HLA-DR Alleles Differ in Their Ability to Present Staphylococcal Enterotoxins to T Cells

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Summary

Staphylococcal enterotoxins (SEs) have been shown to bind to major histocompatibility complex (MHC) class II proteins and stimulate T cells in a $V\beta$-specific manner, and these $V\beta$ specificities for various SEs have been well documented in mice and humans. This study was undertaken in order to examine the ability of human class II molecules to present SEs to human and murine T cell hybridomas. Using a panel of transfectants expressing individual HLA class II antigens, we have shown that HLA-DR alleles differ in their ability to bind and present SEs. Since the HLA-DR proteins share a common α chain, these results indicate that the polymorphic β chain plays an important role in SE binding and presentation to T cells. In addition, we have shown that human class II isotypes markedly differ in their ability to present SEs. The results of this study should provide information on the region of MHC class II molecules that interacts with foreign, and perhaps self, superantigens.

T cells usually recognize peptide fragments of antigen that lie in the antigen-binding pocket of MHC (1–4). The combination of many variable elements of the TCR confers upon the T cell bearing it specificity for peptide antigen and MHC (5, 6). Recently, however, a group of T cell stimulatory antigens have been described that differ in several respects from the classically defined peptide antigens. T cell recognition of these antigens, termed superantigens (7), is dictated predominantly by the $V\beta$ element of the TCR, and the interaction is not strictly dependent on the MHC class II allele. Mls-1ª, Mls-2ª, Mls-3ª, and a B cell–specific product are examples of self-superantigens (8–11) that have been well studied, yet the molecular nature of the self-superantigens has remained elusive.

The Staphylococcal enterotoxins (SEs)[1] stimulate murine and human T cells (12, 13), and appear to mimic self-superantigens in their $V\beta$ specificity (7, 14). T cell stimulation by SE requires MHC class II molecules on an APC (7, 15), and several studies have shown specific binding of SE to MHC class II (16–18). These foreign superantigens do not require proteolytic fragmentation for their activity (14–16),

and SEs are probably not presented by MHC in the same manner as peptide antigens.

The studies in this paper were conducted to examine the ability of different human class II proteins to bind SEs and stimulate human and murine T cells. This analysis showed that different human class II proteins did indeed differ in these assays. The experiments revealed that the allele of the β chain of HLA-DR was crucial in SE binding and stimulation of T cells. These observations provide clues about the interactions between MHC class II proteins, superantigens, and the $V\beta$ component of the TCR.

Materials and Methods

Cell Lines. The generation and characterization of the T cell hybrids used in this study have been described elsewhere (7, 10, 19). All of the T hybrids used in this paper express only a single TCR, as they were generated by fusion of T cells with an $\alpha^-\beta^-$ variant of the BW 5147 thymoma (20).

CH12.1 is a murine B cell lymphoma that expresses I-Aᵏ and I-Eᵈ/ᵏ, and has been used previously for toxin stimulation (7, 21). Raji is a human B lymphoma cell line that expresses HLA-DR3 and DRw10, HLA-DQw1 and DQw2, and HLA-DP7 (22, 23). Jurkat is a human T cell tumor line (24).

Human MHC Class II Transfectants. Full-length cDNAs encoding β chains of class II HLA-DR alleles (DR1, DR2 Dw2,

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[1] *Abbreviations used in this paper:* bio, biotin-labeled; ExF, exfoliating toxin; PE-Av, PE coupled to streptavidin; RSV, Rous sarcoma virus; SE, Staphylococcal enterotoxin; TSST, toxic shock syndrome toxin 1.

DR4 Dw4, DR7, DRw52c, DRw53) and isotypes (-DQw1, DPw2) were introduced into the eukaryotic expression vector RSV.3, as previously described (25). Full-length cDNAs encoding the DRα, -DQw1α, and DPw2α chains were introduced into the eukaryotic expression vector RSV.5. The RSV.5 expression vector contains two expression units: the first one consists of the *Escherichia coli* guanine phosphoribosyl transferase gene encoding resistance to mycophenolic acid, and the second one has the Rous sarcoma virus (RSV) long terminal repeat driving the expression of the class II cDNA (25). Transfections into the adherent murine fibroblastic line were carried out by the calcium phosphate precipitation technique (26). Briefly, DAP-3 cells were cotransfected with 2 μg of each class II α chain in the RSV.5 expression vector and 10 μg of the isotype-matched class II β chains. Mycophenolic acid–resistant cells were selected, and homogeneous populations of cells expressing comparable levels of class II alleles and isotypes were obtained using a single cell cloning deposition system and aseptic cell sorting on the FACstar plus (Becton Dickinson & Co., Mountain View, CA) flow cytometer. Levels of class II were assessed by flow cytometry using an indirect fluorescence assay and a mAb, SG465, that recognizes a monomorphic determinant expressed on all class II alleles and isotypes (27, 28). Transfected cells were maintained in culture in medium containing the selective agent.

Stimulation of T Cell Hybrids. T hybridoma cells, at 10^5/well, were combined with 10^5 APCs and 1 μg/ml SEs in 96-well plates (7, 19). IL-2 production by the T cells was determined by the survival of an IL-2-dependent cell line, HT-2, measured visually or using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (Sigma Chemical Co., St. Louis, MO) (29, 30).

Toxins. The SEs used in this study were staphylococcal enterotoxin A (SEA), staphylococcal enterotoxin B (SEB), staphylococcal enterotoxin C1 (SEC1), staphylococcal enterotoxin C2 (SEC2), staphylococcal enterotoxin C3 (SEC3), staphylococcal enterotoxin D (SED), staphylococcal enterotoxin E (SEE), Toxic shock syndrome toxin 1 (TSST), and exfoliating toxin (ExF). The SEs were obtained from Toxin Technology (Madison, WI). Lyophilized toxins were dissolved in balanced salts solution, filter sterilized, and stored at 4°C until use.

Staphylococcal toxins were biotinylated using a standard procedure. Toxins were dissolved in 0.1 M sodium bicarbonate buffer, adjusted to a concentration of 1 mg/ml, and incubated with a 20-fold molar excess of succinimidobiotin (Sigma Chemical Co.) dissolved in DMSO. The reaction was allowed to proceed at room temperature for 2 h, and then the free biotin was removed by extensive dialysis against PBS.

Toxin Binding Analysis. The binding of toxins to APC was measured using a fluorescence assay as follows. 2 × 10^5 cells, in staining buffer (PBS, 2% FCS, 0.08% sodium azide), were mixed with different concentrations of biotin-labeled toxins (bio-SE) in individual wells of 96-well microculture plates and incubated at 37°C for 45 min. The plates were washed four times with staining buffer before addition of PE coupled to streptavidin (PE-Av; Tago Inc., Burlingame, CA) and incubation on ice for 20 min. The cells were washed four times with staining buffer before cytofluorographic analysis on an Epics C cell sorter (11). Specific binding of the bio-SE was demonstrated by the ability to completely inhibit the fluorescence signal with the addition of 100-fold excess of unlabeled toxin to the initial mixture of cells and bio-SE, and by the lack of staining of nontransfected L cells. The fluorescence intensity was calculated by subtraction of the anti-log of the fluorescence signal obtained by staining the cell line with PE-Av only from the anti-log of the value obtained with bio-SE + PE-Av.

Results

Murine T Cell Hybrids Respond to SEs Presented by Human Class II. Earlier reports have shown that individual SEs bind to human or murine class II molecules, and that this complex stimulates human or murine T cells, respectively, in a toxin- and Vβ-specific manner (7, 14, 19, 31, 32). T cells bearing murine Vβ3, for example, are stimulated by almost all the SEs bound to any murine class II molecule. T cells bearing murine Vβ1, on the other hand, are stimulated only by SEA + murine class II, and T cells bearing murine Vβ6 are not stimulated by any of the SEs bound to murine class II antigens.

In these studies, we wished to assess the ability of different human class II molecules to bind and present SEs to T cells bearing different Vβs. A panel of murine L cell lines transfected with different human class II molecules were therefore tested for their ability to stimulate a human T cell tumor line, Jurkat, in the presence of SEs. To supplement these experiments, and for lack of a large collection of well characterized human T cell lines able to respond to transfected murine L cells, a battery of murine T cell hybridomas were also used in the same assays.

Jurkat and a panel of murine T cell hybrids were tested for their ability to produce IL-2 after stimulation by SEs with murine or human APC. Some sample results are shown in Table 1. The human T cell line, Jurkat, responded to SEA, SEB, SED, and SEE in the presence of Raji cells. This pattern was broader than expected, since we have previously shown that normal T cells bearing human Vβ8, the Vβ expressed on Jurkat, respond well only to SEE, and marginally to SED, presented by normal human mononuclear cells (31). There are several possible reasons for the additional SE responses seen with Jurkat in these experiments. The analysis of toxin responses conducted with bulk human T cells (31) could not distinguish between individual members of the Vβ8 family, and it is conceivable that differences might arise with the study of Jurkat, a clonal T cell line that expresses human Vβ8.1. These additional SE responses by Jurkat in this assay may reflect the contributions of other variable elements of the Jurkat TCR itself, or alternatively, Raji may be a more efficient presenter of SEs than nontransformed human cells. It seems likely that the alleles, isotypes, or relative densities of class II proteins expressed by Raji played an important role, since the response of Jurkat to toxins presented by cells bearing only HLA-DR1 was limited to SED and SEE. Jurkat responded solely to SEE when the toxins were presented by the murine class II–expressing APC, CH12.1.

A T cell hybridoma bearing murine Vβ3, K25-49.16, responded to SEA, SEB, SED, TSST, and ExF in the presence of CH12.1. This result was in close agreement with the data obtained in experiments using bulk murine T cells, where Vβ3-expressing T cells have been shown to respond to these SEs, and additionally, SEC1 (19). The use of human class II–expressing APC (Raji- or HLA-DR1-transfected cells) led to broader toxin responses by K25-49.16, as evidenced by its IL-2 production in response to SEC1 and SEE.

Table 1. *Human and Murine T Cell Responses to SEs Presented by Either Mouse or Human Class II Molecules*

T cell	APC	IL-2 production in response to toxin:							
		None	SEA	SEB	SEC1	SED	SEE	TSST	ExF
					U/ml				
Jurkat[*]	CH12.1[‡]	<10	<10	<10	<10	<10	320	<10	<10
(h Vβ8)	Raji	<10	160	40	<10	>640	>640	<10	<10
	DAP-DR1	<10	<10	<10	<10	>640	>640	<10	<10
K25-49.16	CH12.1	<10	>640	>640	<10	>640	<10	320	>640
(m Vβ3)	Raji	<10	>640	>640	>640	640	>640	>640	>640
	DAP-DR1	<10	>640	>640	>640	>640	>640	640	>640
KMLs-12.6	CH12.1	<10	<10	<10	<10	<10	ND[§]	ND	ND
(m Vβ6)	Raji	<10	<10	<10	40	40	ND	ND	ND
	DAP-DR1	<10	<10	320	>640	>640	ND	ND	ND
KMLs-13.11	CH12.1	<10	<10	<10	<10	<10	ND	ND	ND
(m Vβ6)	Raji	<10	160	<10	320	40	ND	ND	ND
	DAP-DR1	<10	20	80	320	40	ND	ND	ND

[*] Jurkat is a human T cell line expressing Vβ8 (h Vβ8). K25-49.16 is a murine T hybridoma that expresses the Vβ3 element (m Vβ3). KMLs-12.6 and KMLs-13.11 express the murine Vβ6 element.

[‡] CH12.1 is a mouse B cell lymphoma expressing I-A^k and I-E$^{d/k}$. Raji is a human B cell tumor bearing HLA-DR3, -DRw10, -DQw1, -DQw2, -DP7. DAP-DR1 is a mouse fibroblast that expresses human HLA-DR1.

[§] IL-2 production in response to SEE, TSST, and ExF was not measured in this experiment. In five other experiments, Vβ6-bearing T cells failed to produce any detectable amounts of IL-2 when these SEs were presented by these APC.

The most remarkable results shown in Table 1 concern the responses of T cell hybrids bearing murine Vβ6. In previous studies, T cells bearing this Vβ were not stimulated by any of the SEs in the presence of murine presenting cells (7, 19), a result confirmed here when CH12.1 was used as APC. In contrast, five individual Vβ6-bearing T hybrids (two of which are shown in Table 1) responded to SEB, SEC1, SEC2, SEC3, and SED when the toxins were presented by human class II–bearing APC.

Overall, therefore, the data in Table 1 show that SE reactivities by different T cells can be demonstrated using this approach, and considerable cross-species activity occurred. In general, T cells showed a broader range of toxin responses in the presence of human, rather than murine, class II. No reactivities observed with murine class II–bearing APC were lost when human class II–expressing APC were used, and additional SE reactivities were frequently seen with the latter. These additional reactivities elicited by APC bearing human class II could be due to higher affinities of SE for the human rather than murine MHC molecules, leading to increased ligand concentrations and subsequent T cell responses. It should be noted, however, that even though T cells bearing a particular Vβ responded to a broader range of SEs in the presence of human class II, as opposed to murine class II, Vβ specificity for the toxins was maintained, as summarized

in Table 2, which includes data obtained with T cell hybrids bearing a number of different murine Vβs.

SEA Displays a Higher Affinity for Human Class II Proteins than for Murine Ia Antigens. A toxin binding assay, using biotinylated SEA (bio-SEA), Pe-Av, and cytofluorographic analysis, was developed in order to determine whether SEs differ in their binding to human and murine class II molecules. Fig. 1 shows that Bio-SEA bound much better to the human B cell line Raji than to the mouse B cell line CH12.1. The relative affinity of bio-SEA for the class II molecules expressed on Raji was ~100-fold higher than its affinity for the murine class II proteins expressed on CH12.1. This disparity could not be due to differences in levels of MHC ligands present on the two B cell lines, since the level of HLA-DR antigens expressed on Raji was only twofold higher than the amount of I-E molecules present on CH12.1 (data not shown). Similar differences in the binding of biotinylated SEB, SEC1, and SEE to human and murine class II–bearing cells indicate that the other SEs have higher affinities for human MHC molecules (data not shown), and that these binding differences may account for the enhanced SE responses observed with murine T hybrids and APCs expressing human MHC class II antigens.

Human HLA-D Isotypes Differ in their Ability to Trigger Murine T Hybrid Toxin Responses. We wished to investigate whether all the human class II isotypes could present SEs

Table 2. *Changes in Toxin Reactivities of Murine T Cell Hybrids as a Function of the Species Origin of the APCs Class II Molecules*

Murine Vβ	Toxin responses with murine class II	Additional responses with human class II
1	SEA	SEB, SEE, TSST, ExF
3	SEA, SEB, SED, TSST, ExF	SEC1, SEC2, SEC3, SEE
6	None	SEA, SEB, SEC1, SEC2, SEC3, SED
8.1	SEB, SED (SEC3)	SEC1, SEC2, SEC3, ExF
8.2	SEB, SEC1, SEC2, SEC3, SED (SEE)	SEA, TSST, ExF
8.3	SEB, SEC1, SED (ExF)	SEA, SEC2, SEC3, TSST

Weak SE responses observed in the mouse are listed in parentheses.

Figure 1. SEA shows a greater affinity for human MHC class II antigens than for murine class II molecules. The human B lymphoma cell line, Raji, and the murine B cell lymphoma, CH12.1, were tested for binding of bio-SEA. The levels of bio-SEA binding are expressed as fluorescence intensity, which was calculated as described in Materials and Methods.

equally effectively, since preferential binding by some SEs to some isotypes has been reported (17, 33). Since the human B cell lymphoma, Raji, expresses all three isotypes and was therefore not useful for these experiments, we used the L cell transfectants expressing individual isotype molecules.
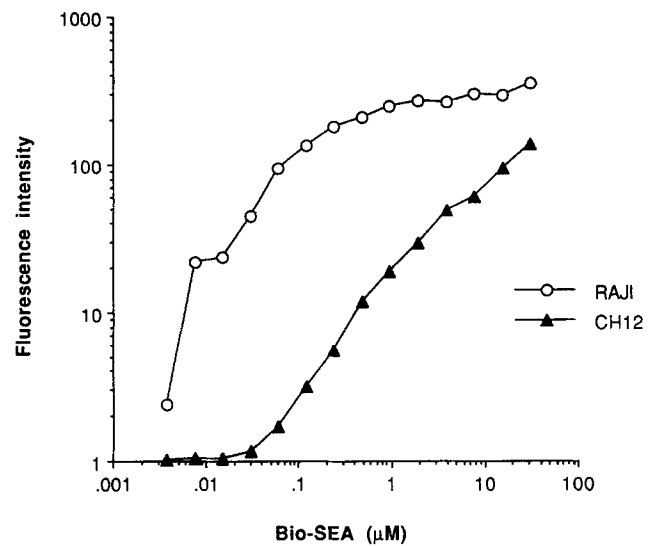
The results are shown in Table 3. Different HLA isotypes did indeed differ in their ability to present SEs. HLA-DR1 was the most efficient presenter, HLA-DQw1 was intermediate, and HLA-DPw2 was the least effective presenting molecule. The Vβ1-expressing T hybrid, KSEA-1.8, for ex-

Table 3. *Human HLA-D Isotypes Differ in Their Ability to Present SEs to T Cells*

T cell	APC	IL-2 production in response to toxin:					
		None	SEA	SEC1	SEE	TSST	ExF
				U/ml			
Jurkat*	DAP-DR1*	<10	20	10	>640	ND‡	ND
(h Vβ8)	DAP-DQw1	<10	<10	<10	80	ND	ND
	DAP-DPw2	<10	<10	<10	160	ND	ND
KSEA-1.8	DAP-DR1	<10	>640	10	>640	40	640
(m Vβ1)	DAP-DQw1	<10	160	<10	<10	<10	<10
	DAP-DPw2	<10	<10	<10	<10	<10	<10
K25-59.6	DAP-DR1	<10	>640	80	80	320	>640
(m Vβ3)	DAP-DQw1	<10	>640	<10	<10	<10	<10
	DAP-DPw2	<10	320	<10	<10	<10	<10
KMLs-12	DAP-DR1	<10	<10	640	<10	<10	10
(m Vβ6)	DAP-DQw1	<10	<10	>640	<10	<10	<10
	DAP-DPw2	<10	<10	<10	<10	<10	<10

The T cell designations are as described in Table I.
* The APC were transfected L cell fibroblasts expressing individual human class II isotypes.
‡ In other experiments, Jurkat failed to produce detectable levels of IL-2 in response to TSST or ExF presented by these APC.

Table 4. *Toxin-specific T Cell Responses Are Influenced by the HLA-DR Allele on the Presenting Cell*

T cell	Toxin	IL-2 production in response to SE + APC:					
		DR1	DR2	DR4	DR6	DR7	DRw53
		U/ml					
Jurkat	None	<10	10	40	40	40	40
(h Vβ8)	SEA	<10	20	40	80	40	40
	SEC1	<10	20	40	40	40	40
	SEE	>640	>640	>640	>640	>640	40
	TSST	20	80	40	40	40	40
KSEA-1.8	SEA	>640	>640	>640	>640	>640	<10
(m Vβ1)	SEC1	40	<10	<10	<10	<10	<10
	SEE	>640	>640	>640	20	10	<10
	TSST	40	<10	10	<10	<10	<10
K25-49.16	SEA	>640	320	160	160	320	320
(m Vβ3)	SEC1	>640	<10	80	<10	<10	<10
	SEE	>640	160	320	160	320	<10
	TSST	640	20	80	<10	<10	<10
KMLs-12.6	SEA	<10	<10	<10	<10	<10	<10
(m Vβ6)	SEC1	>640	320	>640	80	40	>640
	SEE	ND*	ND	ND	ND	ND	ND
	TSST	ND	ND	ND	ND	ND	ND
K16-15.5	SEA	80	10	80	<10	<10	10
(m Vβ8.2)	SEC1	>640	640	>640	>640	>640	>640
	SEE	>640	80	640	80	160	<10
	TSST	<10	<10	<10	<10	<10	20
Mean channel fluorescence:							
SG465 (α-DR, DQ, DP)‡		ND§	184	119	150	138	158

The T cell designations are described in Table 1. The APC were transfected L cell fibroblasts expressing HLA-DR1, HLA-DR2 Dw2, HLA-DR4 (Dw4), HLA-DR6 (w52c), HLA-DR7, and HLA-DRw53, respectively. All of the T cells, with the exception of Jurkat, did not produce detectable levels of IL-2 (<10 U/ml) in response to APC alone.
* See Table 1.
‡ The mAb SG465 recognizes an epitope common to HLA-DR, HLA-DQ, and HLA-DP (27, 28). Fluorescence staining was performed as described in Materials and Methods, using biotin-labeled SG465, followed with PE-Av, and analyzed by cytofluorography. The mean channel fluorescence values are listed here.
§ In a separate experiment using biotin-labeled SG465 + PE-Av, the DAP-DR1 transfectant was shown to express approximately twofold higher levels of HLA-DR than the HLA-DRw53 cell line.

ample, responded to SEA, SEC1, SEE, and TSST with the HLA-DR1 transfectant, but only to SEA with HLA-DQw1 cells, and to no toxins presented by HLA-DPw2. In fact, there were only two cases where the T cells used in this study responded to SEs presented by HLA-DPw2. Jurkat produced IL-2 in response to SEE, and murine Vβ3-expressing T cells responded to SEA with this class II isotype. Similar disparities, either in terms of absolute responses or quantitative differences in magnitudes of response, were observed with the other T cells.

The differences in toxin stimulation seen with these human class II transfectants could not be attributed to the level of surface expression of class II on the cells, since cytofluorographic analysis of these APCs with an antibody that recognizes an epitope common to all three isotypes showed that the HLA-DP transfectant expressed the highest level of class II, and the HLA-DR and HLA-DQ transfectants expressed nearly equivalent levels of MHC molecules (data not shown).

These results indicated that the human class II isotypes differed dramatically in their ability to present SEs. HLA-DR1 was the most effective presenting molecule, whereas HLA-DPw2 was the poorest presenter.

Toxin Responses Are Influenced by the HLA-DR Allele on the APC. A large panel of L cell transfectants expressing different HLA-DR alleles were examined for their ability to present SEs to T cells bearing different Vβs. The results obtained with several of these transfectants are presented in Table 4. The data in Table 4 indicate that the toxin reactivities of a particular T cell are indeed affected by the HLA-DR allele on the APC. For example, K25-49.16 (Vβ3) responded to SEC1 presented by the HLA-DR1- and HLA-DR4-expressing APC, but not to the same SE presented by the HLA-DR2-, HLA-DR6-, HLA-DR7-, and HLA-DRw53-expressing transfectants. Similar results were obtained with the other T cells.

Overall, the differential ability of cells bearing the different HLA-DR alleles to present toxins did not correlate with levels of HLA-DR expressed on the APC (Table 4). The HLA-DR4^{+} line, for example, bore the lowest level of HLA-DR of these APC, yet it was a reasonably efficient SE presenter. Most strikingly, the HLA-DRw53 transfectant expressed one of the highest levels of HLA-DR, and yet it was the poorest presenter. APC expressing this HLA-DR molecule failed to present SEC1, SEE, and TSST to K25-49.16, and yielded similar results with many other T hybrids. The Vβ6-bearing T hybrids were exceptional by virtue of their strong responses to SEC1 presented by HLA-DRw53 (Table 4, and data not shown). Interestingly, HLA-DRw53 was the only HLA-DR molecule that failed to present a particular SE to any of the T hybrids. None of the T cells responded to SEE with the HLA-DRw53-bearing APC. K16-15.5, a Vβ8.2-expressing T hybrid, did not produce detectable amounts of IL-2 in response to SEE and HLA-DRw53, though this hybrid did respond to SEB and the HLA-DRw53 APC.

This marked difference in toxin presentation was tested in more detail by measuring the dose response of K16-15.5
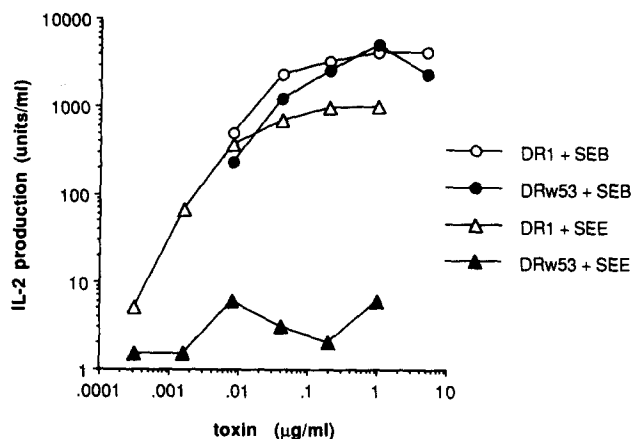
to SEB and SEE with the HLA-DR1 and HLA-DRw53 transfectants (Fig. 2). The SEB response of K16-15.5 was nearly equivalent when HLA-DR1 or HLA-DRw53 were used as presenting molecules. The two HLA-DR antigens dramatically differed, however, in their ability to present SEE to K16-15.5. No detectable IL-2 was produced with the HLA-DRw53 APC, while the titration of SEE with the HLA-DR1 APC gave high levels of IL-2.

This demonstration that HLA-DR alleles differ in their ability to stimulate T cells against SEs implies that the HLA-DR β chain must be a determining factor, since HLA-DR molecules all share the same α chain. A role for the α chain, however, could not be excluded by these findings.

Failure of HLA-DRw53 to Present SEE to T Cells Is Due to Poor Binding of SEE to this Class II Molecule. The apparent failure of HLA-DRw53 to present SEE to the panel of T cells could be the result of lack of toxin binding to this MHC molecule, or could be due to SEE binding to HLA-DRw53 in a manner that does not allow T cell stimulation. The toxin binding assay was used to distinguish between these possibilities. The binding of bio-SEA and bio-SEE to the HLA-DR1 and HLA-DRw53 transfectants is shown in Fig. 3. The toxins showed high levels of binding to the HLA-DR1-expressing cell line, but very low levels of binding of SEA and SEE were seen with the HLA-DRw53 transfectant. The binding of SEA and SEE to HLA-DRw53 was greater than two orders of magnitude less than the binding of these SEs to HLA-DR1. This dramatic reduction of bio-SEE binding to HLA-DRw53 explained why this HLA-DR molecule failed to stimulate with SEE, and this result showed that the HLA-DR β chain determines toxin binding. Interestingly, the binding of bio-SEA to the HLA-DRw53 transfectant was also greatly diminished compared with the binding seen with HLA-DR1.
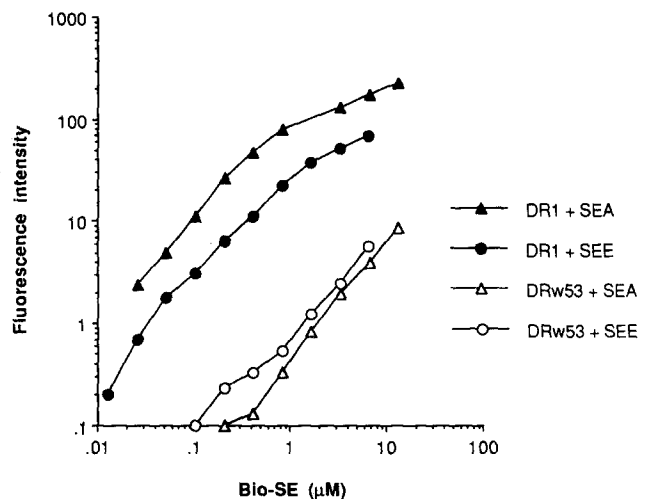

Figure 2. Dose response of a murine Vβ8.2-expressing T cell hybridoma, K16-15.5, to SEB and SEE presented by transfected L cell fibroblasts expressing HLA-DR1 or HLA-DRw53. IL-2 production was assessed by uptake of the vital dye MTT (30) by the IL-2-dependent cell line HT-2. MTT uptake was measured with a Biotek Microplate Autoreader EL-311 (Biotek Instruments, Inc., Winooski, VT) at a wavelength of 570 nm, and the values given have been obtained by calibration with an IL-2 standard. Values of <10 U/ml were not significantly above background.

Figure 3. The HLA-DRw53 molecule binds SEA and SEE poorly. Transfectants expressing HLA-DR1 or HLA-DRw53 were tested for binding of bio-SEA and bio-SEE. The levels of bio-SE binding to the transfectants are expressed as fluorescence intensity, which was calculated as described in Materials and Methods.

This finding may explain why many T cells used in these studies failed to respond to SEA with this transfectant, and why only a few hybrids could respond to this SE in the context of HLA-DRw53 (Table 4, and data not shown). Possibly, the presence of a high affinity TCR could stabilize an apparently weak interaction between SEA and HLA-DRw53 molecules, and the formation of this trimolecular complex would lead to IL-2 secretion by the T hybrid.

Discussion

The experiments presented in this manuscript demonstrate that HLA-DR alleles differentially present SEs to T hybridomas. These results were unexpected, since it had been previously thought that the class II molecule acts as an almost generic platform for SE interactions with Vβ elements, and this appeared to implicate the nonpolymorphic α chain of MHC class II molecules in toxin stimulation. The results of a study on the binding of SEB and TSST to a collection of transfectants expressing HLA class II antigens led the authors to conclude that HLA polymorphisms did not affect binding of these toxins (18). However, the data obtained in this study showed that several alleles of HLA-DR differed in their ability to bind and present several toxins. Since all HLA-DR molecules share a common α chain, these results mapped an important site for MHC-SE, and TCR-MHC-SE, interactions to the β chain of HLA-DR. The finding that class II alleles differentially support SE stimulation of T cells may suggest that class II allelism influences an individual's susceptibility to SE intoxications.

The poorest SE presenter among the HLA-DR alleles tested was HLA-DRw53. This was shown to be due to poor binding of SEA and SEE to this HLA-DR molecule. Comparison of HLA-DR sequences shows that HLA-DRw53 is the least related to the consensus sequence for HLA-DR (34), and it is most likely that these differences in conserved regions of HLA-DR play a role in toxin binding to MHC. This information should provide clues for the region of the MHC molecule that interacts with SE.

The application of a panel of L cells transfected with different isotypes of human class II allowed us to compare the relative abilities of HLA-DR, HLA-DQ, and HLA-DP to present toxins to T hybrids. The hierarchy for supporting SE stimulation (HLA-DR > HLA-DQ > HLA-DP) was similar to that seen with murine isotypes (7, 14), where the structural homologue of HLA-DR, I-E, supported SE responses more efficiently than the HLA-DQ homologue, I-A. In general, this hierarchy of SE stimulation for the human class II isotypes supported the observations made by other workers of SE binding to the Raji B cell line (33). Interestingly, Herrmann et al. (33) were unable to detect ExF binding to Raji, while the results presented here (Table 3) indicate that ExF could be presented by HLA-DR1, but not by HLA-DQw1 or HLA-DPw2. It is perhaps more than coincidental that HLA-DR and I-E are the most efficient presentation molecules for SE. It has been shown that I-E serves as a ligand for self-superantigens, and plays a pivotal role in thymic selection in the mouse (11). One might speculate that I-E, and

HLA-DR have maintained common structural motifs that are recognized by SEs and self-superantigens, a species that has yet to be defined in humans.

These experiments have shown that murine T cells were more likely to respond to a given SE presented by human class II, than the same toxin presented by murine class II. Thus, in every case where a T cell hybrid responded to an SE + murine class II, it would respond to the same SE + human class II. Additional responses were seen, however, when human class II was used to present SEs to murine T cell hybrids. Most strikingly, T cell hybrids bearing Vβ6, which do not respond to any SE presented by murine class II, did respond to some of the SEs, particularly SEC1, presented by human class II molecules.

It is possible that the murine responses to toxins presented by human class II were broader than when murine class II composed part of the ligand, because TCRs have higher affinities for class II antigens of other species, since these T cells have not been selected for tolerance to such xenoantigens. If this increased affinity of TCRs for xenogeneic MHC were the pivotal requirement for broader SE reactivities, one would predict that the human T cells would respond to more SEs presented by murine APC than by human APC. A broader reactivity pattern, however, was not observed with one human T cell line, Jurkat, and xenogeneic MHC. Another explanation is that SE have higher affinities for human than for murine class II, and these differences are manifested by triggering wider T cell responses. The comparison of bio-SEA binding to murine and human B cell lines demonstrated that SEA displays a higher affinity for human class II molecules (Fig. 1). The additional toxin responses, therefore, would simply be due to enhanced toxin binding to HLA-DR, and therefore, an increased concentration of ligand. It should be noted, however, that the SE that has the highest affinity for MHC class II, SEA (16, 35, A. Herman, personal observations), is not the toxin that most often stimulates murine T cells when it was presented by HLA-DR. This would imply that SE specificity still resides, in part, at the level of the TCR and Vβ. In this context, it is also worth noting that *Staphylococcus aureus*, the organism that secretes these SEs, is a human pathogen, and these SEs may therefore have evolved to interact with human rather than murine MHC proteins.

Sometimes, the ability of a particular SE to bind to a specific class II protein did not correlate well with the ability of the SE + MHC complex to stimulate T cells (18, and this study). Although SEA and SEE both bind very poorly to HLA-DRw53, the former toxin, in association with HLA-DRw53, could stimulate some T cells while the latter could not. This finding probably reflects the fact that T cell stimulation in these experiments is the result of the formation of a trimolecular complex of TCR + SE + MHC class II. A TCR with very high affinity for toxin + MHC may be able to stabilize a weak interaction between a SE and an MHC molecule, and thus, T cells bearing particular Vβs may be able to respond to a particular SE + MHC combination, even though the latter interaction cannot easily be demonstrated by binding.

The results obtained from this study may help to elucidate

the mode in which foreign superantigens interact with MHC class II molecules. The SEs have been studied intensively because they share several important properties with self-superantigens, whose structures are, as yet, undefined. The

properties of Vβ-specific interactions, the promiscuous requirement for MHC class II, and their ability to delete T cells in the thymus may make the study of MHC-SE interactions useful for understanding how self-antigens function in vivo.

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