Crystal Structure of Staphylococcal Enterotoxin G (SEG) in Complex with a Mouse T-cell Receptor β Chain*

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Superantigens (SAgs) are bacterial or viral toxins that bind MHC class II (MHC-II) molecules and T-cell receptor (TCR) in a nonconventional manner, inducing T-cell activation that leads to inflammatory cytokine production, which may result in acute toxic shock. In addition, the emerging threat of purpura fulminans and community-associated meticillin-resistant Staphylococcus aureus emphasizes the importance of a better characterization of SAg binding to their natural ligands that may allow the development of reagents to neutralize their action. The three-dimensional structure of the complex between a mouse TCR β chain (mVβ8.2) and staphylococcal enterotoxin G (SEG) at 2.0 Å resolution revealed a binding site that does not conserve the “hot spots” present in mVβ8.2-SEC2, mVβ8.2-SEC3, mVβ8.2-SEB, and mVβ8.2-SPEA complexes. Analysis of the mVβ8.2-SEG interface allowed us to explain the higher affinity of this complex with the others, which may account for the early activation of T-cells bearing mVβ8.2 by SEG. This mode of interaction between SEG and mVβ8.2 could be an adaptive advantage to bestow on the pathogen a faster rate of colonization of the host.

Superantigens (SAgs) are bacterial toxins or viral proteins that bind simultaneously as unprocessed molecules the T-cell receptor (TCR) and MHC-II molecules. As a consequence of this interaction, SAgs activate large numbers of T-cells, promoting a massive release of inflammatory cytokines, such as IL-1, IL-2, TNF-α, and TNF-β. These host cytokines are believed to be responsible for the most severe consequences of SAg intoxication, including capillary leak, renal failure, acute respiratory distress, and death. Systemic intoxication by SAgs can lead to a severe condition known as toxic shock syndrome (reviewed in Ref. 1), which is an acute, multisystem, toxin-mediated illness, often resulting in multiorgan failure. Toxic shock syndrome represents the most devastating expression of a spectrum of diseases caused by SAg-producing strains of Staphylococcus aureus and Streptococcus pyogenes (reviewed in Ref. 2). Bacterial SAgs have also been identified as category B agents of bioterrorism by the U. S. Centers for Disease Control and Prevention due to their extreme virulence and the ease with which they can be produced and disseminated.

Most of the known SAgs share a characteristic three-dimensional structure and bind MHC-II outside of the peptide-binding groove (3–10). However, the complexes formed between SAgs and MHC-II molecules display diversity and the interaction can be through either the α or β chain contacting or not the antigenic peptide (11–15). On the other hand, the binding to the TCR is not yet so well characterized due to the few TCR-SAg complexes that have been crystallized (16–19).

SAgs have been classified into five evolutionary groups or families based on their amino acid sequence (20). All members of group II, or the SEB family, which include SAgs from S. pyogenes SPEA and SSA and S. aureus SAgs SEB and SEC1–3 are known to interact with the mouse TCR Vβ8.2 (mVβ8.2) chain. Because mVβ8.2 was described as the principal TCR involved in experimental autoimmune encephalitis, and taking into account the potential role of SAgs in autoimmune diseases (21–23), it is important to analyze the binding of SAgs that stimulate T-cells carrying this particular β chain. Four members of this family, SEB, SEC2, SEC3, and SPEA, have been crystallized in complex with mVβ8.2, allowing detailed characterization of this interaction. The binding mode

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3 The abbreviations used are: SAg, superantigens; TCR, T-cell receptor; SEB, staphylococcal enterotoxin B; SEC, staphylococcal enterotoxin C; SEU, staphylococcal enterotoxin U; SSA, streptococcal superantigen A; SPEA, streptococcal pyrogenic enterotoxin A; SEG, staphylococcal enterotoxin G; mVβ8, mouse TCR β chain; r.m.s., root mean square; CDR, complementary determining region; HV4, hypervariable region 4.
Crystal Structure of SEG Bound to TCR

To mV88.2 is strictly conserved among the members of the SEB family and occurs through the variable region of the TCR β chain, contacting complementary determining region (CDR) 2, frameworks regions (FR) 2 and 3 and hypervariable region 4 (HV4) (17, 18). The streptococcal SAg SPEA also contacts CDR1 (19). In addition to the crystal structures, an extensive biophysical analysis of SAg mutants identified the energetic hot spots in binding to mV88.2 (24).

The SAgs from S. aureus SEG and SEU are the latest to be included in the SEB family (20). SEG was identified by Munson et al. (25) and is contained in the egc enterotoxin gene cluster with other four SAgs (26). SEG is 233-residues-long, corresponding to a mature protein of 27 kDa, which shares 41 to 46% amino acid sequence identity with other members of the SEB family. SEG has being implicated in toxic shock syndrome and scarlet fever associated with infecting S. aureus strains that lack genes for the classical members of the pyrogenic toxin SAgs, comprising genes sea-see and the tsst-1 (27). In addition, recent reports showed a higher frequency of seg than the classical genes in S. aureus isolated from several sources (28, 29).

We recently reported that, in vivo, SEG stimulates mouse T-cells carrying Vβ7 and Vβ9 TCRs. Stimulation reached a maximum at day 4 or later after injection (30), as usually happens with T-cells stimulated by SEC2–3 or SEB (31, 32). Surprisingly, we also found that SEG stimulates an earlier, stronger, and more widespread stimulation of mV88.2-bearing T-cells, compared with other members of the SEB family, which reached a maximum at day 2 instead of day 4 or later (30). In addition, we analyzed SEG binding to mV88.2 by surface plasmon resonance and isothermal titration calorimetry, which yielded similar results. The affinity (KD = 0.125 μM) by isothermal titration calorimetry and 0.270 μM by surface plasmon resonance) is the highest reported for an interaction of a wild-type SAg and a TCR (33). Sequence alignment of SEB family members revealed that SEG has changes in three highly conserved key amino acid positions, N58S, Y88F, and Q206P (SEG numbering), that were predicted to contact mV88.2. The crystal structure of SEG in unbound form (33) suggested that deletions in the sequence and natural mutations of hot spot residues are responsible for structural remodeling of the putative TCR-binding surface but could not explain the high affinity mentioned above. We also analyzed SEG binding to a mutant of mV88.2 (designated L2CM) that displays high affinity for SEC3 (33, 34). We found that the mutated residues responsible for the increased affinity of L2CM for SEC3, as well as SSA, did not greatly improve the affinity for SEG (33).

Here, we describe the crystal structure of SEG bound to mV88.2 and L2CM and compare these structures with those of other SAgs bound to mV88.2.

EXPERIMENTAL PROCEDURES

Production and Purification of SAgs—The cloning of mouse TCR β chain mV88.2 and the high affinity mutant LC2M was described previously (34). Briefly, Vβ8.2 and L2CM were cloned into expression vector pT7–7 and expressed as inclusion bodies in E. coli BL21(DE3). The inclusion bodies were washed three times with 50 mM Tris-HCl (pH 8.0), 0.1 M NaCl, 2 mM EDTA, and 0.5% Triton X-100, and another three times with the same buffer without Triton X-100, and then solubilized in 50 mM Tris-HCl (pH 8.0), 2 mM EDTA, 5 mM cysteine and 0.5 mM cystamine. After 3 days at 4 °C, the folding mixture was concentrated, dialyzed against 50 mM Tris-HCl (pH 8.5), and applied to a Mono Q anion exchange column (Amersham Biosciences) equilibrated in the same buffer. The protein was eluted with a linear NaCl gradient. Further purification was carried out by size exclusion using a Superdex 75 HR column (Amersham Biosciences) in PBS.

Data Collection—Purified proteins were dialyzed against PBS and mixed in an equimolar ratio for further complex purification by size exclusion chromatography. The exclusion volume containing the complex was concentrated to 7.5 mg/ml of protein prior to dialysis against 0.01 M HEPES (pH 7) and 0.02 M NaCl. Crystallization trials on the SEG-Vβ8.2 and SEG-L2CM complexes were carried out in hanging drops at an initial protein concentration of 3.25 mg/ml in the drop, by mixing 2 μl each of protein solution and mother liquor. Crystals of the SEG-Vβ8.2 and SEG-L2CM complexes grew at room temperature in 1.4 M sodium acetate, 0.1 M sodium cacodylate (pH 6.5), and 0.2 M sodium citrate, 30% isopropanol and 0.1 M sodium cacodylate (pH 6.5), respectively. SEG-Vβ8.2 and SEG-L2CM crystals were transferred to mother liquor containing 10% (v/v) glycerol and frozen in liquid nitrogen. Diffraction data to 2.6 and 2.8 Å resolutions, respectively, were obtained at 100 K on an R-axis IV2+ image plate detector (Rigaku). Higher resolution data (2.0 and 2.6 Å, respectively) were collected using synchrotron radiation at beamline X-25 of the National Synchrotron Light Source, and were processed using the programs DENZO and SCALEPACK (35).

Structure Determination and Refinement—The SEG-Vβ8.2 and SEG-L2CM complex structures were determined by molecular replacement using the program Molrep in the CCP4 program suite (36) with the SEC3-Vβ8.2 and SEC3-L2CM complex structures (Protein Data Bank codes 1JKK and 2AQ3, respectively) (17, 37) as search models. Two SEG-Vβ8.2 and eight SEG-L2CM complexes were found in the asymmetric unit with a Matthews coefficient of 3.65 Å3/Da and 4.03 Å3/Da, respectively (solvent content ~65 and ~70%, respectively). Initial refinement was performed using CNS with positional, simulated annealing and individual B factor refinement. Manual model rebuilding was carried out iteratively in XtalView (38) using σA-weighted 2Fo-Fc maps. After CNS refinement converged, further refinement was carried out with Ref-
Overall Structure of mVβ8.2-SEG Complex—We determined the structure of the complex between SEG and the mVβ8.2 chain to 2.0 Å resolution (Table 1). There are two SEG and two mVβ8.2 molecules in the asymmetric unit. All the regions of the complex are well ordered, with only the β4–β5 loop of SEG lacking electron density. SEG appears to be less ordered than the Vβ domain, as reflected by higher mean temperature (B) factors. Although two complexes are present in the asymmetric unit, their superposition does not indicate significant differences (0.26 and 0.15 Å for the Vβ chain and SEG, respectively). In addition, the same set of hydrogen bonds is observed, the van der Waals contacts involve the same residues, and the buried surface in both complexes exhibit no significant differences.

The overall structure of the mVβ8.2-SEG complex is shown in Fig. 1A. The complex is formed through contacts between CDR2 and FR2 and -3 of the variable domain of the mVβ8.2 chain, which binds in the cleft between the small and large domains of SEG. Although SEG does not contact HV4 of mVβ8.2, the overall binding site of mVβ8.2-SEG is in close agreement with those previously described in the mVβ8.2-SEC2, mVβ8.2-SEC3, mVβ8.2-SEB, and mVβ8.2-SPEA complexes (17, 18, 19). However, detailed interactions at the interface are different, as described below.

The buried surface for mVβ8.2-SEG is 1285 Å², with equal contributions from both molecules. This value is within the range observed in other mVβ8.2 complexes with S. aureus SAgs (17, 18). By contrast, the complex of mVβ8.2 bound to S. pyogenes SAg SPEA displays a higher buried surface (1324 Å²) because SPEA also contacts the CDR1 loop of Vβ (19). The SEG-MHC-II complex has not been crystallized, but sequence and structural alignment of SEG (33) with SEB, the only SAg of the family crystallized in complex with DR1 (11), showed that the putative binding site is conserved and adjacent to the TCR-binding site.

Structure of mVβ8.2-SEG Interface—The “hot spot” residues identified in SEB and SEC3 (24) that make the greatest energetic contribution to stabilization of the mVβ8.2-SAg complex (Asn-24, Tyr-88, and Gln-206; SEG numbering), as well as the energetically less important Asn-58, are strictly conserved in SEC1–3, SEB, SSA, and SPEA. The residues at

mac5 (39), during which solvent molecules were placed in >2σ peaks in the σA-weighted 2Fo − Fc maps with regard to potential interactions with hydrogen bonding partners. Data collection and refinement statistics are summarized in Table 1.

### RESULTS AND DISCUSSION

| TABLE 1 | Data collection and refinement statistics |
|----------------|------------------------------------------|
| SEG/Vβ8.2         | SEG/L2CM                                |
| Space group       | P3,21                                   | P43                        |
| Unit cell dimensions (Å) | a = 91.19, b = 91.19, c = 233.40     | a = 141.21, b = 141.21, c = 252.75 |
| Resolution (Å)    | 78.9-2.0                                 | 50.0-2.6                   |
| Observations      | 867,481                                  | 2,149,930                  |
| Unique reflections | 68,345                                   | 144,940                    |
| Completeness (%)  | 93.7 (60.5)                              | 99.7 (97.2)                |
| Rmerge (%)        | 8.4 (21.6)                               | 11.1 (53.1)                |
| Rfree (%)         | 19.5 (22.3)                              | 21.1 (30.6)                |
| Molecules per asymmetric unit | 2 × SEG/2 × Vβ8.2 | 8 × SEG/8 × L2CM           |
| Protein residues  | 673                                     | 2784                       |
| No. of acetates   | 8                                       |                            |
| No. of water molecules | 490                                       | 186                       |
| Average B factors (Å²) | 35.9                                    | 30.8                       |
| SEG               | 35.9                                    | 30.8                       |
| Vβ8.2             | 30.2                                    | 41.6                       |
| Acetate           | 45.4                                    |                            |
| Waters            | 40.9                                    | 24.2                       |
| r.m.s.d. bonds (Å) | 0.032                                   | 0.032                      |
| Ramachandran plot statistics (%) | 2.42°                                   | 2.66°                      |

* Values in parentheses correspond to the highest resolution shell (1.99–2.05 Å for SEG/Vβ8.2 and 2.60–2.67 for SEG/L2CM).

* Rmerge = Σ(Fo − Fl) / ΣFo where Fo is the ith observation of the intensity of the hkl reflection, and Fl is the mean intensity from multiple measurements of the hkl reflection.

* Rfree = Σ|Fo − Fl| / ΣFo, where Fo and Fl are the observed and calculated structure factor amplitudes for the hkl reflection.

* Rmerge is calculated over reflections in a test set not included in atomic refinement: 3634 reflections, 5.0% for SEG/Vβ8.2; and 7666 reflections, 5.0% for SEG/L2CM.

**FIGURE 1. Structure of the mVβ8.2-SEG complex.** A, overall structure of the mVβ8.2-SEG complex. Colors are as follows: mVβ is green, and SEG is violet. Residues of mVβ and SEG involved in the interaction are colored orange and cyan, respectively. B, interactions in the mVβ8.2-SEG interface. Nitrogen and oxygen atoms are colored blue and red, respectively. Hydrogen bonds are shown as red dashes when Asn-24SEB is involved or as black dashes. mVβ8.2 residues are labeled in orange, and SEG residues are labeled in blue.
Comparison of the mVβ8.2-SEG and mVβ8.2-SEB complex interfaces. Residues of mVβ8.2 involved in the interaction with SEG or SEB are colored orange or green, respectively. SEG residues are colored cyan, and SEB residues are beige. Only the residues with differential features have been labeled for clarity. A, detail of the SAg α1α2 loop interaction. The insertion present in SEG allows the interaction with Gly-58β. Hydrogen bonds are shown as black dashes. B, hydrogen bonds established between Asn-24SEG and Gly-58SEG. C, detail of the interaction between Pro-203SEG and Phe-204SEG with mVβ8.2. Hydrogen bonds are shown as black dashes, and van der Waals contacts are shown as gold dashes. D, superposition of the two mVβ8.2-SAg complex interfaces.

TABLE 2
mVβ8.2-SAg interactions

| Hydrogen bonds | SEG | SEB | SEC3 |
|----------------|-----|-----|------|
| β              |     |     |      |
| His-47         | Phe-171 | Leu-21 | Phe-171 |
| Tyr-50         | Tyr-89 | Tyr-89 | Val-89 |
| Gly-51         | Phe-88 | Tyr-88 | Tyr-88 |
| Ala-52         | Asn-24 | Asn-24 | Asn-24 |
| Gly-53         | Asn-24 | Asn-24 | Asn-24 |
| Ser-54         | Asn-24 | Asn-24 | Asn-24 |
| Thr-55         | Asn-24 | Asn-24 | Asn-24 |
| Glu-56         | Phe-203 | Leu-21 | Phe-203 |
| Lys-57         | Thr-21 | Gly-17 | Thr-21 |
| Gly-58         | Lys-19 | Lys-19 | Lys-19 |
| Tyr-65         | Phe-171 | Leu-21 | Phe-171 |
| Lys-66         | Phe-171 | Leu-21 | Phe-171 |
| Ala-67         | Phe-171 | Leu-21 | Phe-171 |
| Pro-70         | Asn-58 | Asn-58 | Asn-58 |
| Ser-71         | Asn-58 | Asn-58 | Asn-58 |

van der Waals contacts

| β | SEG | SEB | SEC3 |
|---|-----|-----|------|
| His-47 | Phe-171 | Leu-21 | Phe-171 |
| Tyr-50 | Tyr-89 | Tyr-89 | Val-89 |
| Gly-51 | Phe-88 | Tyr-88 | Tyr-88 |
| Ala-52 | Asn-24 | Asn-24 | Asn-24 |
| Gly-53 | Asn-24 | Asn-24 | Asn-24 |
| Ser-54 | Asn-24 | Asn-24 | Asn-24 |
| Thr-55 | Asn-24 | Asn-24 | Asn-24 |
| Glu-56 | Phe-203 | Leu-21 | Phe-203 |
| Lys-57 | Thr-21 | Gly-17 | Thr-21 |
| Gly-58 | Lys-19 | Lys-19 | Lys-19 |
| Tyr-65 | Phe-171 | Leu-21 | Phe-171 |
| Lys-66 | Phe-171 | Leu-21 | Phe-171 |
| Ala-67 | Phe-171 | Leu-21 | Phe-171 |
| Pro-70 | Asn-58 | Asn-58 | Asn-58 |
| Ser-71 | Asn-58 | Asn-58 | Asn-58 |
Pro-70\(^{B}\) contributes to the complex stabilization with a hydrogen bond to Asn-58\(^{SAg}\) in the \(\beta2–\beta3\) loop. This residue is strictly conserved among SAgs of the SEB family except in SEG, which has a Ser in this position. However, SEG has a deletion of three amino acids in the \(\beta2–\beta3\) loop, which positions Ser-58\(^{SEG}\) (Asn-58\(^{SEB}\)) away from the interacting surface with m\(\beta8.2\) (Fig. 2D). Structural superposition revealed 3.5–5.3 Å r.m.s. deviations with the other SAgs in this loop.

The 9 hydrogen bonds in the m\(\beta8.2\)-SEG complex (Fig. 1B) could explain the higher affinity of this interaction compared with the m\(\beta8.2\)-SEB and m\(\beta8.2\)-SEC2–3 complexes, which only have five hydrogen bonds (17, 18). However, the m\(\beta8.2\)-SPEA complex has 11 hydrogen bonds with an affinity similar to the latter complexes and lower than the m\(\beta8.2\)-SEB complex (19). The higher affinity of the m\(\beta8.2\)-SAG complex could be explained by the hydrogen bonding network around Asn-24\(^{SEG}\) (Fig. 2B). This network is not observed in the other three complexes, even though Asn-24\(^{SAg}\) is strictly conserved among bacterial SAgs reactive with m\(\beta8.2\) and is present in most of the known SAgs. This residue was shown to be the major energetic hot spot in binding m\(\beta8.2\) (24). On average, the Asn-24\(^{SAg}\) buried surface is 48 Å\(^2\) in the other three complexes (range 47–54 Å\(^2\)), whereas in the m\(\beta8.2\)-SEG complex, the buried surface of this critical residue is 63 Å\(^2\).

When free m\(\beta8.2\) was superimposed onto m\(\beta8.2\) in complex with SEB, SEC2–3, or SPEA, r.m.s. differences of 0.30, 0.31, and 0.56 Å, respectively, were obtained for all \(\alpha\) carbon atoms. Free m\(\beta8.2\) and m\(\beta8.2\) bound to SEG superposed with an r.m.s. difference in \(\alpha\) carbon positions of 0.41 Å. The most significant conformational change occurs at Glu-56\(^{B}\), whose side chain shows an r.m.s. displacement of 0.86 Å. This movement is essential to contact Asn-24\(^{SEG}\) and Phe-204\(^{SEG}\) through hydrogen bonds (Fig. 2, B and C), which are not present in the other complexes.

Group II SAgs SEB, SEC3, and SPEA engage m\(\beta8.2\) mostly through a conformation-dependent mechanism that is independent of specific V\(\beta\) amino acid side chains (9, 14, 17). However, the SEG-m\(\beta8.2\) complex involves both main and side chains in the hydrogen bond interactions, suggesting a combination of two mechanisms, one conformation-dependent and the other sequence-dependent. Even though the interaction through side chains promotes specificity, this mechanism restricts the V\(\beta\) repertoire that can interact with the SAg.

**Structural Basis for m\(\beta8.2\) Binding Specificity of SEG**—We previously described the *in vivo* selective expansion of murine T-cell subpopulations whereby SEG stimulates T-cells carrying V\(\beta7\) and V\(\beta9\) TCRs in a conventional manner. Surprisingly, we also found that SEG produces an earlier, stronger, and widespread stimulation of m\(\beta8.2\) T-cells, compared with other members of SEB family, which reach a maximum at day 2 instead of day 4 or later as for SEC3 or SEB (30–32). The structure of SEG in unbound form (33) suggested a non-conventional binding site for m\(\beta8.2\) and SEG, but it could not explain by itself the particular behavior of T-cells bearing m\(\beta8.2\) when they are stimulated by SEG. To clarify this issue, the m\(\beta7\) structure (Protein Data Bank code 3HE7) (40) was superposed onto the m\(\beta8.2\)-SEG complex using the program Lsqkab from the CCP4 program suite (Fig. 3A). The r.m.s. difference in \(\alpha\) carbon positions for the 12 residues in the SEG binding site is only 0.43 Å (Fig. 3B). The putative residues involved in the binding surface were analyzed with the program Contact (CCP4 program suite), which showed that Asn-24\(^{SEG}\) would not be able to form the network of five hydrogen bonds as in the m\(\beta8.2\)-SEG complex. The interaction of Asn-58\(^{SAg}\) with Pro-70\(^{B}\) and Ser-71\(^{B}\) (HV4) is highly conserved in the m\(\beta8.2\)-SAg binding site. As described above, SEG does not contact m\(\beta8.2\) HV4 due to a three-residue insertion in the \(\beta2–\beta3\) loop, such that Ser-58\(^{SEG}\) is not present in the interface of m\(\beta8.2\)-SEG complex. However, in the m\(\beta7\)-SEG complex, SEG uses Tyr-59\(^{SEG}\) of the \(\beta2–\beta3\) loop and Ser-31\(^{SEG}\) to contact Val-52\(^{B}\) (CDR2) and Lys-70\(^{B}\) (HV4), respectively (Fig. 3C). In the interaction with m\(\beta8.2\), SEG uses three new residues, Asp-172, Pro-203, and Phe-204. Strikingly, residues 172, 203, and 204 are not part of the binding site in the m\(\beta8.2\)-SEB, m\(\beta8.2\)-SEC3, or the putative m\(\beta7\)-SEG complex.

SEG also interacts with m\(\beta7\). When m\(\beta7\) was superposed onto the m\(\beta8.2\)-SEG complex and the putative bind-
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ing site, mVβ7–SEB did not differ from the mVβ8.2–SEB surface interaction (Fig. 3C). This analysis suggests that SEG contains a binding site for mVβ7 similar to the site found in the other members of the SEB family with mVβ8.2 and with the others Vβ that these SAgS bind (9).

Structure of L2CM-SEG Interface—We also determined the structure of the L2CM-SEG complex to 2.6 Å resolution. L2CM is a variant of mVβ8.2 with 1500-fold higher affinity for SEC3 (34, 37). L2CM also displays higher affinity than Vβ8.2 for other members of SEB family, such as SSA (340-fold). By contrast, L2CM showed only a 2.5-fold increase in its affinity for SEG (33). The structure of the L2CM-SEG complex shows substantial difference with the L2CM-SEC3 interface.

L2CM contains 9 mutations, G17E/A52V/S54N/K66E/Q72H/E80V/L81S/T87S/G96V, but five of them (G17E, E80V, L81S, T87S, and G96V) are outside of the binding site. The other four increase affinity by cooperative or additive interactions (34, 37). The A52V mutation results in the addition of two methyl groups (Cγ1 and Cγ2 atoms) beyond the single methyl group of the wild-type side chain. These methyl groups increase the buried hydrophobic surface with SEG, as in the case of SEC3, which can account for the increase in affinity of L2CM for SEG, compared with mVβ8.2 for SEG. An analysis of the K66E mutation is more complicated because this mutation could positively or negatively affect the affinity in the presence of a second mutation (E80V). Because the binding site of mVβ8.2–SEG is conserved in that region, these mutations may have the same effect in both complexes. In wild-type mVβ8.2, Ser-54β makes an intramolecular hydrogen bond with Glu-56β, precluding the interaction of this residue with the SAg. The mutation S54N promotes a water-mediated hydrogen bonding network that links the Asn-54β and Glu-56β side chains to main chain atoms of Lys-204SEC3. In wild-type mVβ8.2–SEG, this interaction already exists because SEG exhibits a cleft in the β11–π2 region that allows interactions with the TCR (Fig. 2C). The TCR HV4 region is deeply involved in interactions with SAgS of the SEB family, except in the mVβ8.2–SEG complex. The mutation Q72H in HV4 favors the interaction between SEC3 and L2CM. Because SEG does not interact with mVβ8.2 or L2CM HV4 regions, this mutation does not affect its affinity for L2CM. The fact that just one of the nine L2CM mutations, A52V, has a positive effect on mVβ8.2–SEG binding could explain the small increase in affinity that SEG displays for L2CM.

Conclusions—The mVβ8.2 binding surfaces of SEB, SEC2–3, and SPEA, determined by X-ray crystallography of the corresponding complexes, and the putative SSA binding surface, predicted based on the structure of the unbound SAg (7, 14, 17, 18, 41), show conservation of most of the eight contacting residues that constitute the functional epitope. Several of those residues have been identified as hot spots because of their energetic contribution to binding (24). The SEG structure showed that the putative binding surface formed by the eight residues is discontinuous and revealed difference in three of the hot spots, Asn-58, Tyr-88, and Gln-206, which have been replaced by Ser-58, Phe-88, and Pro-206, respectively. Nevertheless, SEG displays the highest affinity de-

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FIGURE 4. The interacting surfaces of SAgS and mVβ8.2. A, outline of the mVβ8.2 binding site of SEG (thick blue line) and SEG (thick yellow line) are superposed on the surfaces of SEG (white) and SEG (gray) superimposed. B, the outline of SEG (thick blue line) and SEG (thick yellow line) binding surfaces are superimposed on the surfaces of mVβ8.2 bound to SEG (white) or SEG (gray) superimposed.
