for the peptide. Earlier epitope stabilization studies (29) have demonstrated that Kbm8 molecules expressed on TAP-deficient cells bind to SSIEFARL in a manner similar, with subtle structural perturbations evident toward the N terminus of the peptide. Subtle changes in the α helices bounding the peptide can impact upon T cell recognition and thymic selection, as highlighted, for example, by our recent studies on the ligand repertoire and conformation of HLA B44 allotypes (2). Moreover, differences in T cell selection by Kb and Kbm8 have previously been documented (15) and suggested that altered peptide repertoire and conformation of MHC-peptide complexes can impact significantly on T cell selection in B6 and Bm8 mice. Consistent with this concept was the ability of certain mAb to differentiate between cells expressing Kb and Kbm8 (17).

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comparable to the wild-type K\textsuperscript{b} molecules. Taken together with the lower thermostability of K\textsuperscript{bm8} complexes, we predict that the stability of these surface-loaded molecules will be much reduced in K\textsuperscript{bm8}, as observed for VSV8 and SEV9 peptides (8). In addition to the conformational changes directly associated with the four polymorphic amino acids, we also see changes in the orientation and mobility of Arg\textsuperscript{62}, a shared residue on the hc $\alpha_1$ helix. Comparison of the structures of previously reported shared viral ligands of K\textsuperscript{b}

**FIGURE 2.** Cut-away view of the Ag-binding clefts of K\textsuperscript{b} (A) and K\textsuperscript{bm8} (B) bound to the SSIEFARL. The 2.0- and 1.8-Å electron density omit maps of SSIEFARL complexed to the respective H-2K molecules are also indicated. Very similar conformations of the peptide were observed, highlighting the exposed Ser\textsuperscript{1}, Glu\textsuperscript{4}, Ala\textsuperscript{6}, and Arg\textsuperscript{7} residues. Analysis of H-bond and van der Waals contacts contributed to by polymorphic amino acids in K\textsuperscript{b} (C) and K\textsuperscript{bm8} (D) are shown in the same orientation as the views in A and B, respectively. These representations were also superimposed (E) to highlight the mobility of the Arg\textsuperscript{62} residue and the rigid-body shift in the $\alpha_1$ helix (residues 62–73). The K\textsuperscript{b} hc is shown in cyan, whereas K\textsuperscript{bm8} is shown in green. The peptide ligands are shown in orange and yellow for K\textsuperscript{b} and K\textsuperscript{bm8}, respectively.
and K<sup>bmn</sup> also reveal variability in the orientation of Arg<sup>62</sup> (8). A role for the positioning and dynamics of Arg<sup>62</sup> in locking in the N termini of bound peptides in HLA B27 complexes has also recently been noted (9). Moreover, a ligand-dependent switch in the orientation of Arg<sup>62</sup> has been observed in structures of HLA B8 complexed to EBV (FLRGRAYGL) and HIV (GGKKKYKL) epitopes (30, 31). The Phe group of the EBV determinant sterically restricts positioning of Arg<sup>62</sup>, forcing it to project into the solvent, whereas the Arg<sup>62</sup> packs down in B8/GGKKKYKL structure in a manner analogous to our K<sup>bmn</sup> structure. Fig. 3A highlights this remarkable variability in the positioning of Arg<sup>62</sup> in selected unligated MHC-peptide complexes.

In all the class-I/TCR complexes that we have examined, where an Arg<sup>62</sup> is present on the MHC helix, it interacts with CDR1<sub>α</sub>. This suggests that, regardless of the positioning of Arg<sup>62</sup> in the unligated state, once the MHC molecule is engaged by the TCR, Arg<sup>62</sup> interacts with the CDR1<sub>α</sub> loop. The flexibility of Arg<sup>62</sup> was observed to accommodate the differing CDR1<sub>α</sub> footprint (Fig. 3B). Thus, we predict that Arg<sup>62</sup> of K<sup>b</sup> and K<sup>bmn</sup> will also interact with the CDR1<sub>α</sub> of anti-HSV TCRs (22). We also propose that Arg<sup>62</sup> functions as an electrostatic guide for TCR docking in general, and thus changes in the dynamics and positioning of Arg<sup>62</sup> can impact on T cell selection.

During infection with HSV, B6 CD8<sup>+</sup> T cells only recognize SSIEFARL bound to cognate K<sup>b</sup> molecules and not to the K<sup>bmn</sup> allelic variant. Conversely, a subset of Bm8 CD8<sup>+</sup> T cells cross-react on K<sup>b</sup> targets presenting the SSIEFARL determinant (6). The ability of T cells to discriminate between complexes suggests the conformational differences observed have functional relevance. Analysis of TCR usage in HSV infection has revealed that Bm8 mice use more diverse V<sub>α</sub>/V<sub>β</sub> combinations, whereas B6 mice use dominant V<sub>α</sub>2, V<sub>β</sub>10, and V<sub>β</sub>8 gene families (Table III). Differences in the structures of the complexes studied here are focused around the N terminus of the ligand and surrounding regions of the α<sub>1</sub> helix and can only, assuming a conserved diagonal TCR docking framework, directly affect the V<sub>α</sub> chain. Any bias in the β-chain most likely reflects the particular V<sub>α</sub>/V<sub>β</sub> pairing. The exaggerated use of V<sub>α</sub>2 in up to 70% of all B6 SSIEFARL-specific T cells may therefore result from differences in this structurally disparate end of the complexes. A distinctive feature of V<sub>α</sub>2-positive TCRs isolated from infected B6 mice is the presence of an acidic residue in the CDR1<sub>α</sub> loop (Table III). Conversely, the CDR1<sub>α</sub> regions used in many of the K<sup>bmn</sup>-restricted CD8<sup>+</sup> T cells such as V<sub>α</sub>5 and V<sub>α</sub>6-1 (6) do not contain an electronegative amino acid in their CDR1<sub>α</sub> loops. Given the conserved nature of CDR1<sub>α</sub>-Arg<sup>62</sup> interaction, this potential salt bridge is available for both K<sup>b</sup> and K<sup>bmn</sup> complexes, and yet only K<sup>b</sup>-restricted CD8<sup>+</sup> T cells demonstrate V<sub>α</sub>2 bias. Moreover, in V<sub>α</sub>2-, V<sub>α</sub>1-, and V<sub>α</sub>5-positive TCRs, the acidic residue is at position 26, a position previously shown to salt bridge to Arg<sup>62</sup> in the Kb5-C20 TCR-H-2<sup>Kb</sup> complex structure (22) and positioned in a region of CDR1<sub>α</sub> that dominantly interacts with Arg<sup>62</sup> in all other ternary complex structures where an Arg<sup>62</sup> is present (32, 33). Therefore, we speculate that the V<sub>α</sub>2 bias in CD8<sup>+</sup> T cells from B6 mice results from selection of TCRs that can efficiently ligate the highly mobile Arg<sup>62</sup> on the α<sub>1</sub> helix of K<sup>b</sup> via salt bridge formation. TCR binding to K<sup>b</sup>/SSIEFARL will have a greater entropic penalty than binding to the equivalent K<sup>bmn</sup> complex because of the mobility of Arg<sup>62</sup> in the K<sup>b</sup> complexes. Given that TCR-MHC interactions are generally enthalpically driven, which reflects the requirement for surface complementation, and that TCR ligation involves entropic penalties associated with the greater order of residues and solvent molecules at the MHC-TCR interface (34, 35), the biased selection of V<sub>α</sub>2 in B6 mice may reflect the repertoire constraints in overcoming a greater entropic penalty for recognition of this complex. This would be consistent with the narrower expansion of SSIEFARL-specific CD8<sup>+</sup> T cells in B6 mice. In contrast, the ordered Arg<sup>62</sup> conformation in K<sup>bmn</sup> complexes imparts much less constraint on the T cell repertoire usage of Bm8 mice, and this leads to more diverse and higher functional avidity of the CD8<sup>+</sup> T cell response. This hypothesis is currently under investigation by studying the thermodynamics of soluble forms of SSIEFARL-specific TCRs.
binding to their cognate MHC-peptide molecules using both BIA-core and calorimetric methodologies in a manner analogous to Davis and colleagues (36).

Our findings indicate the importance of subtle structural variation in MHC-peptide complexes in selecting a suitable diverse antiviral T cell repertoire necessary for protective immunity. Presumably, these subtle thermodynamic and structural constraints are amplified in the environment of the immunological synapse where multiple MHC-peptide-TCR ligation events occur and the influence of coreceptors and adhesion molecules come into play, propagating small advantages and driving extrathymic selection of particular T cell clonotypes. Thus, in addition to events that lead to aggregating small advantages and driving extrathymic selection of particular T cell clonotypes that may impact on protective immunity toward pathogens.

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References

\textsuperscript{4}The CDR1\textalpha sequences of V\textalpha TCRs used dominantly in B6 SSIEFARL-specific TCRs are aligned with other V\textalpha sequences found in Bm8 SSIEFARL-specific TCRs and reveals the pronounced bias for complementary acidic residues (underlined) in the CDR1\textalpha of B6 CTL compared with Bm8. Overlap in Bm8 and B6 V\textalpha sequences is evident in Va275-containing TCRs. Alternate IMGT (International ImMunoGeneTics) classification system is also shown (39).


