Sequences in Both Class II Major Histocompatibility Complex α and β Chains Contribute to the Binding of the Superantigen Toxic Shock Syndrome Toxin 1

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Summary

Class II major histocompatibility complex (MHC) molecules present peptides derived from processed antigen to antigen-specific CD4-positive T cells. In addition, class II molecules bind with high affinity another class of antigens, termed superantigens. T cell stimulation by superantigens depends almost exclusively on the Vβ segment expressed by the T cell receptor (TCR). Mapping of the superantigen binding site on class II molecules should provide valuable information on how MHC and TCR molecules interact. Recombinant mouse I-A class II molecules expressed on transfected L cells were analyzed for their ability to bind the toxic shock syndrome toxin 1. Polymorphic residues in the α helices of both the α and β chains of I-A contributed to quantitative toxin binding, suggesting that the toxin binds to either a combinatorial or a conformational site on class II MHC molecules.

Toxic shock syndrome toxin 1 (TSST-1)[1] is one of a number of related molecules termed superantigens that share the property of being able to activate polyclonal T cells expressing particular Vβ gene segments (1, 2). This T cell activation requires that the superantigen be presented in association with cells bearing class II MHC molecules (1, 3). Superantigens are unlike conventional protein antigens. They do not require active cellular processing (4, 5), they do not occupy the MHC antigen-binding groove (6, 7), and their presentation is not restricted by a particular class II MHC molecule (1, 3, 8). Nonetheless, not all class II MHC molecules are able to bind and present each of the superantigens (6–14). TSST-1 binds effectively to the human class II HLA-DR but not the HLA-DP molecule (6, 10). In the mouse, avid binding to I-A^b but not to I-A^k has been demonstrated, while binding to I-A^d is intermediate (12). Mapping of the site on MHC to which superantigens bind is not yet complete. For example, synthetic peptides from the α helix of the β chain can interfere with the binding of staphylococcal enterotoxin A (SEA) (14). However, the pattern of TSST-1 binding to recombinant human class II molecules demonstrates that the α1 domain contains important sequences for this toxin/MHC interaction (6).

[1] *Abbreviations used in this paper:* SE, staphylococcal enterotoxin; TSST-1, toxic shock syndrome toxin 1.

To better map the region of the MHC molecule to which TSST-1 binds, a convenient system was developed in which an antiserum to TSST-1 was used to quantitate the relative amount of TSST-1 binding to I-A-positive cell lines. This assay was then used to analyze the ability of TSST-1 to bind to various wild-type and recombinant class II MHC molecules expressed by transfected L cells.

Materials and Methods

Cell Lines. L cell transfectants expressing the wild-type molecules I-A^b, I-A^d, and I-A^k, and recombinant I-$A^{b/k}$ were previously described (15, 16). The recombinant molecules are named using the convention $A\alpha^{\alpha\alpha\alpha}A\beta^{\beta\beta}$ to indicate the allelic origins of the three hypervariable regions (αHV1, αHV2, and αHV3) of the Aα chain and the allelic origins of the β strands and α helix, respectively, of the Aβ chain. In the Aβ chain, amino acids 1–49 constitute the β strands and amino acids 50–96 constitute the α helix. For the Aα chain, αHV1 refers to amino acids 1–38, αHV2 refers to amino acids 39–65, and αHV3 refers to amino acids 66–end. $A\alpha^b$ and $A\alpha^k$ differ at amino acids 11 and 15 in αHV1; 53, 55, 56, and 59 in αHV2; and 69, 70, 75, and 76 in αHV3. Therefore, the αHV1 differences are in the predicted β strands and the αHV2 and αHV3 differences are in the α helix.

FACS® Analysis of I-A Surface Expression and of TSST-1 Binding and Data Reduction. I-A surface expression was determined by separately incubating L cell transfectants (3×10^5) with saturating amounts of 34-5-3 (monoclonal anti-I-$A^{b,d}$) (17) and 10-2.16 (monoclonal anti-I-A^k) (18) followed by 50 μl of a 1:50 dilution

of FITC-conjugated F(ab')$_2$ fragment of goat anti-mouse Ig (Cooper Biomedical, Malvern, PA). The degree of staining of these antibodies has been shown to accurately reflect the level of surface I-A expressed by these transfectants (15). The nonbinding antibody served as a negative control. To determine the degree of TSST-1 binding, cells were first incubated with 100 μl of TSST-1 (Toxin Technology, Madison, WI) at a final concentration of 10 μg/ml, followed by 100 μl of a 1:100 dilution of rabbit anti-TSST-1 (Toxin Technology), and finally incubated with 50 μl of a 1:70 dilution of FITC-conjugated F(ab')$_2$ fragment of goat anti-rabbit Ig (Cooper Biomedical). These conditions were shown in preliminary experiments to be saturating. For controls, the cells were incubated with the antiserum and the second antibody, but not the toxin. All reagents were diluted in PBS (Mediatech, Washington, DC) containing 2% FCS (HyClone Laboratories, Logan, UT) (PBS-2%). All incubations were at 4°C for 30 min and cells were washed twice with PBS-2% FCS between each reagent and at the end. Cells were analyzed using a FACScan® flow cytometer (Becton Dickinson & Co., Mountain View, CA). Mean fluorescence was calculated by the C30 program (Becton Dickinson & Co.). The relative TSST-1 fluorescence is defined as the ratio of (TSST-1 fluorescence − control)/(anti-Ia fluorescence − control). In each experiment, relative TSST-1 fluorescence was corrected by a constant multiplier so that the relative TSST-1 fluorescence for one of the I-A^b-expressing transfectants (NABB.1F) was 0.5 (range of actual values was 0.2–0.65). These corrected values were used to calculate the mean TSST-1 fluorescence for each recombinant molecule, the standard errors of these values, and p values for all pair-wise combinations using the two-tailed student's t test.

Results and Discussion

The results of a typical experiment are shown in Fig. 1. As has been observed (12), there was high level TSST-1 binding to I-A^b, intermediate binding to I-A^d, and little to no binding of TSST-1 to I-A^k. If one calculates the relative TSST-1 fluorescence, a value is obtained that reflects the ability of each MHC molecule to bind the toxin. In this experiment, the relative TSST-1 fluorescence for I-A^b was 0.44, for I-A^d was 0.22, and for I-A^k was 0.01. This value varied little over a threefold range of surface I-A expression (data not shown). The relative TSST-1 fluorescence value does not necessarily indicate the percentage of I-A molecules that bind the toxin. However, it can be used as a measurement of the relative binding of the toxin to the different cells. Thus, approximately twice as many TSST-1 molecules bind to cells expressing I-A^b as compared with I-A^d, indicating that the interaction of the toxin with I-A^b is of higher affnity or avidity than the interaction of toxin with I-A^d (or I-A^k). The lack of TSST-1 binding to I-A^k is not due to a general lack of interaction between this class II allele and all exotoxins, since SEA, SEB, SEC, SEE, and exfoliating toxin all bind to I-A^k (7; and D. Karp, unpublished observations).

A panel of L cell transfectants expressing a variety of mutant I-A molecules derived by the exchange of genomic or cDNA sequences between I-A^b and I-A^k alleles has been developed. These molecules allow the dissection of MHC functions associated with particular Aα and Aβ hypervariable segments and have proven useful in mapping molecular interactions within the MHC molecule that control I-A heterodimer expression and conformation (15, 16). For each of these recombinant molecules, several different transfectant lines and clones, each expressing a different level of surface I-A, were analyzed to determine the relative TSST-1 fluorescence. A compilation of the results from five experiments is shown in Fig. 2. The data reveal, first, that the toxin binds to all transfectants expressing I-A molecules that contain the α helix from Aβ^b, while it does not appreciably bind to molecules that contain the α helix from Aβ^k. In particular, the toxin does not bind to the molecule Aα^{bbb}Aβ^{bk}, which contains k residues in the Aβ chain α helix but is otherwise I-A^b, even though the toxin binds well to the wild-type I-A^b molecule. This indicates that polymorphisms in the Aβ chain α helix are important for toxin binding. Furthermore, at least in this allelic combination, the allelic origin of the Aβ chain β strands does not affect toxin binding (compare Aα^{bbb}Aβ^{bb} [Fig. 2, no. 1] with Aα^{bbb}Aβ^{kb} [no. 2]). In addition to the role of

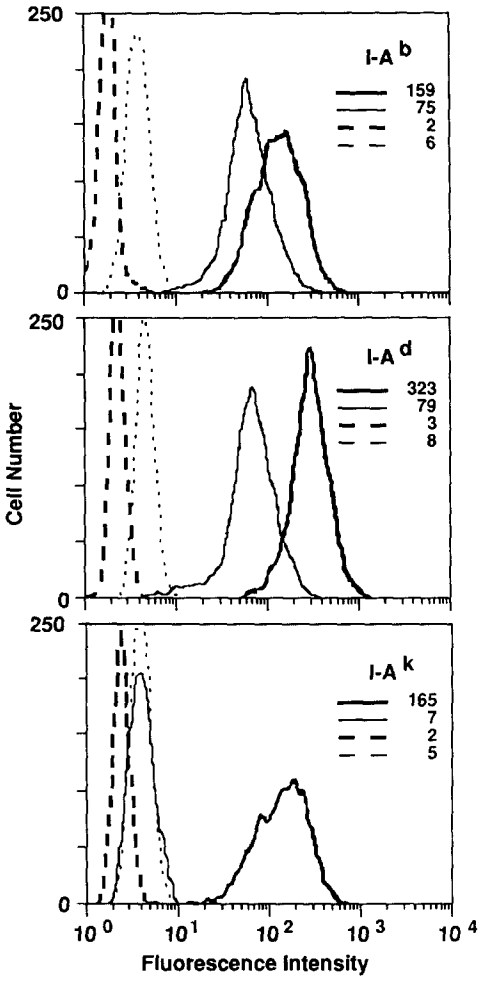
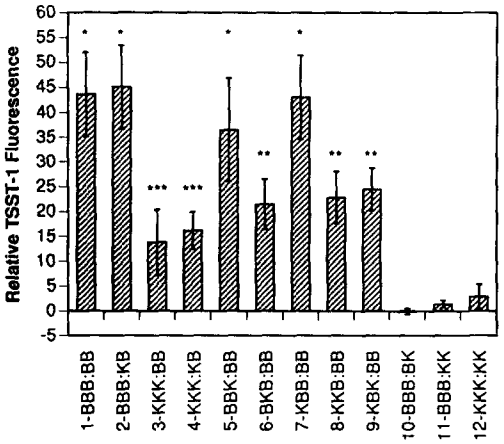


Figure 1. Binding of TSST to I-A^b, I-A^d, and I-A^k. The transfected L cell line is stained either for I-A surface expression (*heavy solid line*) or for the amount of TSST-1 bound (*thin solid line*). The dotted lines are controls for I-A (*heavy line*) or TSST-1 (*thin line*) staining. Fluorescence intensity is expressed in arbitrary units. The calculated mean fluorescence for each of the histograms is shown.



Recombinant MHC molecules

	α Chain			β Chain	
	sheets	helix			
Cell #	HV1	HV2	HV3	Sheets	Helix
1	B	B	B	B	B
2	B	B	B	K	B
3	K	K	K	B	B
4	K	K	K	K	B
5	B	B	K	B	B
6	B	K	B	B	B
7	K	B	B	B	B
8	K	K	B	B	B
9	K	B	K	B	B
10	B	B	B	B	K
11	B	B	B	K	K
12	K	K	K	K	K

Figure 2. Relative TSST-1 binding to recombinant I-A molecules. The relative TSST-1 fluorescence was calculated as described using two to four different cell lines expressing each of the indicated MHC molecules. In each experiment, relative TSST-1 fluorescence was corrected by a constant multiplier so that the relative TSST-1 fluorescence for the I-A^b-expressing transfectant (NABB.1F) was 0.5 (range of actual values was 0.2–0.65). The means and SEs of these corrected values as obtained in six separate experiments are depicted for each recombinant molecule. For molecules 1–9, which bound the toxin to some degree, n ranged from 7 to 17; for the other molecules, which did not bind to toxin, n ranged from 2 to 6. The three groups indicated with either one, two, or three asterisks represent three statistically separate groups ($p < 0.05$) of toxin binding using a two-tailed student's t test. B and K denote the allelic origins ($H\text{-}2^b$ or $H\text{-}2^k$) of each of the segments of the MHC molecule in the following order: αHV1 αHV2 αHV3:βHV(1+2) βHV(3+4). The αHV1 and βHV(1+2); regions are in the MHC β pleated sheet. The αHV2, αHV3, and βHV(3+4) regions are in the two α helices.

MHC β chain polymorphic residues, it appears that, as previously reported for HLA-DR molecules, residues in the α chain affect toxin binding (6). Thus, the molecule $A\alpha^{bbb}A\beta^{bb}$ (no. 1) binds the toxin better than the molecule $A\alpha^{kkk}A\beta^{bb}$ (no. 3); similarly, $A\alpha^{bbb}A\beta^{kb}$ (no. 2) binds the toxin better than $A\alpha^{kkk}A\beta^{kb}$ (no. 4; $p < 0.005$ for cell line nos. 1 or 2 vs. 3 or 4). Note that $A\alpha^{kkk}A\beta^{bb}$ (no. 3) and $A\alpha^{kkk}A\beta^{kb}$ (no. 4) bind the toxin similarly ($p > 0.40$), demonstrating that even when binding is suboptimal, κ polymorphisms in the β chain β strands do not influence toxin binding.

One potential caveat in this analysis is the use of two different antibodies to detect surface I-A expression. The antibody 34-5-3 detects all I-A molecules in which the α helix of the Aβ chain derives from either the b or d haplotype. This antibody has similar overall activity and/or affinity for all recombinant MHC molecules containing either of these molecular segments (15). The antibody 10-2.16 detects I-A molecules in which the α helix of the Aβ chain derives from the k haplotype. Comparisons of the TSST-1 fluorescence between cells stained with 34-5-3 and those stained with 10-2.16 could be difficult to evaluate. However, 10-2.16 was only used to quantitate relative I-A surface expression for cell nos. 5–7 (Fig. 2). The toxin minimally binds to the I-A molecules expressed by these cells. For all the other cells in Fig. 2, 34-5-3 was used to quantitate relative I-A surface expression. Therefore, all comparisons of relative TSST-1 fluorescence in which any toxin binding was observed always involved the use of the 34-5-3 antibody to monitor surface I-A expression. Furthermore, use of the antibodies Y3P and 39J to determine the levels of cell surface I-A expression gave the same results (data not shown). These antibodies principally detect polymorphisms in the Aα chain and allow a direct comparison of relative TSST-1 fluorescence for MHC molecules composed of different Aβ chain α helices.

To further map the Aα chain polymorphisms important for TSST-1 binding, the relative TSST-1 fluorescence was determined using transfectants expressing the wild-type $A\beta^b$ chains paired with recombinant Aα chains. The results, also shown in Fig. 2, were obtained using the antibody 34-5-3 to determine cell surface I-A expression as all of these cell lines express I-A molecules detected by this antibody. The substitution of $A\alpha^k$ residues in either αHV1 (no. 7) or αHV3 (no. 5) has little or no effect on TSST-1 binding ($p > 0.10$ for cell line nos. 5 vs. 1 or 2; $p > 0.30$ for cell line nos. 7 vs. 1 or 2), whereas the substitution of $A\alpha^k$ residues in αHV2 (nos. 6 or 8) diminishes toxin binding similar to what is obtained with the wild-type $A\alpha^k$ chain ($p < 0.005$ for cell lines nos. 6 or 8 vs. 1 or 2; $p < 0.05$ for cell lines nos. 6 or 8 vs. 5 or 7). Thus, it would appear that αHV2 is the most important of the Aα polymorphic segments for TSST-1 binding. Nonetheless, it is also apparent that the presence of $A\alpha^b$ residues in αHV2 is not sufficient for TSST-1 binding as the molecule $A\alpha^{kbk}A\beta^{bb}$ (no. 9) only binds intermediate levels of the toxin and is statistically similar to cell lines nos. 6 ($p > 0.05$) and 8 ($p > 0.20$).

The data indicate that residues in both MHC surface α helices contribute to the binding of TSST-1. This could indicate either that there is one TSST-1 binding site on the MHC molecule and that this site is influenced by the combination of polymorphic residues in the two MHC α helices, or that TSST-1 binds to two independent sites on the MHC molecule, one in the α helix of the Aβ chain and the other in the α helix of the Aα chain. The 65% decrease in relative TSST-1 fluorescence when toxin binding to $A\alpha^kA\beta^b$ is compared with binding to $A\alpha^bA\beta^b$ is consistent with either interpretation. However, if there were two independent sites, one would also expect a similar partial decrease in relative TSST-1 fluorescence when toxin binding to $A\alpha^bA\beta^k$ is compared with binding to $A\alpha^bA\beta^b$. This is not observed as there is essentially no detectable binding of TSST-1 to $A\alpha^bA\beta^k$. Therefore, we conclude that residues in the two helices together define the TSST-1 binding site and that there is only one TSST-1 binding site per I-A molecule.

The data demonstrate that polymorphic residues in the α helix of Aβ are essential for TSST-1 binding and that high level toxin binding is dependent on polymorphisms in both

the α and β chain α helices. Whereas αHV2 is the single most important α chain polymorphic region in contributing to the TSST-1 binding site, high level toxin binding requires the presence of other *b* polymorphisms in either αHV1 or αHV3. The αHV2 region most likely contributes to a conformational or combinatorial toxin binding site because residues in this region interact with the α helix of class II β chains (16, 19). Models for class II structure also predict the presence of a salt bridge between αHV3 and the α helix of β chains (20, 21), but this bridge does not involve polymorphic residues in either chain except in the case of the mutant Aβ chain expressed by NOD mice (22). Furthermore, functional analyses indicate that the αHV3 region is involved in interactions with polymorphisms in the β strands of the Aβ chain but not the β chain's α helix (16). Thus, it is most likely that TSST-1 binds to I-A at a combinatorial αHV2/βHV4 site or binds to a conformational site that is controlled by this αHV2/βHV4 interaction.

Given the overall homology of function between the various toxins and the common feature of Vβ preference in the stimulation of T cells by these toxins, it is likely that, for each of the superantigens, the functionally relevant interactions between superantigens, MHC, and TCR would be analogous. Analyses of superantigen binding to MHC molecules, however, has not identified a single region of the MHC molecule as the locus for all toxin binding. In a previous study, it was shown that DR α1 residues but not DP α1 residues allow TSST-1 binding to human class II MHC molecules (6). Others have shown that the binding of SEB, SEC 1, and exfoliating toxin to MHC was insensitive to the substitution of alanines throughout the α helix of the Aα chain (7). Analysis of SEA binding to mouse class II molecules has shown that this can be inhibited by a peptide derived from the α helix of the Aβ chain. The abilities of synthetic peptides to inhibit SEA or TSST-1 binding to mouse and human cell lines was shown to vary with the toxin, the peptide, and the cell type (14). Furthermore, although TSST-1 and SEB appear to have different binding sites on class II molecules (9), the binding of both toxins can be competed by SEA (23). Most recently, it has been shown that SEA (24, 25) and SEE (25) binding to HLA-DR molecules depends on residue 81 in the DRβ chain. This residue is in βHV4, the region of the β chain known to contribute to an interchain interaction with the αHV2 segment.

These reports may suggest that many of the superantigens bind to unique and distinct sites on MHC. The data in this report suggest an alternate explanation. That is, the toxins may all bind to the same conformationally sensitive region of MHC and the specific sites for the individual toxins may partially overlap. Consequently, depending on the individual toxin, the MHC allele or isotype analyzed, and the affinity of the toxin-MHC interaction, toxin binding may appear to preferentially depend on sequences in one α helix or the other, and individual toxins may only partly inhibit each other's binding to MHC.

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