The Vβ Complementarity Determining Region 1 of a Major Histocompatibility Complex (MHC) Class I-restricted T Cell Receptor Is Involved in the Recognition of Peptide/MHC I and Superantigen/MHC II Complex

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Summary

We investigated the role of the complementarity determining region 1 (CDR1) of T cell receptor (TCR) β chain both in antigen/major histocompatibility complex I (MHC I) and in superantigen (SAg)/MHC II complex recognition. Residues 26 to 31 of the Vβ10 domain of a TCR derived from an H-2K^d-restricted cytotoxic clone were individually changed to alanine, using site-directed mutagenesis, and the mutated TCR β chains were transfected along with the wild-type TCR α chain into a TCRα$^-$β$^-$ T hybridoma. These mutations affected antigen/H-2K^d complex recognition, although to a different extent, as estimated by interleukin 2 production. Certain mutations also affected differently the recognition of two Staphylococcal toxins, exfoliative toxin and Staphylococcal enterotoxin C2, presented by HLA-DR1. Whereas mutation of residues D30 or T31 affect the recognition of both toxins, residues T26, L27, and H29 are critical for the recognition of only one of the SAgs. These observations demonstrate the participation of the CDR1 region in the recognition of peptide/MHC class I as well as SAg/MHC II complexes.

Unlike Igs, which can recognize antigen in its native form, the TCR recognizes a peptide that is the product of antigen processing and that is trapped in the groove of the MHC molecule on the plasma membrane of a presenting cell (for a review, see references 1 and 2). However, the organization of rearranging gene elements and the primary amino acid sequence of the TCR are similar to those of Igs (3, 4). The conservation in α and β TCR chains of residues found to be crucial for the structure of the Ig variable domains, suggests that the three-dimensional structure of the TCR is similar to the known atomic structure of the Fab moiety (5). By analogy to the antigen binding site on Ig, three regions of TCR α and β chains, which are hypervariable and which correspond to the loops formed between β sheets, are named CDR1, CDR2, and CDR3. The last one is encoded by the V-(D)-J junction in α and β TCR chains and is therefore the most variable one, whereas CDR1 and CDR2 are encoded in the genome by the V segment. A fourth hypervariable region (CDR4) was identified in the TCR β chain (6, 7).

Based on this model of TCR structure and the known crystal structure of several HLA molecules, different groups (4–6) have proposed that the less variable CDR1 and CDR2 regions might make direct contact with the MHC molecules whereas the CDR3 region might make direct contact with the antigenic peptide. Consistent with this model, mutations of the CDR regions, especially in CDR3, cause a loss of recognition of the antigen/MHC complex (8–12). However, to our knowledge, all of the data available until now concern TCR restricted to class II MHC molecule.

In the past few years, a distinct group of antigens has been described and termed superantigens (SAg)[1] (for a review see reference 13). Several exotoxins of *Staphylococcus aureus* have superantigenic properties: activation of subsets of T cells expressing particular Vβ families and presentation in a site of the MHC class II molecule distinct from the peptide-binding pocket, in the absence of processing and of absolute allotype restriction (14). Several studies have led to map critical residues for SAg recognition to the lateral solvent-exposed face of the

[1] *Abbreviations used in the paper:* ExT, *S. aureus* exfoliative toxin; SAg, superantigen; SEC2, *S. aureus* enterotoxin C2; SN, supernatant; wt, wild type.

Vβ domain, mainly in the CDR4 region (15–19). However, there is recent evidence that other components of the TCR may also contribute to the interaction with the SAg/MHC complex (20–23). Moreover, although it is generally assumed that the surface of the TCR that engages MHC in the complexes with SAg is the same one that contributes to MHC binding in conventional peptide/MHC complexes, experimental data for this point are scarce.

The results obtained in this study identified residues in the Vβ10 chain CDR1 region of a H-2K^d–restricted TCR that are important both for specific peptide/MHC class I and/or SAg/MHC class II complex recognition.

Materials and Methods

Transfecting Cells and Plasmids. The 58 TCR $\alpha^-\beta^-$ cell line derived from DO.11.10 T hybridoma was previously described (24). It was transfected by electroporation with plasmid pCA208 containing the Lyt2.2 gene and the *gpt* selective marker (25). Selection was done in the presence of mycophenolic acid (2.0 μg/ml), xanthine (250 μg/ml) and hypoxanthine (15 μg/ml). After cloning and subcloning, one CD8$^+$ clone denoted 3D1 was selected for transfection with cDNAs encoding the wild type (wt) or mutated (see below) Cw3/1.1 TCR α and β chains. A full-length cDNA corresponding to the TCR α chain present in Cw3/1.1 cytotoxic clone (26) was subcloned into the SalI/BamHI opened plasmid pHβAPr-1-neo (27). Similarly, a full-length cDNA corresponding to the TCR β chain found in Cw3/1.1 was subcloned into SalI/BamHI opened plasmid pHβAPr-1-neo. To facilitate further handling of the TCR Vβ region, an XhoI site was engineered at the Jβ/Cβ junction. This process resulted in the substitution of an isoleucine (ATA) by a leucine (CTC) at position 116A (see Fig. 1). Linearized plasmids pHβAPr-1-neo/Cw3α and pHβAPr-1-neo/Cw3β were cotransfected with plasmid pCA262 containing the cDNA of murine CD3 ζ chain (28). Transfectants were selected in 1.6 mg/ml of geneticine G418-sulfate (GIBCO BRL, Gaithersburg, MD) and screened for TCR expression by flow cytometry with a FITC-conjugated anti-CD3 mAb (2C11, Pharmingen), using a FACScan® (Becton Dickinson & Co., Mountain View, CA). Subclones expressing high levels of TCR were isolated by flow cytometry sorting using 2C11-FITC anti-CD3 mAb (Pharmingen, San Diego, CA) in a FACStar® (Becton Dickinson & Co.).

Electroporation Conditions. 10^7 T hybridoma cells, resuspended in 0.8 ml Hepes buffered saline, were given a single pulse (240 V, 192 Ω, 900 μF), in 4-mm cuvettes at room temperature, using a Cellject (Eurogentec S.A., Sart Tilman, Belgium) apparatus. Cells were immediately resuspended in 40 ml of complete 10% FCS RPMI 1640 (GIBCO BRL). Selection medium was added 48 h later, plating 10^5 cells/well in 24-well plates (Costar Corp., Cambridge, MA).

Site-directed Mutagenesis of TCR Vβ. The full-length Cw3 TCR β cDNA was introduced into the SalI-BamHI open plasmid pBSK$^+$ (Stratagene, La Jolla, CA). The Vβ10-Dβ1-Jβ1.2 segment was then excised using SalI and XhoI restriction enzymes, from plasmid pBSK$^+$Cw3β. The purified fragment served as template in recombinant PCR, as previously described (29), for the generation of the single residue Ala-substituted mutants of the CDR1 β region. The SalI/XhoI–digested PCR products were cloned into the SalI/XhoI–digested and –purified pBSK$^+$Cw3β, containing the TCR β constant region. After sequencing, mutant (as well as wt) Cw3 TCR β cDNAs were subcloned into the SalI/BamHI site of the expression vector pH APr-1-neo (27).

Antibodies. 2C11-FITC (anti-CD3) mAb and H57-biotinylated (anti-Cβ) mAb were purchased from Pharmingen, and 53-6.7-FITC anti-Lyt2 mAb from Becton Dickinson & Co. Anti-Vβ10 and anti-Vα8 stainings were done with supernatants of B21.5 and B21.14 hybridomas, respectively (30), using fluorescein-conjugated mouse anti–rat antiserum from Immunotech (Marseille, France), as secondary reagent.

Activation of Transfected Clones. Transfectants were grown in complete 10% FCS RPMI 1640 (GIBCO BRL) without G418 for 48 h before assay. 10^5 irradiated H-2K^d expressing P815 cell line (10,000 rad) were plated into 96-well plates (Costar Corp.) and incubated for 3 h with 10^{-4} M Cw3(170-179) peptide (Neosystem Laboratories SA, Strasbourg, France) at 37°C. 10^5 transfected TCR$^+$ clones were then added in triplicates. Alternatively, 10^5 transfected clones were plated into 96-well microculture plates (Costar Corp.) previously incubated at 4°C overnight with purified 2C11 (anti-CD3) or H57 (anti-Cβ) mAbs at the indicated concentrations, or with 10 μg/ml of mouse anti–rat antiserum (Immunotech), washed three times with PBS, and then incubated for 1 h with complete medium (10% FCS) or with anti-Vβ10 (B21.5) rich supernatant, respectively. In some cases, PMA (Sigma Chemical Co., St. Louis, MO) was added in a final concentration of 10 ng/ml. The T hybridoma 9.4, product of the fusion of the CD8$^+$ cytotoxic clone, Cw3/1.1 with the 58 TCR $\alpha^-\beta^-$ hybridoma was used in SAg-induced activation assays. L cells transfected with HLA-DRB1*1102 (L625.7), DRB1*1101 (L581.13), DRB*1301 (L597.2), DRB1*1101 F671 (L620.2), DRB1*1104 F67I (L663.5), and DRB1*1302 (L650.2) (31) were a gift of R. W. Karr (Monsanto Co., St. Louis, MO). DRB1*1101 F67I and DRB1*1104 F67I are mutants in which phenylalanine at position 67 in the DR β chain was changed into isoleucine (Karr, R. W., unpublished observations). L cells transfected with HLA-DRB1*0101 (DAP3-DR1) (32) were provided by E. Long (National Institutes of Health, Rockville, MD). L cells transfected with HLA-DR were used in SAg-induced activation at 10^5 cells/well. *S. aureus* enterotoxin C2 (SEC2) and *S. aureus* exfoliative toxin (ExT) (Toxin Technology Inc., Sarasota, FL) were added at 10 μg/ml final concentration, followed by 10^5 transfected T hybrids clones. Cultures were maintained at 37°C in a 5% CO_2 humid atmosphere. Supernatants (SN) were harvested 24 h later and freezed before testing.

IL-2 Assay. 10^4 CTLL-2 cells per well were cultured in 100 μl of media containing 20% of conditioned SN or different doses of a rIL-2–rich SN in 96-well microculture plates (Costar Corp.). Proliferation was assessed by [^{3}H]thymidine incorporation and IL-2 U estimated as previously described (33).

Normalization of the Activation Responses. The relative antigen (or SAg) recognition index, (estimated by IL-2 production) was calculated as previously described (11) with some modifications. Briefly, the following antigenic recognition index (ARI) was calculated: ARI = equivalent [mAb] antigen (or SAg)/[mAb] 50% maximum, where equivalent [mAb] antigen (or SAg) is the equivalent concentration of the anti-TCR mAb (2C11, anti-CD3 or H57, anti-Cβ) that gives the same IL-2 release as the antigenic (or superantigenic) stimulus, and [mAb] 50% maximum, is the concentration of the anti-TCR mAb that gives 50% of maximal response. The results are expressed as the percentage of the above calculated ARI index for the mutant transfectant in relation to the calculated ARI index for the wt.

Results

The Cw3/1.1 TCR and the T Hybridoma Transfection System. The TCR studied here was derived from a CD8$^+$ cytotoxic clone, Cw3/1.1, originally obtained by immunizing a DBA/2

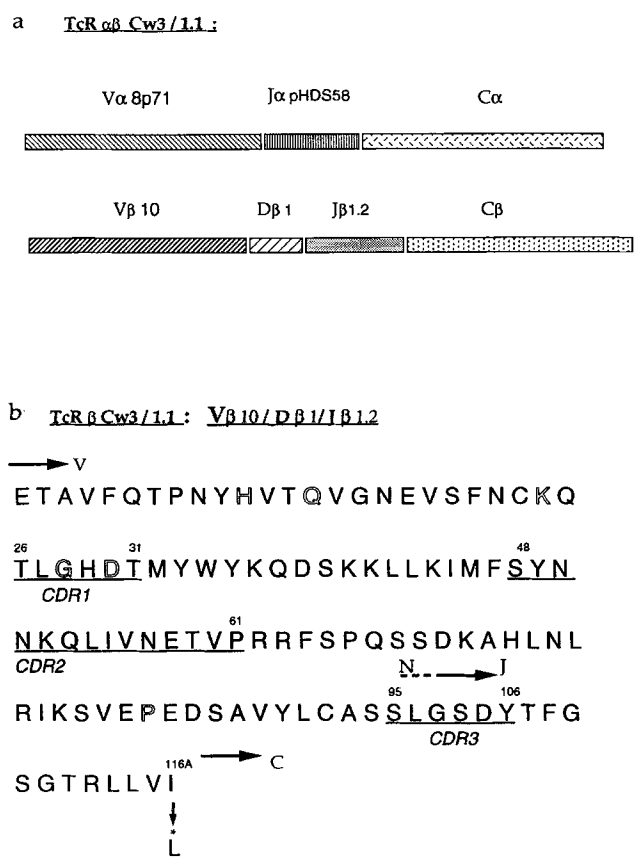


Figure 1. Outline structure of the Cw3/1.1 TCR. (*A*) Schematic representation of the TCR α and β chains. (*B*) Partial protein sequence of the TCR-β chain. CDR1, CDR2, and CDR3 regions are underlined. Single-letter code for amino acid residues. (*Arrows*) Borders of the V, J, and C regions; (*dotted line*) probable N-region sequences.

mouse (H-2^d) with a P815 cell line transfected with HLA-Cw3 (P815-Cw3) (26). The fine specificity of the Cw3/1.1 TCR for the Cw3 (170-179) peptide presented by H-2K^d has been described (26). Based on the predicted structure of the TCR V region (5), the CDR1 region of the Cw3/1.1 TCR Vβ chain should lie between residues 26 and 31 (numbered according to reference 34). The relevant features of the Cw3/1.1 TCR, including the amino acid sequence of its Vβ10 segment, are displayed in Fig. 1. Note that the last residue of the Jβ1.2 segment, an isoleucine, was replaced by a leucine in order to create an XhoI site, which allowed the excision of the VDJβ segment for mutagenesis. This conservative change is not likely to affect the specificity of the Cw3/1.1 TCR, inasmuch as leucines are found at very high frequency at this position (34).

The expression of the accessory molecule CD8α was shown to be necessary, in some cases, to endow a recipient T hybridoma with the specificity of the transfected CTL-derived TCR (25). To optimize specific recognition, we therefore transfected the genomic DNA of the CD8α molecule into the T hybridoma 58 $\alpha^-\beta^-$ (24). The cDNAs corresponding to the wt or mutated TCR β chain were then transfected into the established CD8$^+$ TCR $\alpha^-\beta^-$ 3D1 subclone, together with a wt TCR α chain cDNA.

The CD3 ζ chain constitutes a limiting factor for the transport of the TCR complex to the plasma membrane (35). To reach high levels of TCR expression in the 3D1 subclone, and because 58 $\alpha^-\beta^-$ expresses low levels of endogenous CD3 ζ (Malissen, B., unpublished observation), a plasmid driving the expression of the CD3 ζ chain was systematically cotransfected with the ones driving TCR α and β chains expression (data not shown).

The surface expression of CD8α and of the TCR $\alpha\beta$/CD3

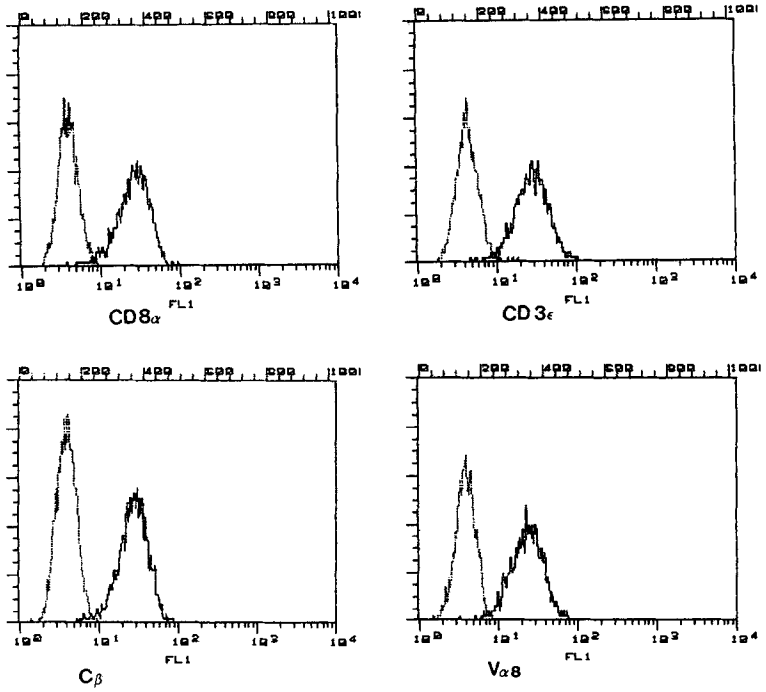


Figure 2. Surface expression of CD8 and TCR/CD3 molecules in the T hybridoma transfectants as assessed by flow cytometry. Recipient (*dotted lines*) and Cw3/1.1 TCR transfected cells (*solid lines*) were stained with antibodies: (*top left*) 53-6.7-FITC (anti-CD8); (*top right*) 2C11-FITC (anti-CD3ϵ); (*bottom left*) H57-biotinylated (anti-Cβ), followed by labeling with streptavidin-phycoerythrin; (*bottom left*) B21.14 (anti-Vα8), followed by fluorescein-conjugated mouse anti-rat antiserum.

complex was analyzed by flow cytometry, using several mAbs: 2C11 (which recognize an epitope on the CD3 ϵ chain), H57 (which is specific for the C region of the β chain), and an anti-Vα8: B21.14 (30). Fig. 2 shows the FACS® profiles of the WT.27 clone expressing a wt TCR after staining with mAbs directed to the CD8α, CD3ϵ, Cβ, or Vα8 molecule domains. Clones expressing the same or higher amounts of CD8 and TCR than the wt TCR transfectants, were kept for further assays.

We also used a fourth mAb, B21.5, which discriminates between the two allelic forms of mouse Vβ10 (30). This mAb recognizes $V\beta10^b$, which differs from $V\beta10^a$ at positions 11, 14, 24, 28, 30, and 84 (36) (numbered according to reference 34). All the $V\beta10^b$ CDR1 mutants described here are recognized by the anti-$V\beta10^b$ mAb (data not shown). This observation suggests that the alanine substitutions do not cause major change in the Vβ10 structure and that the CDR1 residues 28 and 30 do not contribute to the epitope defined by mAb B21.5.

The Effect of Alanine Substitution in the Cw3/1.1 TCR β Chain on Anti-Vβ10 mAb-induced Activation. To check their functional capabilities, mutants were first tested for IL-2 production induced by different doses of plate-immobilized mAb. All mutants could be activated in this way to produce IL-2 when induced by an anti-Cβ mAb (H57). Moreover, the different CDR1 β mutants, with the exception of G28A, displayed a similar pattern of activation when induced by anti-Cβ or anti-Vβ10 mAbs, comparable with that observed for the wild type TCR transfectants, as shown in Fig. 3. Although mutant G28A is recognized by B21.5 mAb as well as the other mutants (data not shown), it was found to be more sensitive to anti-Vβ10 mAb-induced activation. These results further support the notion that the alanine substitutions did not grossly disrupt the structure and function of the mutated TCR.

Specific Peptide/MHC I Recognition by the Cw3/1.1 TCR β Mutants. The mutants were further tested for IL-2 production upon presentation of the Cw3(170-179) peptide by the H-$2K^d$–expressing P815 cell line. Only T26A and L27A mutants gave detectable responses (Table 1). The amount of IL-2 secreted by mutants G28A, H29A, D30A, and T31A were <0.07–0.16 U/ml, the minimum detectable level, depending on the experiment. As detailed in Materials and Methods, we normalized the response to the Cw3 peptide/K^d of each tested clone in relation to its response to one anti-CD3 (2C11) or one anti-Cβ (H57) mAb. In an attempt to maximize the T hybridoma response, 10 ng/ml of PMA was added to the cultures. This increased the IL-2 secretion in response to antigen or anti-TCR mAbs 2–10-fold. Nevertheless, IL-2 production by the G28A, H29A, D30A, and T31A mutants in response to Cw3 peptide/K^d was not detectable even in the presence of PMA (Table 1). In summary, alanine substitutions at CDR1 β positions 26 or 27 partially reduced the peptide/MHC recognition, whereas substitutions at positions 28, 29, 30, and 31 abolished recognition in our system.

Superantigen-induced Activation of Cw3/1.1 TCR β Mutants. We next tested the effect of the alanine substitutions

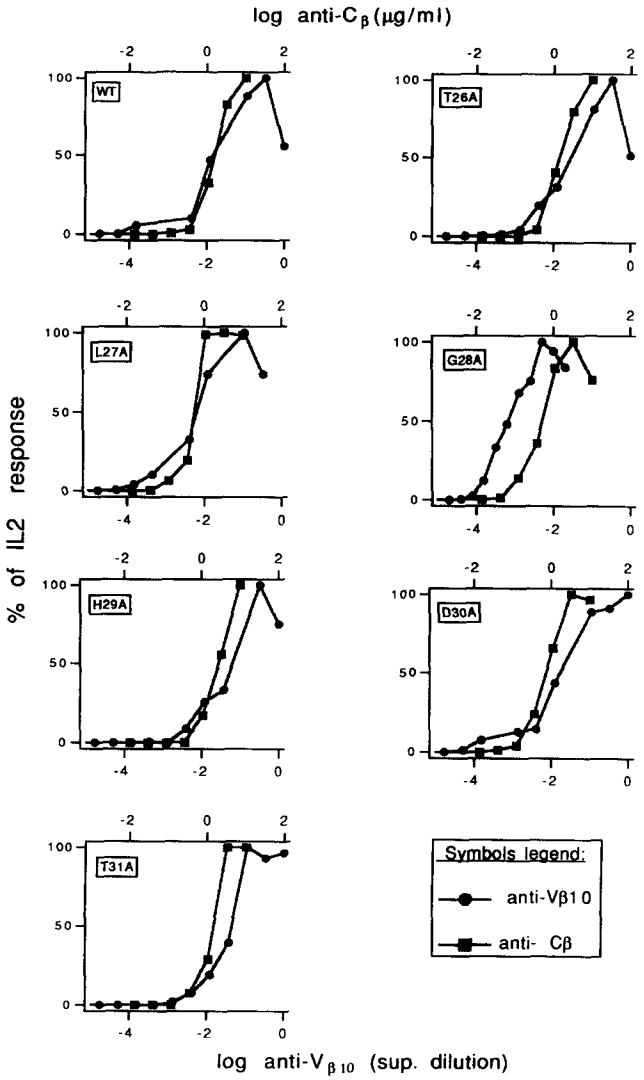


Figure 3. Responses to anti-Cβ and anti-Vβ mAbs by wt and CDR1β mutant transfectants. The IL-2 secretion by clones expressing the indicated TCR was measured in response to the indicated amounts (*top*) of immobilized H57 anti-Cβ mAb (*squares*) or of B21.14 anti-Vβ10 mAb (*circles*, *bottom axis*). Results are representative of three experiments.

on SAg/MHC II recognition. Three exogenous SAg have been described as activators of Vβ10 in the mouse (14): SEA, SEC2, and ExT, all of them *S. aureus* toxins. In our hands, SEC2 and ExT, but not SEA, induced IL-2 secretion in wt Cw3/1.1 TCR-bearing clones, when presented by L cells transfected with HLA-DR1 molecule. The results obtained are shown in Table 2. Responses to SAg were normalized with respect to the activation induced by anti-Cβ mAb, as above. All the CDR1 mutants, with the exception of G28A, have a reduced response to at least one of the superantigenic stimuli. It is interesting to note that whereas the alanine substitutions at positions 26 or 29 severely affected SEC2 recognition, substitution at position 27 almost abolished the response to ExT, without affecting SEC2 recognition. On the other hand, the recognition of SEC2 and ExT by mutants D30A

Table 1. *Recognition of Cw3 peptide (170-179)/K^d Complex by CDR1β Mutants**

	Type	Clone	Anti-TcR giving 50% of max. IL-2 release	Anti-TCR equivalent to Cw3/Kd response	Relative response§
			μg/ml	*μg/ml*	%
Exp. 1	T26A	21.8	5.84‡	0.76	16.9
	wt	wt.27	21.29	16.34	100.0
Exp. 2	T26A	21.24	1.54	0.10	15.4
	wt	wt.20s4	1.03	0.43	100.0
Exp. 1	L27A	32.5s4	17.49	1.53	11.6
	L27A	32.5s33	11.88	1.52	16.9
	wt	wt.20s4	8.16	6.16	100.0
Exp. 2	L27A	32.25s7	0.29	0.01	14.3
	wt	wt.27	5.72	1.68	100.0
Exp. 1	G28A	51.5s1	1.85	NS	NS
	G28A	51.5s14	1.68	NS	NS
	G28A	51.29s6	5.20	NS	NS
	G28A	51.29s11	5.00	NS	NS
	wt	wt.27	5.72	1.68	100.0
Exp. 2	G28A	51.5s15	1.63	NS	NS
	G28A	51.29s13	5.81	NS	NS
	wt	wt.20s4	3.42	2.51	100.0
Exp. 1	H29A	4.1/31s5	1.85	NS	NS
	H29A	4.1/31s4	16.70	NS	NS
	wt	wt.27	21.29	16.34	100.0
Exp. 2	H29A	4.1/20	6.65	NS	NS
	H29A	4.1/56	9.73	NS	NS
	H29A	4.1/3	12.51	NS	NS
	wt	wt.27	6.72	14.50	100.0
Exp. 1	D30A	62.16	5.82	NS	NS
	D30A	62N13s19	6.71	NS	NS
	wt	wt.27	5.72	1.68	100.0
Exp. 1	T31A	72.N2	1.95	NS	NS
	wt	wt.20s4	1.95	1.20	100.0

* 10^5 CDR1β mutated or wt transfectants were cocultured with 10^5 K^{d+} P815 cells, in the presence of 10^{-4} M of Cw3 (170-179) peptide and 10 ng/ml of PMA, for 24–36 h. IL-2 production was estimated in a CTLL-2 assay.
‡ Anti-Cβ (H57) or anti-CD3 (2C11) mAbs were plated at 30.0–0.0137 μg/ml, after a threefold dilution.
§ The relative antigenic response was calculated as described in Materials and Methods.

and T31A is reduced to the same extent, D30A having more pronounced effects. A summary of the data is shown in Fig. 4.

SAg Presentation by Different HLA-DR Alleles. To obtain more information about TCR/SAg–MHC II interaction, presenting cells expressing other MHC II molecules were used for SAg presentation. Fibroblast L cell lines transfected with different alleles of HLA-DR (DRB1*1101, DRB1*1102, DRB1*1301, DRB1*1302), or with mutated HLA-DR (DRB1*1101 F67I and DRB1*1104 F67I) (31) were tested for ExT and SEC2 presentation to the wt Cw3/1.1 TCR-bearing hybridoma 9.4. Results are shown in Table 3. Three of these HLA-DR molecules were ineffective (DRB1*1301, DRB1*1302) or very poor presenters (DRB1*1102) for SEC2 and ExT to the $V\beta10^+$ 9.4 hybridoma, although all of

Table 2. *SAg/MHC Complex Recognition by CDR1β Mutants**

		Anti-TCR giving 50% of max. IL-2 release	Anti-TCR equivalent to SEC2 response	Anti-TCR equivalent to ExT response	Relative response to SEC2 and ExT, respectively	
		ng/ml	*ng/ml*	*ng/ml*		%
Exp. 1	T26A	1,647‡	149.0	149.0	37	87§
	wt	1,881	454.0	194.0	100	100
Exp. 2	T26A	1,490	32.0	291.0	4	62
	wt	2,448	136.0	776.0	100	100
Exp. 1	L27A	548	128.0	0.8	97	1.4
	wt	1,881	454.0	194.0	100	100
Exp. 2	L27A	95	14.0	1.4	112	14
	wt	963	131.0	103.0	100	100
Exp. 1	G28A	2,460	1,130.0	1,290.0	181	189
	wt	1,457	370.0	400.0	100	100
Exp. 2	G28A	760	272.0	133.0	158	190
	wt	1,472	333.0	136.0	100	100
Exp. 1	H29A	3,010	423.0	402.0	58	130
	wt	1,881	454.0	194.0	100	100
Exp. 2	H29A	4,100	524.0	963.0	23	74
	wt	2,448	136.0	776.0	100	100
Exp. 1	D30A	833	9.1	10.7	5	12
	wt	1,881	454.0	194.0	100	100
Exp. 2	D30A	624	1.5	10.3	1	5
	wt	2,448	136.0	776.0	100	100
Exp. 1	T31A	5,959	ND	370.0	ND	23
	wt	1,457	370.0	400.0	100	100
Exp. 2	T31A	3,106	383.0	144.0	54	50
	wt	1,472	333.0	136.0	100	100

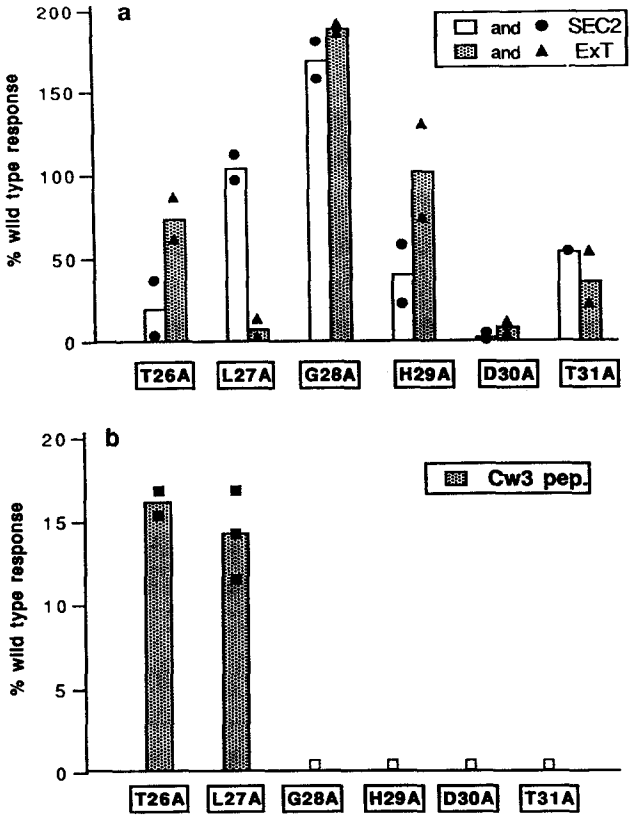
* 10^5 CDR1β mutated or wild type transfectants were cocultured with 10^5 DR-transfected L cells, in the presence of 10 μg/ml of SEC2 or ExT toxins, for 24–36 h. IL-2 production was estimated in CTLL-2 assay.
‡ Anti-Cβ (H57) or anti-CD3 (2C11) mAbs were plated at 30.0–0.0137 μg/ml, after a threefold dilution.
§ The relative superantigenic response was calculated as described in Materials and Methods.

the HLA-DR transfectants were effective in stimulating the Vβ1$^+$ and Vβ8.2$^+$ DO.11.10 T hybridoma (data not shown). The analysis of the polymorphic residues lying on the α-helices of the MHC II molecules shows a correlation between the presence of arginine at position 71 and a significant level of IL-2 secretion, suggesting that the arginine at position 71 is critical for the recognition of the complex HLA-DR/ExT or SEC2 by the Cw3/1.1 TCR.

An Ig V_H Model for the TCR Vβ Domain. Using the alignment of Chothia et al. (5), the mutated residues of the Vβ10 domain were mapped on the known structure of the V heavy domain of the D1.3 Ig (37), as shown in Fig. 5. The residues that specifically affect the recognition of SEC2 or ExT (T26, L27, and H29) are predicted to lie in the part of the CDR1 loop that faces the CDR4 region, whereas residues D30 and T31 which affect recognition of both toxins, are at the COOH-terminal end of the CDR1 region, closer to the CDR2 and CDR3 loops.

Discussion

In the absence of direct structural data, several groups (4–6) have proposed models of the TCR/peptide/MHC complex, in which the more variable CDR3 loops of the α and β TCR chains contact the peptide lying in the groove of the MHC molecule, whereas the CDR1 and CDR2 loops contact the



α-helices of MHC. Several studies support a role for critical contacts between peptide and CDR3 α and β residues of the TCR (for a review see reference 2). Indirect evidence suggesting contacts between TCR and MHC molecules has been obtained by mutating MHC residues (38–42). Multiple residues on one or both α-helices of the MHC molecules are likely to be simultaneously recognized by the TCR, but the sites of these interactions are not fixed, since different TCRs specific for the same peptide/MHC complex can be differentially affected by mutations of MHC residues (38, 40, 42). The nature of the MHC/TCR interaction has also been investigated by mutating the CDR regions of the TCR (10–12). Recently, Nalefski et al. (11) have systemically mutated the CDR1, CDR2, and CDR3 regions of the α chain of a class II MHC-restricted TCR. Residues important for peptide/MHC recognition were identified in all three CDR α regions. A concurrent model (43) predicts that SAgs bind

Figure 4. Mutations of CDR1β residues affect peptide and SAg recognition to a different extent. The SAg (*a*) or antigen (*b*) recognition index of the CDR1 β mutant transfectants was calculated as described in Materials and Methods and expressed as a percentage of the wt index. Bars indicate the mean of independent experiments and symbols the relative index of each experiment SEC2 (*filled circles* and *open bars*), ExT (*filled triangles* and *filled bars*), Cw3 (170-179) peptide (*filled squares* and *filled bars*). (*Single open square*) Experiments in which no antigen response was detected. Results are averaged from the same experiments as those described in Tables 1 and 2.

Table 3. *SAg Presentation by Different HLA-DR Alleles**

APC‡	(HLA-DR allele)	DR β chain polymorphic residues§ 58	67	70	71	86	IL-2 secretion‖ ExT¶	SEC2
							U/ml	
DP3-DR1	(DRB1*0101)	**A**	**L**	**Q**	**R**	**G**	32.3	30.2
L625.7	(DRB1*1102)	E	I	D	E	V	4.1	1.2
L650.2	(DRB1*1302)	**A**	I	D	E	**G**	<0.4	0.4
L597.2	(DRB1*1301)	**A**	I	D	E	V	<0.4	<0.4
L663.5	(DRB1*1104 F67I)	E	I	D	**R**	V	28.2	44.2
L620.2	(DRB1*1101 F67I)	E	I	D	**R**	**G**	33.4	41.2
L581.13	(DRB1*1101)	E	F	D	**R**	**G**	36.1	38.9

* The murine 9.4 T hybridoma expressing the wild type Cw3/1.1 TCR (Vβ10+) was activated by toxin SAg, as described in Materials and Methods. The results of one out of three independent experiments is shown. The murine T hybridoma DO.11.10 (Vβ1+ and Vβ8.2+), that express equivalent levels of the TCR/CD3 complex, respond to ExT and SEC2 secreting significant amounts of IL-2 (>50 and >290 U/ml, respectively) presented by all the HLA-DR alleles tested.

‡ L fibroblasts transfected with different HLA-DR alleles (31) were used as presenting cells. All L cell lines express similar levels of HLA-DR (data not shown).

§ Only polymorphic residues lying on the α-helices of the DRβ chain are shown. Sequences are from reference 54.

‖ IL-2 production was measured using the CTLL-2 assay, as described in Materials and Methods. The IL-2 secreted in response to L cells alone equal CTLL background in all experiments.

¶ ExT and SEC2 were used at 10 μg/ml final concentration.

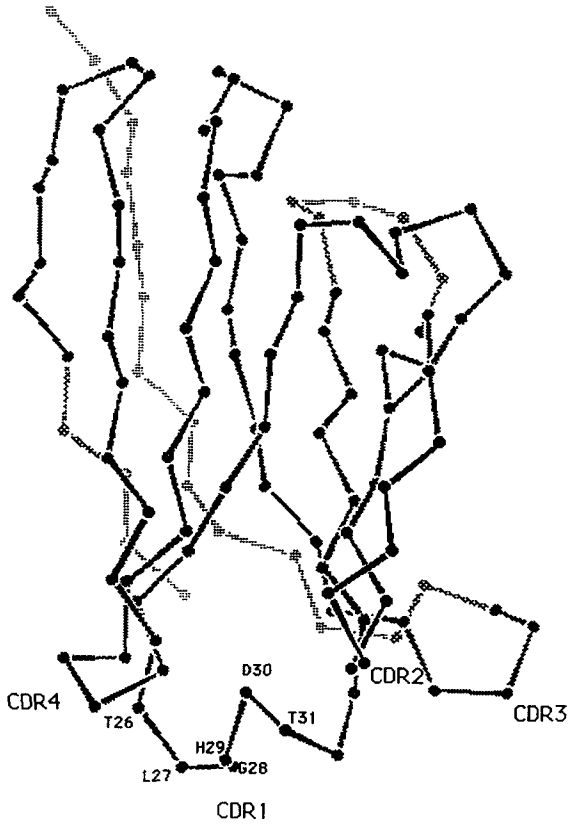


Figure 5. The predicted localization of the mutated Vβ10 residues based on Ig. The diagram of the α carbon skeleton of the V_H domain was generated with the coordinates of the x-ray crystallographic analysis of the D1.3 mAb (37), using MolDraw software (53). The predicted positions of the Vβ10 mutated residues were obtained using the alignment of Chothia et al. (5).

laterally to the MHC II molecule (at a distance from the peptide binding groove) and to the lateral exposed face of the Vβ domain, promoting contact between TCR and MHC surfaces. Experimental data on exogenous SAg binding to MHC molecules (44) and on the role of the Vβ CDR4 region in SAg recognition (for reviews see references 45 and 46) have provided some support to these predictions. Evidence for the involvement of other components of the TCR during SAg/MHC II complex recognition has also been reported (20–23).

The role of the TCR β CDR1 region in the recognition of both peptide/MHC I and SAg/MHC II complexes by a class I–restricted TCR thus far had not been evaluated, and was the purpose of this study. By site-directed mutagenesis, we prepared alanine substitutions at all CDR1 positions of a Vβ domain and the mutants were transfected into an appropriate hybridoma. All mutants could be expressed at a comparable level, and it appears unlikely that the alanine substitutions in the CDR1 β region disrupted the overall conformation of the TCR for several reasons. In general, protein structures appear quite stable with regard to alanine single point substitutions, especially in loop structures (47, 48). The most profound effects on ligand binding are invariably found with mutations alterating ligand-contacting residues (47). The fact that all the CDR1 mutants (except G28A) responded to an anti-Vβ10 mAb in the same dose-dependent way as to an anti-Cβ mAb, strongly suggests that the overall structure of the TCR is conserved. Further evidence that alanine mutation in residues T26, L27, and H29 is not causing conformational changes, is the fact that mutations at these positions have no effect on the recognition of at least one of the SAg tested.

We first analyzed the recognition of the complex made of K^d and a specific peptide. This revealed that all the residues of the CDR1 β region are substantially involved, in particular residues 28 to 31, the mutation of which completely disrupted recognition. A role for the charged (H29 and D30) and polar (T31) residues is compatible with previous observations based upon the occurrence of individual amino acids in presumptive functional subregions of the TCR α and β chains and class I MHC molecules (49). The results suggested that the interaction between TCR and MHC molecules would be mediated mainly by charged and polar aromatic residues. Histidines display a remarkably high occurrence in the CDR1 β region (49). It is unlikely, however that these histidines are essential for the local conformation of the binding loop, at least in the TCR studied here, because the alanine substitution at residue H29 did not affect response to the ExT SAg. Glycine 28 may confer a greater flexibility to the CDR1 loop. Note that, although the G28A mutation abolished the specific peptide/MHC I recognition, it significantly increased the responsiveness to an anti-Vβ10 mAb and the recognition of both toxin-SAg/MHC II complexes. This result shows the different participation of the G28 residue during the engagement of one or the other type of complex.

We then studied the recognition of SAg/MHC complexes by exposing the hybridomas expressing the various mutants to HLA-$DR1^+$ cells and either SEC2 or Ext superantigens. All mutations (except G28A) diminished the recognition of at least one of the SAg/DR complexes. There are at least two possible explanations for which the CDR1 β region could influence toxin recognition. First, recognition of SAg may be critically dependent on concomitant recognition of the MHC II molecule by the TCR. Second, the CDR1 region may contact SAg directly. The two hypotheses are not mutually exclusive, and some residues of the CDR1 β region may contact the MHC II molecule whereas others may contact the SAg.

The SEC2 toxin has high homology to other *S. aureus* enterotoxins, for which more data on MHC II binding and TCR recognition are available (15, 45). For the exfoliating toxins, however, the matter of relatedness is much less clear (14), and data on MHC II and TCR binding are scarce. SEC2 and ExT may bind to the same or overlapping sites on the MHC II molecules as demonstrated for other toxins (50). It is interesting to note that the analysis of SAg recognition by the mutants described here indicates that the recognition of SEC2 and ExT by the Cw3/1.1 Vβ10 TCR does not involve the same CDR1 β residues. In fact, whereas the recognition of one of the SAg was not affected by mutations at residues T26,

L27, and H29, the recognition of the other toxin was significantly diminished by the same mutation. The unrelatedness between SEC2 and ExT can explain these results. The direct contact between the CDR1 β region and toxin SAg was also suggested in a recent study (23). In addition, evidence for different contact residues in the hypervariable region 4 of Vβ8.2 TCR for unrelated endogenous Mls^a and bacterial SAg has recently been obtained (20).

L cells transfected with the different HLA-DR alleles were used to present the ExT and SEC2 SAg to the wt Cw3/1.1 TCR-bearing hybridoma 9.4. Both SAg could be presented to the Vβ10$^+$ 9.4 hybridoma by DRB1*0101, DRB1*1104-F67I, DRB1*1101-F67I, and DRB1*1101, but not by DRB1*1102, DRB1*1301, or DRB1*1302 (Table 3), although all of the different HLA-DR alleles were effective in presenting the same SAg to the Vβ8$^+$/Vβ1$^+$ DO.11.10 hybridoma (data not shown). Similar results were previously reported by Herman et al. (51), who have shown that some class II molecules can present SAg to some T cells but not to others that have the proper TCR Vβ element. Since all HLA-DR molecules share a common α chain, these results mapped an important site for TCR/MHC II/SAg interaction to the β chain of HLA-DR. Moreover, the usage of L cells transfected with different HLA-DR alleles to present ExT or SEC2 is evidence in favor of the role of arginine 71 of the β chain of HLA-DR in TCR/MHC II interaction during SAg recognition (Table 3). Accordingly, the recently resolved crystal of the DR1 molecule (52) indicates that R71 might be oriented towards the peptide binding site and might also be accessible to the TCR.

The observation that mutations at residues D30 and T31 affect to an equal extent the recognition of both toxins, D30 having more drastic effects, is compatible with the view that these CDR1 β residues interact with the MHC II molecule. Residue D30 is a candidate for the interaction with R71 on the DR molecule, and we are presently investigating this possibility. The great similarity of MHC class I and II molecules, and the fact that TCR bearing the same Vβ elements can recognize both classes of molecules (2), make it possible that the same CDR regions of a TCR may be involved in both type of interactions. So, it is possible that the same CDR1 β residues that may contact MHC II in the presence of the SAg, may also contact MHC I in the recognition of peptide/K^d complex. Indeed, mutations on D30 and T31 affect both toxin-SAg recognition and also disrupt the recognition of the Cw3 peptide/K^d complex. It seems unlikely to us that these two residues would be directly involved both in SAg and peptide contact. We thus suggest that in our system these residues are essential for interaction with both classes of MHC molecules. Whether all the CDR1 residues are contacting only the MHC class I residues located on the top of α helices, or also antigenic peptide residues in our system, needs further investigation.

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