T Cell Receptor–Major Histocompatibility Complex Class II Interaction Is Required for the T Cell Response to Bacterial Superantigens

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Summary

Bacterial and retroviral superantigens (SAGs) stimulate a high proportion of T cells expressing specific variable regions of the T cell receptor (TCR) β chain. Although most alleles and isotypes bind SAGs, polymorphisms of major histocompatibility complex (MHC) class II molecules affect their presentation to T cells. This observation has raised the possibility that a TCR–MHC class II interaction can occur during this recognition process. To address the importance of such interactions during SAG presentation, we have used a panel of murine T cell hybridomas that respond to the bacterial SAG Staphylococcal enterotoxin B (SEB) and to the retroviral SAG *Mtv-7* when presented by antigen-presenting cells (APCs) expressing HLA-DR1. Amino acid substitutions of the putative TCR contact residues 59, 64, 66, 77, and 81 on the DR1β chain showed that these amino acids are critical for recognition of the SAG SEB by T cells. TCR–MHC class II interactions are thus required for T cell recognition of SAG. Moreover, *Mtv-7* SAG recognition by the same T cell hybridomas was not affected by these mutations, suggesting that the topology of the TCR–MHC class II–SAG trimolecular complex could be different from one TCR to another and from one SAG to another.

Superantigens (SAGs)[1] stimulate a high proportion of T cells bearing specific Vβ regions of the TCR (reviewed in 1). This activation requires APCs expressing MHC class II molecules (2–4). The SAG family includes retroviral products of the mouse mammary tumor virus, and bacterial toxins produced mostly by *Staphylococci* and *Streptococci* (reviewed in 1). Unlike conventional antigens (Ags), SAGs bind outside of the polymorphic antigen binding groove of MHC class II molecules (5). Site-directed mutagenesis has shown that Staphylococcal enterotoxin A (SEA) interacts with histidine 81 of the MHC class II β chain (6, 7) while amino acids 36 and 39 of the DRα chain are critical for toxic shock syndrome toxin 1 (8) and SEB binding (9, and our unpublished observations).

Experiments suggested at first the absence of TCR–MHC class II contact during SAG recognition. This conclusion was inferred from the fact that most class II molecules can present SAGs to a given T cell (4, 10–12). Moreover, $CD8^+$ T cells expressing TCRs restricted to MHC class I molecules recognize the MHC class II–SAG complex (13–15). Finally, the TCR can interact with SAGs in the absence of MHC class II positive cells although this interaction does not result in T cell activation (16, 17). These results have led to the hypothesis that MHC class II molecules serve only as a scaffold for SAG presentation and are not directly in contact with the TCR. A more systematic analysis of SAG presentation suggests that TCR–MHC class II interactions are required for T cell stimulation by SAGs. Stimulation of T cell clones is affected by MHC class II polymorphisms (18–20) and a skewing of TCR-α chain expression among T cells reactive to retroviral and bacterial SAGs was reported (21–23). Moreover, fine specificities of TCR for particular MHC class II–SAG complexes are influenced by the non-Vβ (Dβ, Jβ, Vα, and Jα) elements of the TCR suggesting that these regions might interact with MHC class II molecules (24, 25). The crystal structure of the bacterial toxin SEB supports this hypothesis since the TCR and MHC class II binding regions are adjacent thus permitting a direct contact between the three molecules (26).

To address a possible role for an interaction between TCR and MHC class II during SAG recognition, we have used mutant MHC class II molecules at putative TCR contact

[1] *Abbreviations used in this paper:* SAG, superantigen; SEA or B, Staphylococcal enterotoxin A or B.

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residues to present bacterial and retroviral SAGs to different murine T cell hybridomas. These substitutions have allowed us to show that distinct TCR contact residues on MHC class II molecules affect the response of each hybridoma to SEB. These mutations do not affect *Mtv*-7 SAG presentation suggesting that the same T cells interact in a different manner with the MHC class II molecules during SEB and *Mtv*-7 SAG recognition. These results provide a strong evidence for an interaction between the TCR and MHC class II molecules in the T cell response to SAGs.

Materials and Methods

Cell Lines. Kmls 13.11, Kmls 12.6, and RG 17 are murine T cell hybridomas expressing the TCR Vβ6 chain and are stimulated by SEB and *Mtv*-7 SAG (27, 28). 3DT52.5.8 is a CD4 negative murine T cell hybridoma specific for D^d and expressing the TCR Vβ1 and Vβ8.1 genes (29). This T cell hybridoma recognizes SEA and SEB but not *Mtv*-7 SAG (30). KR3 is a $CD4^+$ murine T cell hybridoma that expresses the TCR Vβ8.1 chain and recognizes SEB and *Mtv*-7 SAG (31). DAP-3 is a MHC class II negative murine fibroblastic cell line (32). The hybridomas Kmls 13.11 and Kmls 12.6 were grown in DMEM (GIBCO BRL, Gaithersburg, MD) supplemented with 5% FCS (GIBCO BRL), 4 mM dextrose (Sigma Chemical Co., St. Louis, MO), 2 mM L-glutamine (GIBCO BRL), essential and nonessential amino acids (GIBCO BRL), 1 mM sodium pyruvate (GIBCO BRL), 10 μM β-mercaptoethanol (Sigma Chemical Co.), and 20 μg/ml gentamycin (GIBCO BRL). 3DT52.5.8, RG17, and KR3 were grown in RPMI 1640 (GIBCO BRL) supplemented with 5% FCS, 2 mM L-glutamine, 10 μM β-mercaptoethanol, and 20 μg/ml gentamycin. DAP-3 cells were cultured in DMEM supplemented with 5% FCS, 2 mM L-glutamine, and 20 μg/ml gentamycin.

Generation of Mutant HLA-DR Molecules. The SmaI–BamHI cDNA fragment that encodes DR1 (*DRB1*0101*) β chains was cloned between the filled-in SalI site and the BamHI site of the eukaryotic expression vector RSV.3 (33, 34). The cDNA fragment that encodes the DRα chain was also subcloned in the RSV.3 expression vector (35). Site-specific mutagenesis of the DRβ chain cDNA was performed as previously described (7) using the PCR overlap extension technique (36). The full-length PCR fragments carrying the mutations on the DRβ chain cDNA were digested with SacI and StuI. This 454-bp fragment was shuffled into the SacI- and StuI-digested wild-type DR1 cDNA cloned in the RSV.3 eukaryotic expression vector. Generation of the DRα mutant 39, 42, 46 has been described elsewhere (9). All of the mutants generated by PCR were confirmed by nucleotide sequencing.

DNA Transfections. DNA transfections were performed using the calcium phosphate coprecipitation technique as previously described (7). Homogeneous populations of DAP-3 cells expressing comparable levels of MHC class II molecules were obtained by two rounds of aseptic cell sorting on a FACStar® Plus (Becton Dickinson Immunocytometry Systems, San Jose, CA). Stable transfections of *Mtv-7 sag* gene in DAP-3 fibroblasts expressing wild-type or mutated MHC class II molecules were performed as previously described (9).

Cytofluorometric Analysis of DAP-3 Cells Transfected with HLA-DR Molecules. Cells were stained with either L-243 or 50D6, a mouse anti–human MHC class II antibody that recognizes all DR alleles except DR7 and DRw53, followed by goat anti–mouse (GAM)-FITC (GIBCO BRL). As negative control, the different transfectants were stained with the secondary antibody alone. MHC class II expression was analyzed by flow cytometry using a FACScan® (Becton Dickinson Immunocytometry Systems).

Stimulation of Murine T Cell Hybridomas. Stimulation of the different T cell hybridomas with recombinant SEB (rSEB; a generous gift of J. Kappler and P. Marrack, National Jewish Hospital, Denver, CO) was carried out as follows. 75×10^3 T cells/well were added to 2×10^4 DAP-3 fibroblasts expressing wild-type or mutated HLA-DR molecules. Different concentrations (0–1 μg/ml) of bacterial toxin SEB were added to the coculture in 96-well plates and incubated for 24 h at 37°C, 5% CO_2. Triplicates were performed for each experimental condition. T cell stimulation was determined by IL-2 production. IL-2 production was assayed by the capacity of the culture supernatants to support the proliferation of the IL-2–dependent cell line CTLL.2, as measured using the hexosaminidase colorimetric assay (37). Presentation of *Mtv*-7 SAG by mutated MHC class II molecules was performed as previously described (9).

Toxin Binding Analysis. 20 μg of rSEB were iodinated using 0.5 μg of iodogen (Pierce Chemical Co., Rockford, IL) coated to tubes and 250 μCi of ^{125}I (Amersham Corp., Toronto, Canada). Free iodine was removed from the labeled toxin using Sephadex G-10 exclusion chromatography. The ability of different HLA-DR mutants to bind SEB was determined as follows: 10^6 DAP-3 cells expressing the wild-type or mutated HLA-DR molecules were incubated with 100 ng of ^{125}I-toxins in 200 μl of binding buffer (DMEM + 2% FCS + 0.1% NaN_3) for 4 h at 37°C. Tubes were agitated every 15–20 min. Duplicates were performed for each condition. After incubation, the cells were pelleted through an oil cushion (84% silicone oil and 16% mineral oil); pellets were cut from the tube and were then counted on a gamma-counter. Nonspecific binding was determined in the presence of a 100-fold excess (10 μg) of cold toxin.

Results

Mutations of Putative TCR Contact Residues on HLA-DR1β Chain Affect rSEB Presentation to Vβ8.1 Murine T Cell Hybridomas. To test the importance of TCR–MHC class II interactions for the recognition of SAG by T cells, we have substituted several of the putative TCR contact residues on the β chain of HLA-DR1 by alanines (Table 1). These mutated class II molecules were then used to stimulate two SEB-responsive $V\beta8.1^+$ T cell hybridomas (Fig. 1). Stimulation of 3DT52.5.8 by rSEB was observed with all the APCs expressing the different mutated MHC class II molecules. Transfected cells expressing the wild-type DR1 molecule stimulated IL-2 production by both $V\beta8.1^+$ T cell hybridomas even at concentrations of SEB as low as 1 ng/ml, whereas untransfected fibroblasts did not present SEB. Efficient presentation of rSEB by the MHC class II molecules mutated along the α-helix of the β1 domain (DR1 59.64, DR1 66.68, DR1 77.78, DR1 81A, and DR1 81Y) was observed with differences in the dose-response curve for each mutant (Fig. 1). Most of the mutations involving these residues led to an effect on the dose-response curve for both Vβ8.1 hybridomas tested, namely 3DT52.5.8 (Fig. 1, *A* and *B*) and KR3 (Fig. 1, *C* and *D*). The only mutant that seemed not to affect the presentation of rSEB involved the H81Y substitution. Indeed, cells expressing DR1 81Y were as efficient as wild-type DR1 molecule in presenting rSEB. In contrast, mutant DRα39.42.46, which fails to bind SEB, does not

Table 1. *Description of the HLA-DR1 Mutants*

Cell line	Mutation	Mean fluorescence value	
		L-243	50D6
DAP-3	–	5	4
DR1	–	120	106
DR1 59.64	βE59>A, Q64>A	57	36
DR1 66.68	βD66>A, 68>A	76	76
DR1 77.78	βT77>A, Y78>A	93	75
DR1 81A	βH81>A	38	19
DR1 81Y	βH81>Y	127	N.D.
DRα39.42.46	αK39>A, V42>A, E46>K	5	88

N.D., Not done.

present the toxin to both hybridomas even at the highest concentration tested (Fig. 1). These results thus indicate that the putative TCR contact residues β59, β64, β66, β77, and β81 are involved in the presentation of rSEB to the Vβ8.1 T cells, 3DT52.5.8 and KR3.

Mutations of Putative TCR Contact Residues on HLA-DRβ1 Chain Abolish Presentation of SEB to Vβ6 Murine T Cell Hybridomas. The effect of these mutations was then assessed on the presentation of rSEB to Vβ6$^+$ hybridomas, Kmls 13.11, Kmls 12.6, and RG 17. The latter will respond to rSEB only when presented by human MHC class II molecules (18). As also shown in Fig. 2 *A*, they require 10-fold higher levels of rSEB to produce detectable levels of IL-2 when compared with Vβ8.1 hybridomas (Fig. 1). A significant decrease of the T cell response to SEB was observed when class II molecules mutated at TCR residues 59 and 64 were used to present rSEB to Kmls 13.11 (Fig. 2 *A*); IL-2 production required a 10-fold higher concentration of SEB when compared with

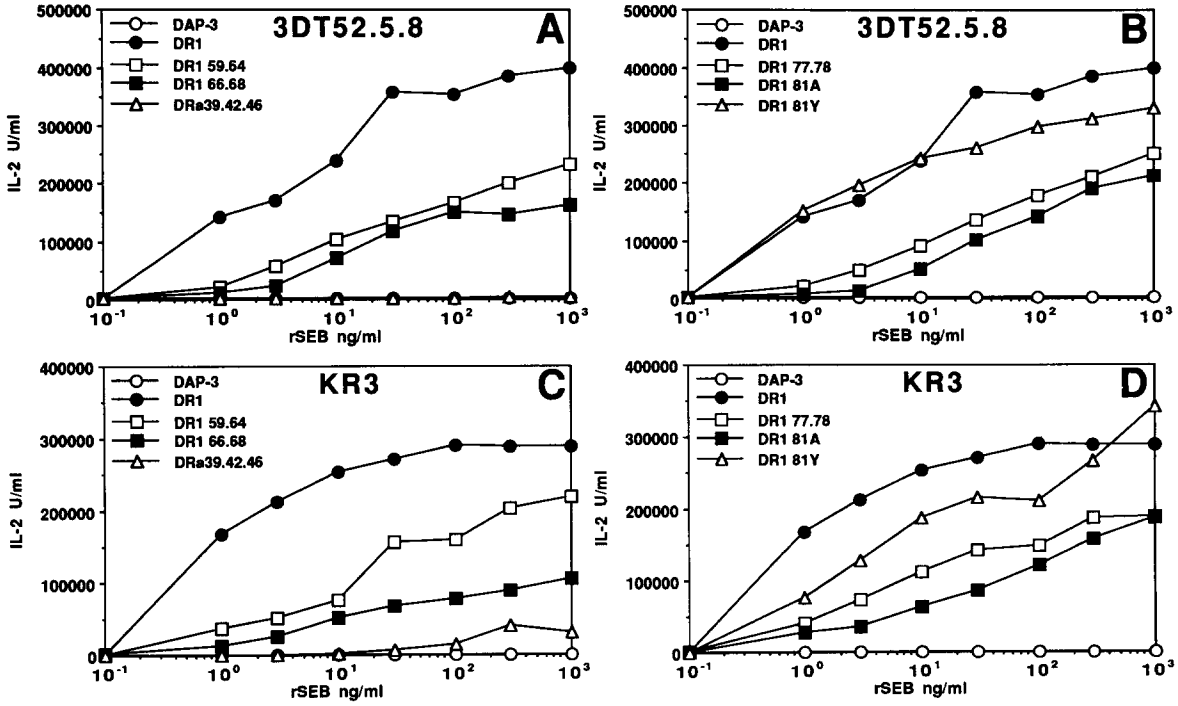

Figure 1. Dose-response curve of 3DT52.5.8 (*A* and *B*) and KR3 (*C* and *D*) to DAP-3 cells transfected with wild-type and mutant MHC class II molecules. T cell hybridomas were incubated 24 h at 37°C with DAP-3 cells expressing wild-type or mutated MHC class II molecules in the presence of 0–100 μg/ml of toxins. Each condition was done in triplicate. Stimulation of the T cell hybridomas is indicated by IL-2 production and was determined by the ability of culture supernatants to support the proliferation of the IL-2–dependent cell line CTLL.2.

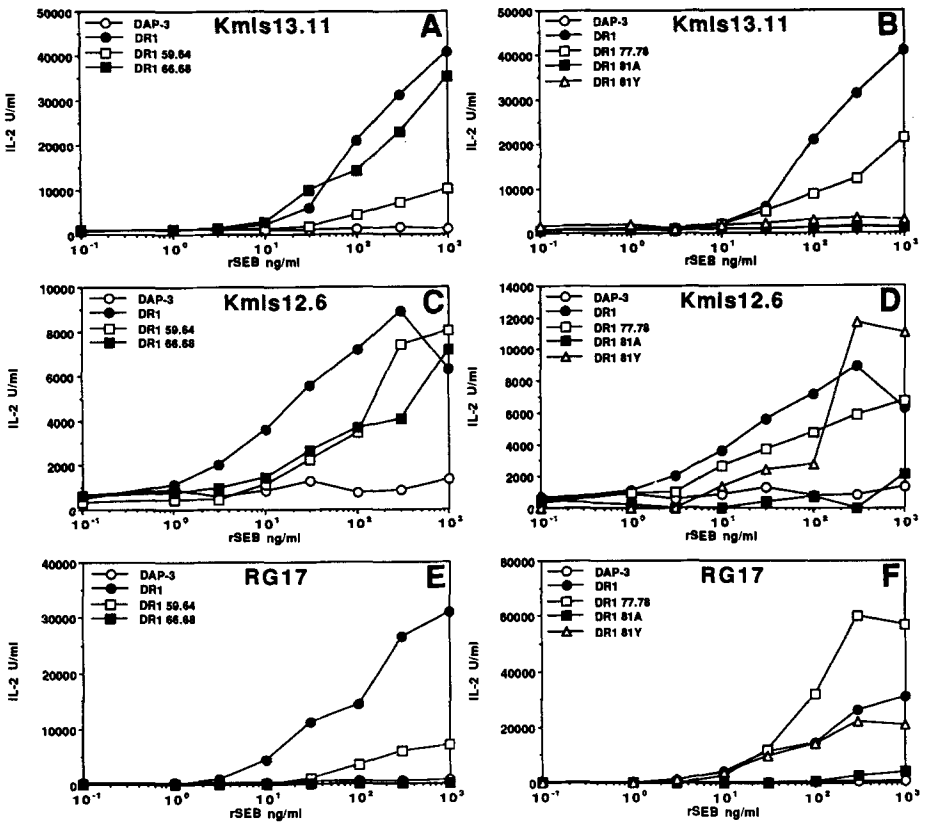

Figure 2. Dose-response curve of Vβ6$^+$ T cell hybridoma to Kmls 13.11 (*A* and *B*), Kmls 12.6 (*C* and *D*), and RG17 (*E* and *F*) to DAP-3 cells transfected with wild-type and mutant DR1 molecules. The cocultures and IL-2 measurements were performed as described in Fig. 1.

wild-type class II molecules. In contrast cells expressing mutants DR1 66.68 were as efficient in presenting rSEB as cells expressing the wild-type DR1 molecule. Moreover, as shown in Fig. 2 *B*, mutation of residue β81 to either an alanine (DR1 81A) or a tyrosine (DR1 81Y) complete abrogated presentation of rSEB to Kmls 13.11 even though they express high and comparable levels of MHC class II molecules (Table 1). Mutation of the other putative TCR contact residues β77 (DR1 77.78) (Fig. 2 *B*) had no effect on rSEB presentation to Kmls 13.11. These results indicate that TCR residues β59, β64, and β81 are critical for SEB recognition by Kmls 13.11 T cells in the context of DR1.

The effect of the same mutations was also determined for two other Vβ6$^+$ murine T cell hybridomas. Interestingly, presentation of rSEB to Kmls 12.6 involved different putative TCR contact residues on MHC class II molecules than what was observed with Kmls 13.11. As illustrated in Fig. 2 *C* mutants β59.64 and β66.68 had a moderate effect on SEB presentation in quantitative dose response curve assays. Similar results were obtained for mutants 77.78 (Fig. 2 *D*). However mutation of residue β81 to an alanine but not to a tyrosine abolished rSEB presentation to Kmls 12.6 (Fig. 2 *D*). These results demonstrate the critical role of residue 81 in the presentation of SEB to this TCR. They also suggest a role for allelic polymorphism in the presentation of bacterial toxins to different TCRs since distinct amino acids at position 81 lead to different outcome.

A distinct pattern was observed with the other Vβ6$^+$ T cell hybridoma RG 17. Mutations at positions 59.64 and 66.68 lead to drastic reduction of the capacity of these class II molecules to present SEB (Fig. 2 *E*). Moreover the alanine substitution at position β81 of DR1 affected the stimulation of RG 17 (Fig. 2 *F*). It is also interesting to note a significant enhancement in the stimulation of RG 17 by mutant DR1 77.78. It is possible that presence of small side chains at these positions facilitates the interaction of the DR1–SEB complex with the TCR. This effect was not observed with the other hybridomas. These results suggest that TCR–MHC class II interactions are required for efficient stimulation of T cells with rSEB. Moreover, the topology of the interaction between the TCR and MHC class II molecules is different from one TCR to another since distinct TCR residues affect the response of each hybridoma.

Mutation of Putative TCR Contact Residues on HLA-DR1β Chain Does Not Affect SEB Binding. Quantitative binding assays were performed to verify if mutations of putative TCR contact residues on the β chain of DR1 affected the interaction with SEB. Fig. 3 shows that all the mutants efficiently bound rSEB. The variation in the amount of ^{125}I-rSEB binding was always correlated with the level of MHC class II expression on the different transfectants (Fig. 3). Specificity of the binding is shown using DAP-3 untransfected cells which do not bind ^{125}I-rSEB. Moreover, mutant DRα39.42.46 fails to bind ^{125}I-rSEB in this assay and had previously been shown to lose the capacity to present SEB to T cells (9).

Mutation of residue β81 (either to an alanine or a tyrosine) on HLA-DR1 affects rSEB recognition by three out of five murine T cell hybridomas. The results indicate that

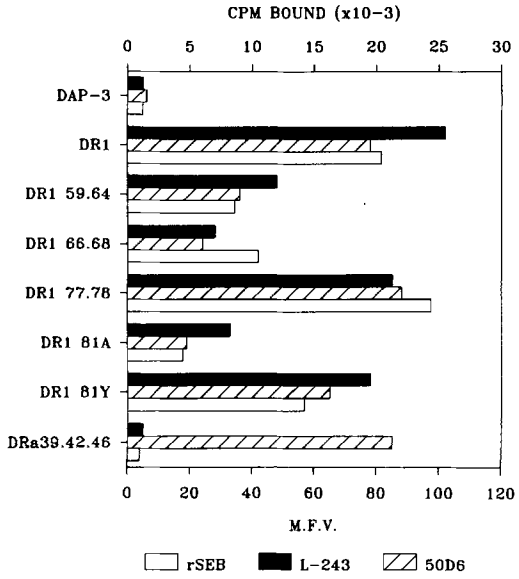

Figure 3. Binding of rSEB on transfectants expressing wild-type or mutated HLA-DR molecules. Binding of 100 ng of ^{125}I-rSEB. Cells were incubated in the presence of radiolabeled toxins for 4 h at 37°C (*white bars*). Binding is expressed as bound cpm. The different transfectants were also stained with L-243 and 50D6 mAbs to determine levels of MHC class II expression by flow cytometry. M.F.V., mean fluorescence value.

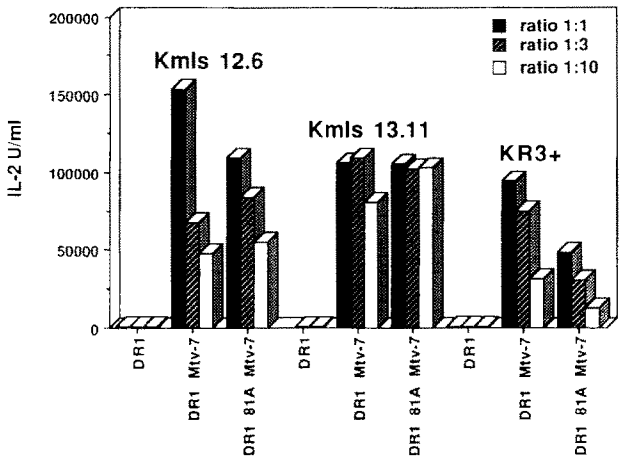

Figure 4. *Mtv*-7 SAG presentation to different murine T cell hybridomas by HLA-DR1 mutated at putative TCR contact residue β81. Presentation of *Mtv*-7 SAG to KR3$^+$ (Vβ8.1$^+$), Kmls 13.11 (Vβ6$^+$), and Kmls 12.6 (Vβ6$^+$) T cells by DAP DR1 and DAP DR1 81A. Fibroblasts expressing wild-type or mutated HLA-DR1 molecules were transfected with the *Mtv*-7 sag gene and then used at different stimulator/effector ratios (1:1, 1:3, 1:10) to stimulate the different murine T cell hybridomas. The cocultures and IL-2 measurements were performed as described in Fig. 1. Class II expression was monitored using the 50D6 antibody and the M.F.V. were: DR1, 178; DR1 Mtv-7, 120; DR1 81A Mtv-7, 140.

SEB binding is not affected by these mutations. DR1 81A or DR1 81Y bind rSEB proportionally to the levels of class II molecules expression at the cell surface (Fig. 3). Moreover, the binding of toxic shock syndrome type 1 is not impaired suggesting that these mutations do not alter the conformation of HLA-DR1 (not shown). As expected, binding of SEA is completely abolished by mutation of residue β81 (not shown) thus confirming our and others results (6, 7).

Mtv-7 SAG Presentation to Different Murine T Cell Hybridomas by HLA-DR1 Mutated at the Putative TCR Contact Residue β81. The requirement for TCR–MHC class II interactions in bacterial SAG recognition raised the possibility that it could also play a role in presentation of endogenous retroviral SAG such as *Mtv*-7. Mutation of residue β81 of HLA-DR1 to an alanine affects the rSEB response of the Vβ6$^+$ hybridomas to rSEB. To test a possible effect of this mutation on *Mtv*-7 SAG presentation, DR1 and DR1 81A were transfected with the *Mtv-7 Sag* gene and used to stimulate the murine T cell hybridomas that efficiently respond to *Mtv*-7 SAG. As shown in Fig. 4, the two Vβ6$^+$ hybridomas (Kmls 13.11 and Kmls 12.6) responded as efficiently as the Vβ8.1$^+$ hybridoma KR3 to *Mtv*-7 SAG presented by wild-type DR1 or DR1 81A. Similar results were previously reported with another Vβ8$^+$ hybridoma stimulated with DR1 81A cells transfected transiently with *Mtv*-7 Sag (9). This result is in sharp contrast to SEB presentation where Vβ8$^+$ hybridomas responded well while Vβ6$^+$ cells were not stimulated using class II molecules DR1 81A (Figs. 1 and 2). These results suggest that stimulation of T cells with retroviral or bacterial SAGs requires different TCR–MHC class II interactions.

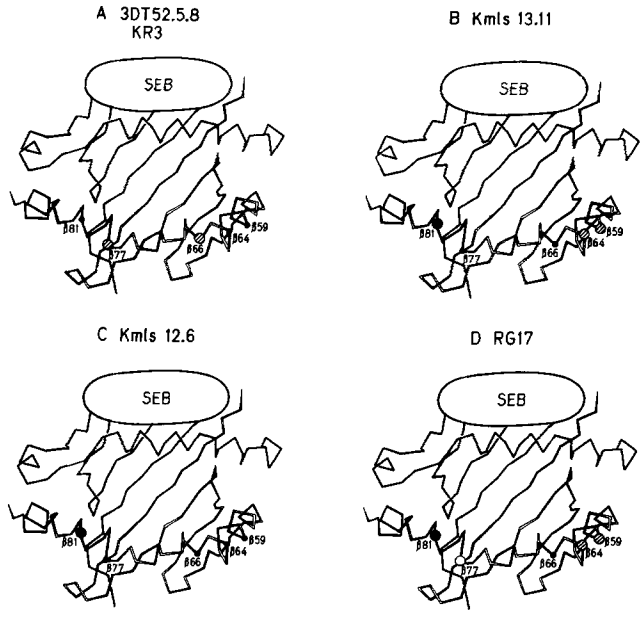

Figure 5. Position of the residues that have been substituted on DR1β chain. The DRα chain is drawn in black and the DRβ chain in white. The MHC class II molecule drawing is adapted from the crystallographic structure of HLA-DR1 described by Brown et al. (41). Mutated residues are indicated by circles. *Small dots* (.) represent residues that do not affect rSEB presentation. *Solid circles* (●) represent residues that lead to a total abrogation of rSEB presentation; *hatched circles* (⌀) represent residues that practically affect presentation of rSEB; and empty circles (O) represent residues that enhance rSEB presentation.

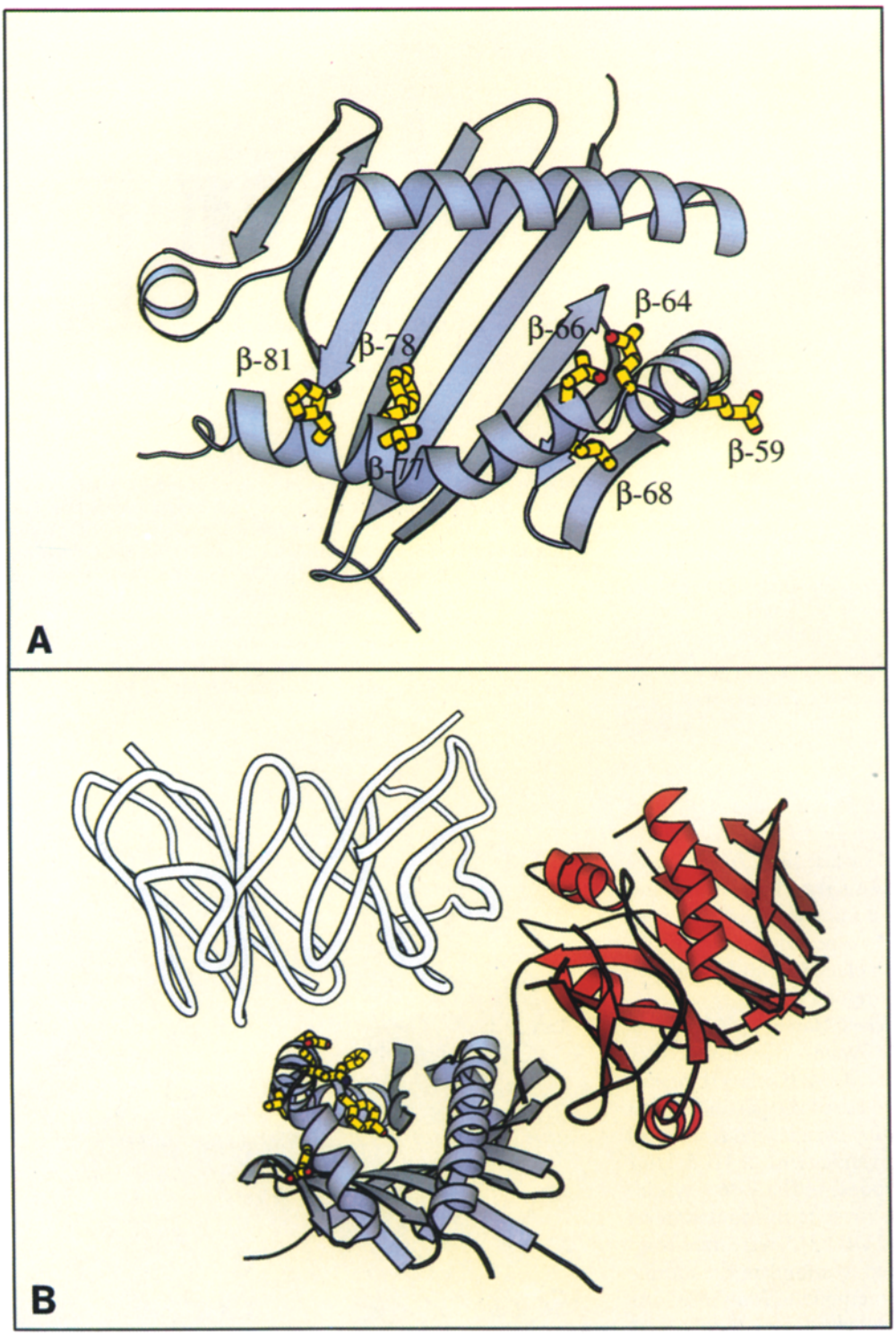

Figure 6. (*A*) Top view of the HLA-DR1α1 and β1 domain. The DR1α1 domain is on the top and the DR1β1 domain is below. Residues shown in *yellow* are from the DR1β1 domain and affect T cell stimulation by SEB, but not SEB binding. (*B*) A model for ternary complex formation between DR1, SEB, and TCR based on the DR1:SEB crystal structure analysis and DR1β chain mutation that affect T cell stimulation but not SEB, DR1α1 β1 domains and peptide are shown in *blue*. SEB is shown in *red* and DR1 residues that affect T cell stimulation but not SEB binding are shown in *yellow*. A model of the Vα and Vβ regions of the TCR, based on known immunoglobulin structures, is shown in *white*. The TCR model is positioned based on mutational data for both *S. aureus* toxins and TCRs as described (40). The model is consistent with the occurrence of direct interactions between DR1β chain residues and TCR residues in SAG-mediated stimulation of T cells.

Discussion

In this study, we have clearly demonstrated that residues that are not involved in the binding of bacterial SAGs are important for the stimulation of T lymphocytes by these molecules. Different murine T cell hybridomas that respond efficiently to rSEB and *Mtv*-7 SAG when presented by HLA-DR1 were used. Our results indicate that histidine 81 is critical for the formation of the trimolecular complex involving the TCR of the different Vβ6$^+$ murine T cell hybridomas since substitution of histidine β81 of DR1 to either a tyrosine or an alanine completely abrogated or strongly reduced SEB presentation to these T cells. This effect is not due to a decreased binding of this toxin on the mutated MHC class II molecules (Fig. 3). The lack of serological differences be-

tween mutated and wild-type DR molecules using a panel of 13 DR-specific mAbs indicates that these mutations did not lead to gross conformational modifications (data not shown). The latter is supported by the fact that DR1 molecules having the mutation β81Y are still able to present antigenic peptides to six of eight DR1-restricted T cell lines (6) and SAGs to the Vβ8.1 hybridomas used in the present study. Interestingly, substitution of residue β81 of HLA-DR1 to a tyrosine does not abolish the SEB response of Kmls 12.6 and RG 17 although alanine substitution strongly affected this response. It is possible that substitution of the histidine β81 to an alanine, which has a very small side chain, does not allow an interaction with this particular TCR but that substitution to a tyrosine, which also possesses an aromatic side chain, permits a more efficient contact. Although the crystal structure of class II suggests that H81 might interact with peptide (38), our results clearly show that residue β81 is involved in an interaction with the TCR (Fig. 5). Indeed a recent report has demonstrated a critical role for this residue in presentation but not binding of class II–restricted peptides (39). It is however possible that unconventional interactions between TCR and MHC occur during presentation of bacterial toxins since SEB binds on the class II α chain and thus obscures TCR contact residues on this side of the groove (40). With the knowledge that residue β81 is directly involved in the binding of SEA (6, 7), it is more than likely that in SEA recognition the TCR will interact with other residues on MHC class II molecules.

The role of TCR–MHC class II interactions in the response to SAG was further confirmed by the differential effect of β66 on T cell hybridomas. Mutation of this residue almost abolished the presentation of rSEB to RG 17 and Kmls 13.11 but had little effect on other T cells. Although this mutation was done in conjunction with the mutation of residue β68, which is pointing into the antigen binding groove, we do believe that the effect observed is due to mutation of the putative TCR contact residue β66 (Fig. 6). Mutations of the putative TCR contact residues β59 and β64 affect the presentation of rSEB to four out of five hybridomas used in these experiments. These results suggest that different murine T cell hybridomas see the SAG–MHC class II complex in distinct ways (Fig. 5). Moreover, our results indicate that a particular TCR does not interact with all putative TCR contact residues located on the MHC class II molecules. In contrast to the negative effect of mutation 59, 64, 66, and 81A and 81Y, substitution of βT77A, Y78A led to a significant enhancement of RG17 response to SEB. This effect is more likely to be due to the mutation of residue 77 which is pointing out toward the TCR whereas the aromatic ring and lateral side chain of residue β78 is oriented toward the peptide binding groove (Fig. 6).

Recently, Ehrich et al. (39) have reported that mutation of putative TCR contact residues on the α and β chain of I-E^k affect SEA presentation to T cells. Interestingly, not all murine T cell hybridomas were affected by mutation of a particular TCR contact residue confirming our results. Moreover, they showed that the same T cell hybridoma interact with different TCR contact residues in Ag- and SAG-specific responses (39). Our results also showed that the same T cell hybridoma interact with different TCR contact residues for SEB and *Mtv-7* SAG recognition. This different topology of interaction could result from the use of different SAG binding sites on MHC class II molecules (9). The localization of the SAG on the MHC molecule will probably determine the orientation of the CDR4 loop and by the same way the overall topology of the TCR–class II.

The results presented here favor a model in which TCR–MHC class II–SAG interactions occur. The fact that SAG presentation is not truly MHC restricted does not rule out a TCR–MHC class II interaction, since the putative TCR contact residues on MHC class II molecules are highly conserved throughout evolution (41, 42). 8 of 10 putative TCR contact residues of the class II β chain are conserved between wild mice, laboratory mice, and humans. Interestingly, the presence of an histidine at position β81 is conserved among all human and murine MHC class II alleles and isotypes with the exception of DRw53 and I-A^u. Other TCR contact residues are also identical within a class II isotype as all HLA-DR alleles share the same TCR contact residues. Moreover, some of these amino acids (5 out of 24) are conserved between MHC class I and class II molecules, which could explain the recognition by $CD8^+$ T cells of SAG–MHC class II complexes. Consistent with this view, $CD4^+$ and $CD8^+$ T cells use the same pool of V regions to recognize MHC and Ags (43–46) further supporting the possibility that TCRs expressed by $CD8^+$ T cells can interact with MHC class II molecules.

The importance of TCR–MHC class II interactions will probably be influenced by the affinity of the TCR for the SAG. Weak SAGs or TCRs with low affinity for SAGs will probably show a pattern of MHC restriction that will be influenced by putative TCR contact residue of the MHC class II molecule. Thus, it is interesting to note that the Vβ8.1 hybridomas, which are less dramatically affected by the MHC mutation, respond to much lower concentration (10–30-fold) of SEB and are likely to have a higher affinity for the toxin than the $V\beta6^+$ T cells. The existence of a direct contact between the TCR and MHC class II molecules in SAG recognition may have important implications during the in vivo response to SAG. Bacterial and retroviral SAGs have been associated with the pathogenesis of several diseases (1, 47–54). MHC class II polymorphisms of TCR contact residues may have an important role to play in the susceptibility to SAG-related diseases and on the level of T cell expansion, deletion, and unresponsiveness observed after SAG encounter.

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