Different Superantigens Interact with Distinct Sites in the Vβ Domain of a Single T Cell Receptor

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Summary

CD4 T cell receptors (TCRs) recognize antigenic peptides presented by self major histocompatibility complex (MHC) class II molecules as well as non-self MHC class II molecules. The TCRs can also recognize endogenous retroviral gene products and bacterial toxins known collectively as superantigens (SAGs) that act mainly on the Vβ gene segment–encoded portion of the Vβ domain; most SAGs also require MHC class II for presentation. We have studied the interaction of the TCR from a well-characterized CD4 T cell line with SAGs by mutational analysis of its Vβ domain. This appears to separate viral (v)SAG from bacterial (b)SAG recognition. T cells having a TCR with a glycine to valine mutation in amino acid residue 51 (G51V) in complementarity determining region 2 of the TCR Vβ domain fail to respond the bSAGs staphylococcal entertoxin B (SEB), SEC1, SEC2, and SEC3, whereas they retain the ability to respond to non-self MHC class II molecules and to foreign peptides presented by self MHC class II molecules. It is interesting to note that T cells expressing mutations of both G51V and G53D of Vβ regain the response to SEB and partially that to SEC1, but do not respond to SEC2 and SEC3, suggesting that different bacterial SAGs are viewed differently by the same TCR. These results are surprising, because it has been generally believed that SAG recognition by T cells is mediated exclusively by hypervariable region 4 on the exposed, lateral face of the TCR Vβ domain. Response to the vSAG Mtv-7 was generated by mutation in Vβ residue 24 (N24H), confirming previously published data. These data show that the vSAG Mtv-7 and bSAGs are recognized by different regions of the TCR Vβ domain. In addition, various bSAGs are recognized differently by the same TCR. Thus, these mutational data, combined with the crystal structure of the TCR β chain, provide evidence for distinct recognition sites for vSAG and bSAG.

The TCR can recognize superantigens (SAGs)[1] derived from bacterial or endogenous retroviral genes (bSAG or vSAG). Unlike conventional antigen recognition, where the CDR3 regions of the TCR α and β chains play a critical role, response to these substances involves primarily the Vβ gene segment–encoded portion of the Vβ domain of the TCR. As 2–10% of T cells express the product of a given Vβ gene segment, SAGs stimulate T cells of many different specificities. Recognition of bSAGs and vSAGs by the TCR is believed to involve regions distinct from the antigen recognition site, comprising the CDR 1, 2, and 3 loops of both chains of the TCR, as predicted earlier (1). Many reports have shown that a region known as hypervariable region 4 (HVR4) between the E and F strands of the TCR Vβ domain is a crucial site for SAG recognition, but is not involved in responses to foreign peptide–self MHC (2–4). Although several reports (5–8) suggest that TCR sites other than the HVR4 of the TCR Vβ domain affect recognition of SAGs, all of these studies nevertheless point to the lateral face of Vβ as the most crucial factor in SAG recognition. Recently, the x-ray crystal structure of the bSAG Staphylococcal enterotoxin B (SEB) bound to the human MHC class II HLA-DR1 molecule has been solved (9). This shows that SEB binds to the α-helical region of the HLA-DR α chain; if the TCR Vβ domain recognizes this complex, the TCR Vα domain must sit on top of the α-helical region of the HLA-DR β chain. In fact, two studies showed that the V region of the TCR α chain interacts with the α-helical region of the HLA-DR β chain during SEB recognition (10, 11). Previously, we have reported that during non-self MHC recognition, the CDR1 and/or CDR2 of the TCR Vα domain interacts with the α-helical region of the I-A α chain, leading us to conclude that the TCR β chain must interact with the I-A β chain (12). This TCR–MHC inter-

[1] *Abbreviations used in this paper:* bSAG, bacterial superantigen; CA, conalbumin; HVR4, hypervariable region 4; SAG, superantigens; SEB, Staphyloccal enterotoxin B; TSST-1, toxic shock syndrome toxin 1.

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action appears to be in a distinct orientation from the TCR–SEB–MHC interaction.

In this report, we have examined SAG recognition by the TCR of the well-characterized CD4 T cell line D10.G4.1 (13). We have examined the role of several amino acid residues, including those in the CDR1 and CDR2 equivalents of the TCR β chain, in recognition of both bSAG and the vSAG Mtv-7, as well as in recognition of foreign peptide–self MHC and non-self MHC class II molecules. One mutation at residue 51 from glycine to valine (G51V) in CDR2 of the TCR Vβ domain, which does not affect non-self MHC recognition, has a profound effect on responses to the bSAGs SEB, SEC1, SEC2, and SEC3. It is interesting to note that T cells expressing this mutation plus that of a neighboring residue (Dlθαg51v,g53dβ), recover responsiveness to SEB and SEC1, but not to SEC2 and SEC3, whereas cells having Dlθαg53dβ respond well to all bSAGs. In agreement with the earlier studies (2), a mutation at residue 24 (N24H) of the TCR β chain results in a gain of response to the vSAG Mtv-7. Transfectants expressing mutations in both Vα and Vβ retain the characteristic responses of each chain when stimulated separately either with non-self MHC molecules or with SAG.

Materials and Methods

Animals. The mice used in these studies were obtained from The Jackson Laboratory (Bar Harbor, ME) or from the Core Mouse Breeding Facility maintained at Yale. The following mouse strains were used: B10.BR ($I\text{-}A^k$), AKR/J ($H\text{-}2^k$, $Mls\text{-}1^a$), and C57BL/6J ($I\text{-}A^b$).

Antibodies. The following mAbs were used: monoclonal anti-CD3ε YCD3-1 (14) and monoclonal anti-CD4 GK1.5 (15).

Cell Lines. The T cell line D10.G4.1 (13) has been described previously. The class II MHC–expressing cell line CH27 has been described previously (16). The 4G4, TCR α and β negative T cell hybridoma was kindly provided by Ed Palmer (Basel Institute for Immunology, Switzerland). This cell line allows expression of transfected α/β heterodimeric TCR.

Mutagenesis of TCR β Chain. Mutagenesis of a TCR β chain cDNA construct was performed using the overlapping extension method (17) with the PCR. PCR conditions were 25 cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C using wild-type D10 TCR β chain cDNA as a template DNA. The following PCR primers were used. 5′ primer GTATCCTGAGAGGAAGCATG and 3′ primer CTGCTCGGCCCCAGGCCTCT were the two external primers to generate the mutation. Mutagenic primers were as follows: N24H, coding sequence, GAGCTGTCACCAGACTAATA, noncoding sequence, TATTAGTCTGGTGACAGCTC; H29E, coding sequence, TGTAATCAGACTAATAACGAGAACAACATG, noncoding sequence, CATGTTGTTCTCGTTATTAGTCTGATTACA; G51V, coding sequence, CATTATTCATATGTTGCTGGCAGCACT, noncoding sequence, AGTACTGCCAGCAACATATGAATAATG; G53D, coding sequence, TCATATGGTGCTGACAGCACTGAGAAA, noncoding sequence, TTTCTCAGTGCTGTCAGCACCATATGA; and G51V/G53D, coding sequence, CATTATTCATATGTTGCTGACAGCACTGAG, noncoding sequence, CTCAGTGCTGTCAGCAACATATGAATAATG.

Production and Analysis of Transfectants. Transfected T cell hybridomas expressing mutant Vβ8.2 chains were generated and analyzed as previously described (12).

Peptides and SAGs. Peptides were synthesized as described (18). Purified SEB, SEC1, SEC2, and SEC3 were purchased from Toxin Technology, Inc. (Sarasota, FL).

Analysis of T Cell Responses. Experiments were carried out on 96-well flat-bottom plates in 200 μl/well with $1–5 \times 10^4$ TCR transfectants and APCs. When spleen cells were used as APCs, $\sim 1–3 \times 10^5$ cells/well were used. When B cell lines were used as APCs, $\sim 1–5 \times 10^4$ cells/well were used. These conditions produced the most optimal stimulation without increasing nonspecific IL-2 secretion. The TCR transfectants were incubated for 24 h, and the IL-2 content of the supernatants was measured by a CTLL assay.

CTLL-2 Stimulation Assays. All IL-2 assays were performed in 96-well flat-bottom plates with CTLL-2 responder cells. 50 μl/well of supernatant from experimental plates was transferred to a clean plate, frozen in the liquid nitrogen freezer, and thawed to kill any viable cells. Approximately $5–10 \times 10^3$ CTLL cells/well 50 μl were added and allowed to proliferate for 20 h; they were then pulsed with high specific activity [^{3}H]thymidine (1 μCi/well) for 4 h, and harvested onto filter mats. The incorporated radioactivity was counted on a β-plate counter.

Results

The D10 TCR and the Rationale for Mutagenesis. The TCR of the cloned T cell line D10 recognizes a peptide fragment of conalbumin (CA) (18) presented by the self MHC molecule $I\text{-}A^k$, as well as the non-self MHC molecules $I\text{-}A^{b,v,p,q, \text{ and } d}$ (19, 20) and the bSAGs SEB, SEC1, SEC2, and SEC3 (1). In an earlier study, we showed that the NH_2-terminal half of the TCR Vα domain played a critical role in the recognition of non-self MHC class II I-A molecules (12). These studies suggested that the CDR1 and/or CDR2 equivalent of the Vα domain interacts with the α-helical region of the I-A α chain. We have identified Vα residue 30 as the primary distinction between these two TCR molecules (Hong, S.-C., D.B. Sant'Angelo, G. Waterbury, and C.A. Janeway, Jr., manuscript submitted for publication).

To further analyze the individual amino acids responsible for the recognition of SAGs, we initiated a mutational analysis of the D10 TCR Vβ domain. The V domain of the β chain of the D10 TCR is encoded by Vβ8.2 (12). Because mice have three closely related Vβ8 genes, we used the nine amino acid differences between TCR Vβ8.1 and TCR Vβ8.2 to guide our mutagenesis strategy. These differences are located between the NH_2 terminus and CDR1, in CDR1 residues 30 and 31, in CDR2 residues 51 and 53, and in strand F between CDR2 and CDR3 (Table 1). The majority of T cells expressing TCR Vβ8.1 recognize a vSAG, Mtv-7, whereas only a small fraction of T cells expressing TCR Vβ8.2 do so (2, 21). On the other hand, SEB strongly stimulates T cells expressing Vβ8.2 but only weakly stimulates T cells expressing TCR Vβ8.1. It is known that the CDR3 regions of both Vα and Vβ are involved in peptide recognition (12, 22–24). Moreover, as CDR3 is thought to lie in the center of the TCR recogni-

Table 1. *Amino Acid Differences between Vβ8.1 and Vβ8.2*

Amino acid residue	Vβ8.1	Vβ8.2
10	S	N
24	H	N
CDR1 (25-33)		
30	D	N
31	Y	N
CDR2 (48-56)		
51	V	G
53	D	G
83	S	T
84	L	P
88	A	S

tion site, mutations in CDR3 are not informative as to SAG recognition (data not shown). We have therefore focused our analysis on mutagenesis of CDR1 and CDR2 of the TCR Vβ domain. Several independent mutations were generated by overlapping PCR mutagenesis (17). The wild-type and mutant D10 TCR β chain cDNAs were cotransfected with D10 TCR α chain cDNA as well as mouse CD4 cDNA into a murine hybrid thymoma called 4G4. These transfectants expressed comparable levels of the TCR and CD4 molecules as detected by FACS® analysis (Table 2). All the transfectants were tested for their response to anti-TCR or anti-CD3 mAb, to antigen presented by self MHC, to allogeneic MHC molecules, and to both bSAG and vSAG.

vSAG Mtv-7 Is Recognized by Residue 24 which Contributes to HVR4 of the TCR Vβ Domain. According to the crystal structure of the TCR Vβ8.2 chain (25), residue 24 of the TCR β chain is adjacent to the HVR4 loop, and is exposed on the lateral surface of Vβ8.2 together with two adjacent amino acids in HVR4, 73 and 74, that are known to affect Mtv-7 recognition (Fig. 1 *a*) (2). When asparagine

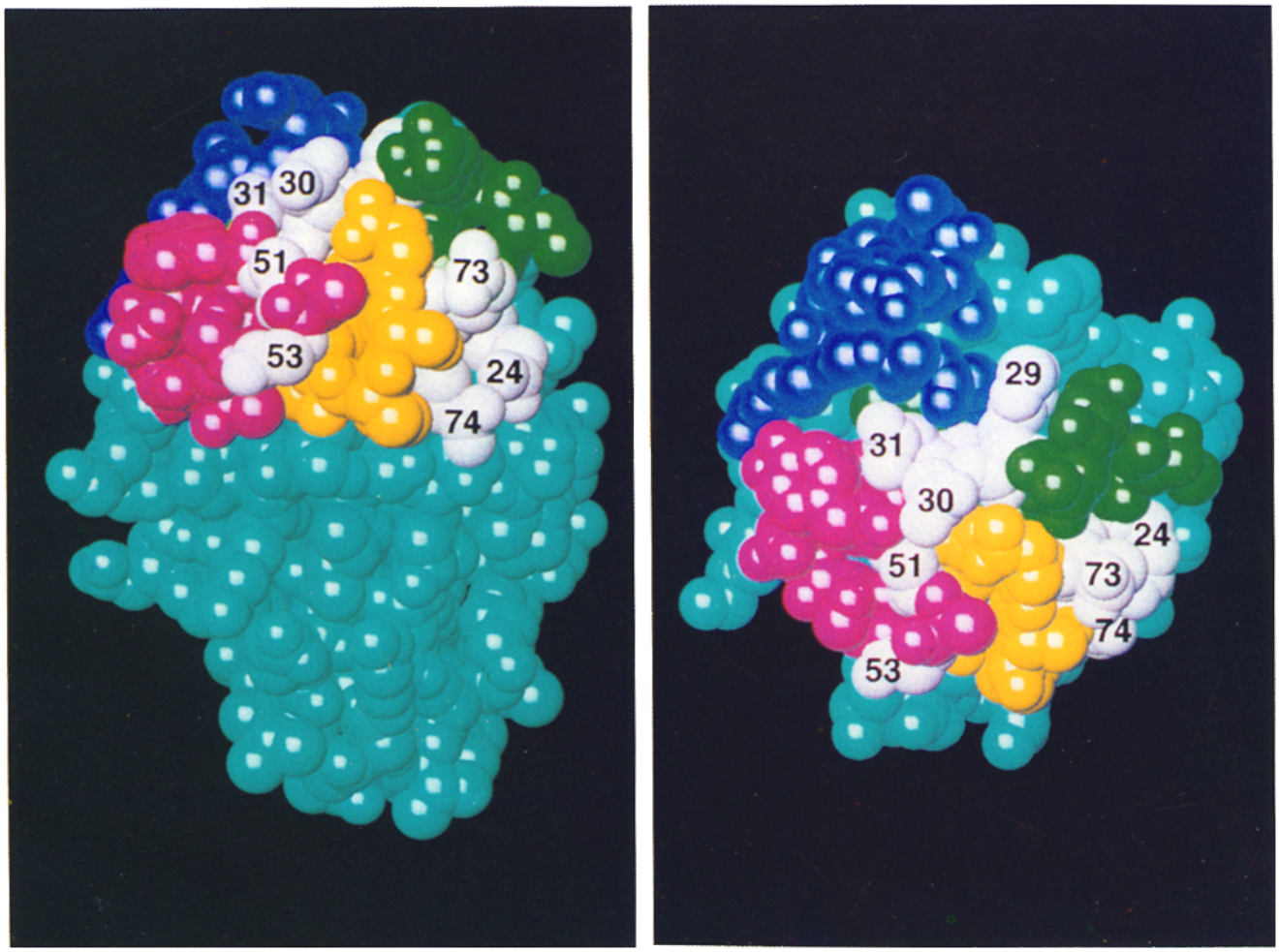

Figure 1. Space-filling model of TCR Vβ8.2 chain. (*a*) Space-filling model of the putative vSAG (Mtv-7) binding face of the Vβ8.2 domain. Mutation of residue 24 confers reactivity to Mtv-7 on this Vβ, when mutation at residues 51 and 53 affect responses to SEB and SEC1, 2, and 3. (*b*) Space-filling model of CDR loops facing the MHC–peptide complex showing the amino acid residues examined. (*Green*) CDR1; (*magenta*) CDR2; (*blue*) CDR3; and (*yellow*) HVR4. (*Silver*) Mutated amino acids with the residues mutated shown by a number.

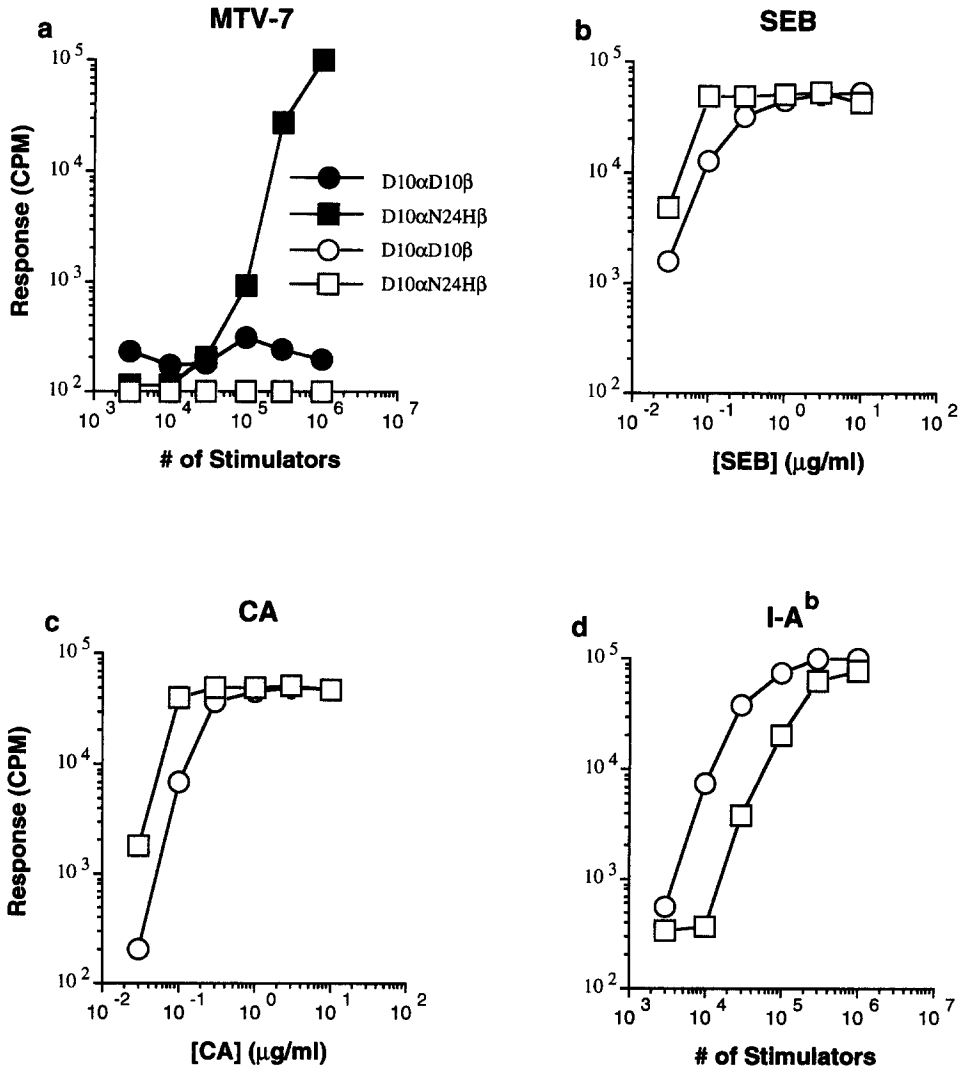

Figure 2. The responses of the transfectants to various stimuli. Mutation N24H confers reactivity to Mtv-7 (*a*) but does not alter recognition of SEB (*b*), antigen (*c*), or allogeneic MHC class II (*d*). (*a*) Mtv-7 response of the transfectants. Responses of transfectants to Mtv-7$^+$ strain AKR/J (●, ■) and to Mtv-7$^-$ strain B10.BR (○, □). (*b*) SEB response of the transfectants presented by 10^5 CH27 cells. (*c*) Response of the transfectants to an antigenic peptide of conalbumin (*CA*) presented by 10^5 CH27 cells. (*d*) Response of the transfectants to non-self class II MHC I-A^b, stimulated with spleen cells from C57BL/6J mice. 1–3 × 10^4 transfectants were used for the IL-2 release assay.

24β in the D10 TCR (Vb8.2) was replaced by the histidine (N24H) found at this position in an inbred mouse, Vβ8.1, reactivity to Mtv-7 resulted (Fig. 2 *a*). As expected, cells bearing the TCR D10αN24Hβ responded strongly to antigen, to SEB, and to allogeneic MHC molecules (Fig. 2, *b–d*). These data confirm that recognition of the vSAG, Mtv-7, is mediated by the lateral face of HVR4 of the TCR Vβ domain.

Effect of Amino Acids in the CDR2 Loop of the TCR Vβ Domain on Recognition of bSAG. When T cells expressing various mutations in TCR Vβ8.2 were tested for their responses to the bSAG, SEB, all T cells except those with a mutation in CDR2 residue 51 (D10αG51Vβ) responded equally to SEB (Fig. 3 *a*). Cells bearing D10αG51Vβ responded to anti-CD3 mAb stimulation and to allogeneic MHC molecules equivalently to D10αD10β transfectants, but showed a diminished sensitivity to antigenic peptide (Fig. 3, *b–d*). We also tested other bSAGs that are recognized by the murine TCR Vβ8.2. As expected, all of these bSAGs stimulated transfectants expressing D10αD10β, but none stimulated transfectants expressing the mutant D10αG51Vβ; this suggests that all of these recognition events are affected by this mutation (Fig. 3, *e–g*). We also tested many other T cells expressing other mutant TCR β chains, and all of these responded to SEB as well as to anti-CD3 mAb stimulation, with the exception of those having the G51Vβ mutation (Table 2).

To further analyze this loss of function mutation, T cells expressing β chains with double mutations, including G51Vβ, were produced. T cells expressing the double mutations N24H,G51Vβ, and H29E,G51Vβ, together with the D10 TCR α chain, responded well to anti-CD3 mAb stimulation (Fig. 4 *a*), but failed to respond to stimulation with SEB (Fig. 4 *b*). This again confirms the role of G51Vβ in SEB recognition. In addition, T cells expressing N24H, G51Vβ responded well to Mtv-7 (Fig. 4 *c*), segregating vSAG and bSAG recognition by the same TCR Vβ domain.

Differences in the Recognition of bSAGs by the Same TCR. T cells expressing the mutation G51Vβ lost response to all the bSAGs tested (Figs. 3 and 4), whereas additional mutations in other residues in Vβ, such as the β strand–associated N24H,G51Vβ and the CDR1 loop H29E, G51Vβ did not alter responses to SEB from those observed with the original G51Vβ mutation. However, when T cells express-

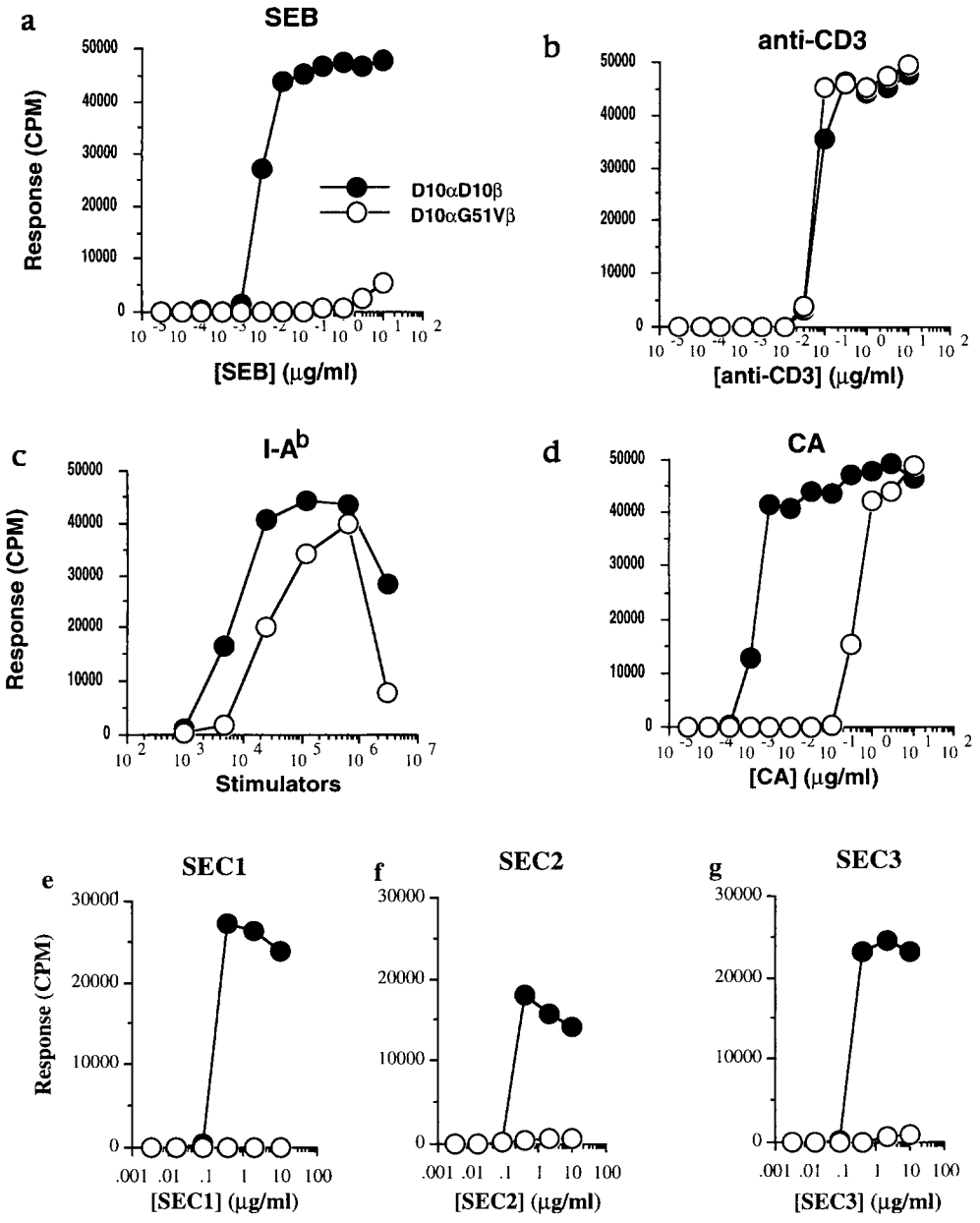

Figure 3. The response of transfectants expressing the D10αD10β (●) or the mutant D10αG51Vβ (○) TCR to various stimuli: (*a*) SEB, (*b*) anti-CD3, (*c*) I-A^b spleen, (*d*) CA peptide plus I-A^k spleen cells, (*e*) SEC1, (*f*) SEC2, and (*g*) SEC3. Responses were elicited as in Fig. 2. For anti-CD3 and bSAG stimulation, different amounts of anti-CD3 mAb (YCD3-1) or bSAG were presented by CH27.

ing a double mutation in CDR2, replacing residues 51 and 53 with those naturally found in inbred TCR Vβ8.1 (G51V,G53Dβ), the resulting transfectants (D10αG51V, G53Dβ) were found to have recovered responses to SEB and to high concentrations of SEC1, but not those to SEC2 and SEC3 (Fig. 5). These data again demonstrate that the CDR2 loop affects the recognition of all of these bSAGs and that SEB and the SECs are recognized similarly, but not identically, by this TCR.

Responses to Allogeneic MHC Class II Molecules and to bSAG Are Independent Recognition Events by the Same TCR. We tested whether the TCR recognizes MHC class II molecules in the same way during responses to allogeneic MHC class II molecules and to bSAG. To do so, we made a transfectant that expresses a well-characterized mutant TCR α and β chain. The TCR, AK8:D10αD10β, contains seven mutations in the NH_2-terminal half of the TCR α chain that result in a gain of response to I-A^d and a loss of response to I-A^b from the original wild-type D10αD10β TCR (12). We made a transfectant expressing this TCR α chain paired with a β chain containing the G51V mutation, and tested its reactivity. This T cell, called AK8:D10αG51Vβ, did not respond to SEB, which is characteristic of the TCR β chain mutation G51Vβ, but maintained its response to anti-CD3 mAb (Fig. 6, *a* and *b*). In addition, this double mutant TCR lost its usual response to I-A^b and gained a response to I-A^d, which is characteristic of the mutant TCR α chain AK8:D10α (Fig. 6, *c* and *d*). This suggests that TCR recognition of allogeneic MHC molecules and bSAG can occur independently.

Table 2. *Transfectants Tested for their SEB Recognition*

TCR-α	TCR-β	SEB response	CD3 response	MFI of transfectant with Anti-TCR	MFI of transfectant with Anti-CD4
D10	D10	+++	+++	93	117
D10	N27D	+++	+++	103	100
D10	N27Q	+++	+++	99	110
D10	N28D	+++	+++	115	88
D10	N28Q	+++	+++	123	87
D10	H29E	+++	+++	197	115
D10	H29Q	+++	+++	ND	ND
D10	N30D	+++	+++	ND	ND
D10	N31Y	+++	+++	ND	ND
D10	G51A	+++	+++	ND	ND
D10	G51V	−	+++	131	125
D10	G53A	+++	+++	ND	ND
D10	G53D	+++	+++	147	119

MFI, mean fluorescence intensity.

Discussion

The TCR of the CD4 T cell clone D10.G4.1 recognizes a peptide fragment of antigen presented by self MHC class II molecules, allogeneic MHC class II molecules, and vSAG and bSAG presented by self MHC class II molecules. In this study, we made site-directed mutations of the Vβ domain in an attempt to understand the nature of these various TCR–ligand interactions. We have shown for each mutant tested in this manuscript, a retention of normal or slightly decreased recognition of peptide plus self MHC class II and/or of allogeneic MHC class II. Thus, we believe that the mutations we have introduced do not significantly perturb MHC class II recognition by this receptor.

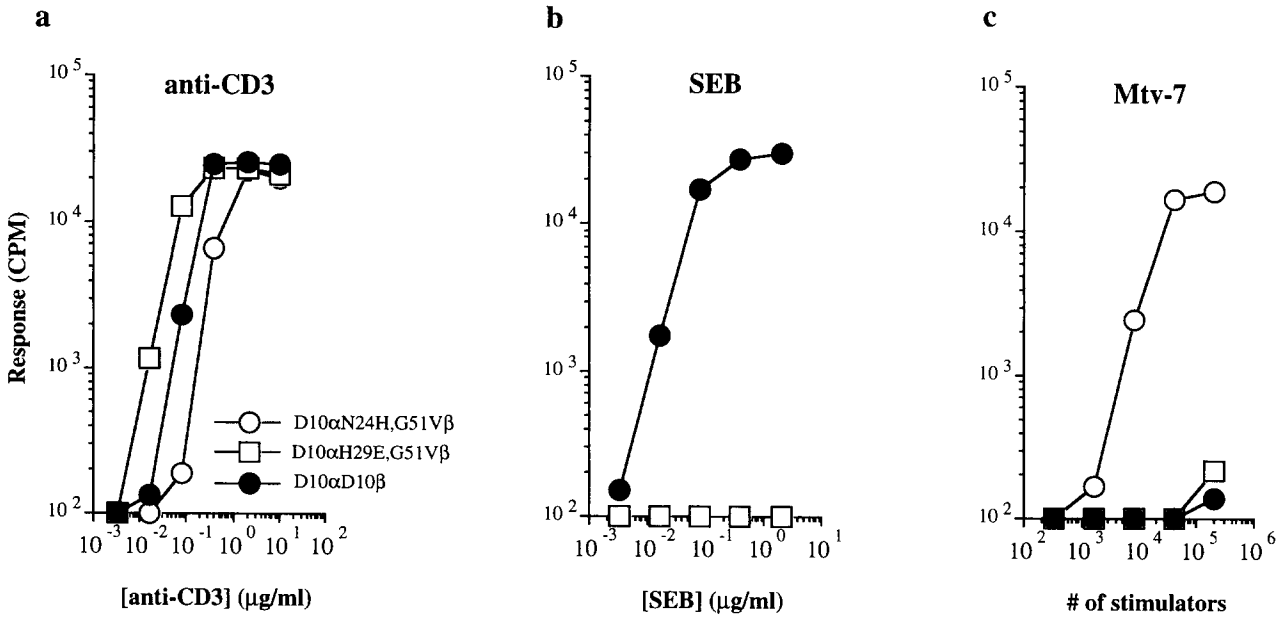

Figure 4. The responses of transfectants expressing the D10αD10β wild-type receptor (●), the double mutant N24H,G51Vβ paired with D10α (○) and the double mutant H29E,G51Vβ paired with D10α (□) to various stimuli. (*a*) Response of the transfectants to anti-CD3 mAb (YCD3-1) presented by 10^5 CH27 cells. (*b*) Response of the transfectants to SEB presented by 10^5 CH27 cells. (*c*) Response of the transfectants to Mtv-7; increasing numbers of spleen cells from an Mtv-7^+ (AKR/J) mouse were used to stimulate the T cells. 1–3 × 10^4 TCR transfectants were used for the IL-2 release assay.

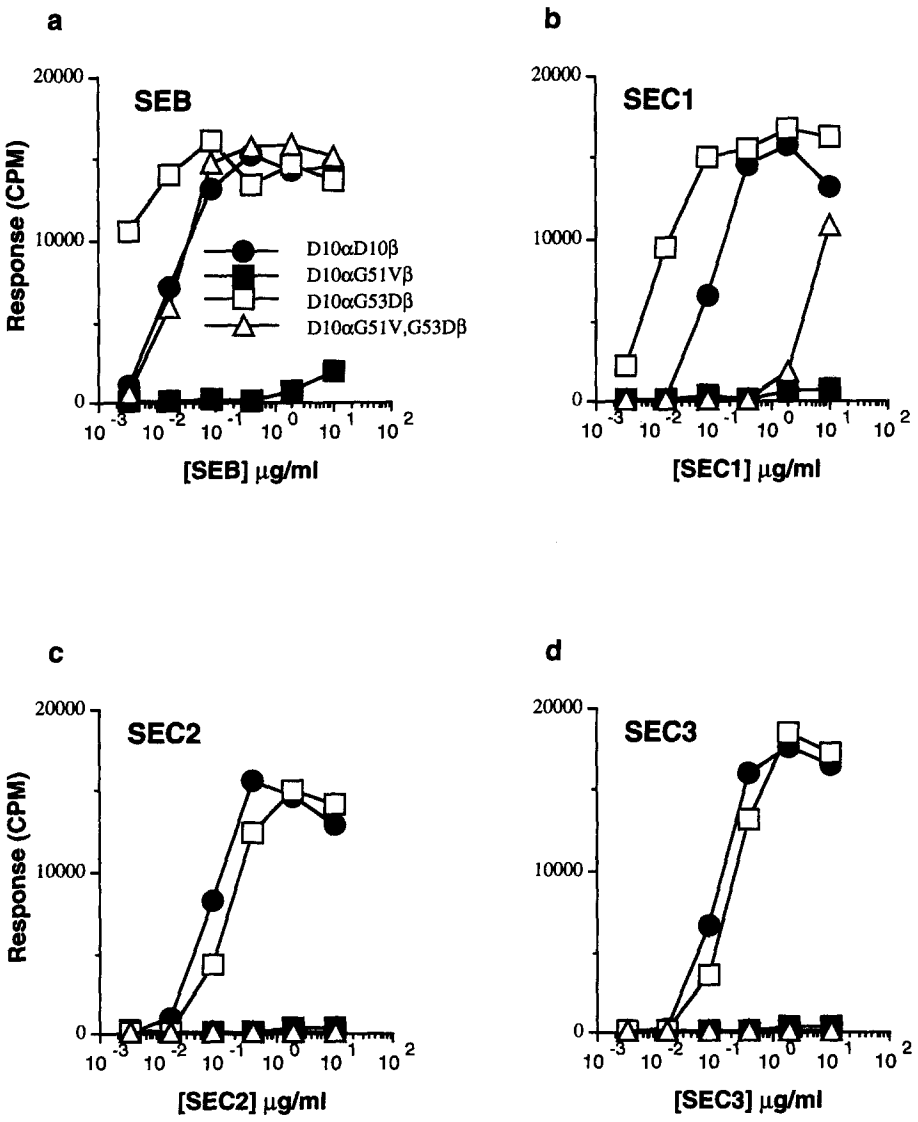

Figure 5. The response of various TCR transfectants to different bacterial superantigens. Wild-type D10αD10β (●), D10αG51Vβ (■), D10αG53Dβ (□), and D10αG51V,G53Dβ (△) were stimulated with the bSAGs SEB (*a*), SEC1 (*b*), SEC2 (*c*), and SEC3 (*d*) presented by CH27.

Although the resolution of a crystal structure of this receptor–ligand pair should provide major insights into this crucial process, the variability of both the TCR and its ligands, and the complexity of signaling through the receptor, require detailed functional analysis of this interaction as well. Moreover, the low affinity of TCR–ligand interactions (26, 27) suggests that obtaining such complexes may be extremely difficult; indeed, it has been suggested (28–30) that binding of the TCR alone is not sufficient for T cell activation, and that a conformational change in the TCR may occur as an essential part of recognition. If this is the case, then crystallographic analysis may be even more difficult. Therefore, we have approached this problem by making a detailed structure–function analysis of this interaction an alternative approach. We have made several TCR mutations in both Vα and Vβ of the D10 TCR to try to determine which residues mediate each interaction of the TCR with its various MHC class II ligands. Since the Vα and Vβ CDR3 loops of the TCR are said to interact with different residues of a foreign peptide presented by self MHC molecules, the information acquired by CDR3 mutation may be limited to the interaction of the TCR with specific antigen (22). To understand TCR–MHC and TCR–SAG interactions, we and others (2, 3, 12, 31, 32) have used mutational studies of CDR1, CDR2, and HVR4, each of which defines a loop of the α and β chains of the TCR. Here, we will discuss how our new data may further the understanding of this interaction.

TCR recognition of SAG has been well characterized. Because of the polyclonal in vivo deletion of T cells expressing certain Vβ chains in mice expressing endogenous vSAG and the polyclonal expansion of the same T cells by vSAG and bSAG in vitro and in vivo (33), this recognition event is believed to be mediated by specific polymorphic sites in the Vβ gene segment–encoded part of the β chain. Mutational and transfection studies of TCR support the Vβ-mediated recognition of SAG, especially the role of HVR4 in the TCR Vβ domain (2–4). We have confirmed the

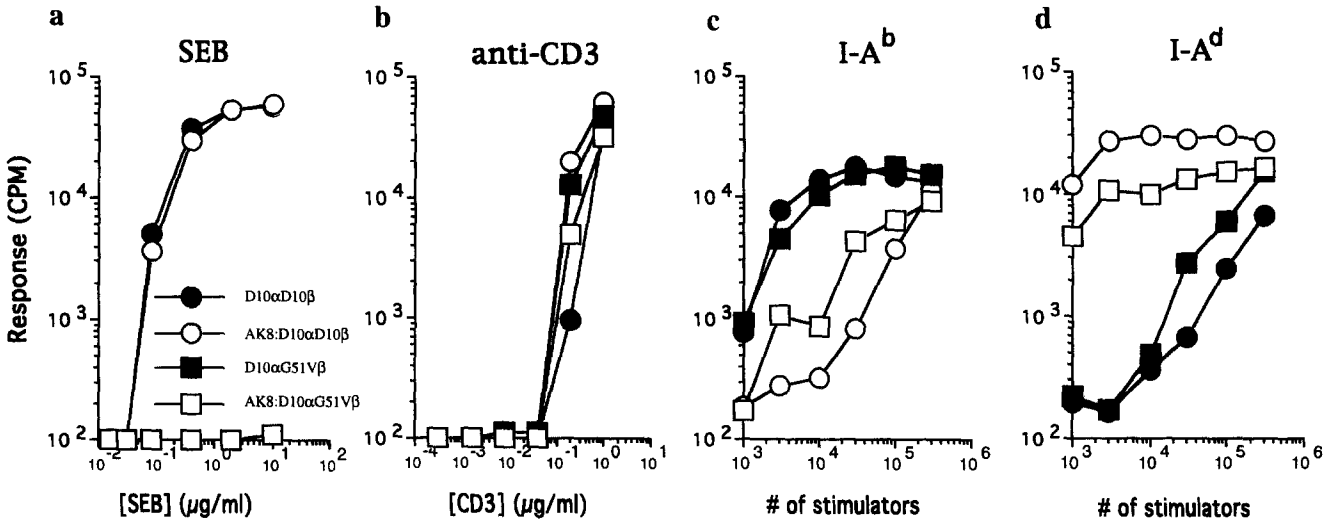

Figure 6. The response of various TCR transfectants to various stimuli. Transfectants were tested for their response as in Fig. 2. Spleen cells from BALB/c mice were used for the allogeneic response to I-A^d. TCR transfectants: (●) D10αD10β, (○) AK8:D10αD10β; (■) D10αG51Vβ, and (□) AK8:D10αG51Vβ.

finding reported by Pullen et al. (2) that mutation in residue 24 (here, N24H) in the Vβ domain can confer Mtv-7 reactivity on a Vβ8.2 TCR. It is clear from the crystal structure of the TCR Vβ8.2 chain (Fig. 1, *a* and *b*) that amino acid residue 24 on the lateral face of Vβ associates with residues 73 and 74 which are known to be involved in Mtv-7 recognition. In addition, these three amino acid residues are located away from the peptide–MHC interaction site. This may facilitate the direct interaction of Mtv-7 with this site, as predicted (1). Glycosylation of asparagine residues 24 and 74 may interfere with the interaction of wild-type TCR Vβ8.2 with Mtv-7. Substitution of one of these two asparagine residues seems to be sufficient for Mtv-7 interaction, since mutation of amino acid residue 74 from asparagine to glutamic acid also restores Mtv-7 reactivity (2).

Our data on the role of residue 51 of CDR2β, shown by the mutant G51V, support other studies for TCR recognition of bSAG (31, 32) that describe evidence that CDR1 or CDR2 of the TCR Vβ chain can affect bSAG recognition. The crystal structure of the TCR Vβ8.2 chain (Fig. 1, *a* and *b*) clearly demonstrates that amino acid residue 51 is distant from the amino acids critical for a response to Mtv-7. In addition, this amino acid, which is facing towards the peptide–MHC, is not carried on the lateral face of Vβ like the Mtv-7–binding site. Recent crystal structures of SEB bound to HLA-DR1 (9) and of toxic shock syndrome toxin-1 (TSST-1) bound to HLA-DR1 (34) show that SEB contacts HLA-DR1 at two sites on the DR1 α chain along one edge of the peptide-binding groove. The TSST-1–binding site, on the other hand, extends over almost half of the peptide-binding site, so that TSST-1 contacts the α-helical region of the DR1 α chain, the bound peptide, and part of the α helix of the DR1 β chain. This suggests that TSST-1 binding may be peptide dependent. Furthermore, it is likely that the recognition of TSST-1–DR1 by the TCR is very different from that of SEB-DR1 or peptide-DR1. Both of these crystal structures suggest that bSAG recognition by the TCR may be mediated by either CDR1 or CDR2 of the TCR, rather than the lateral part (HVR4) of the TCR, as has been shown in responses to the vSAG Mtv-7.

T cells expressing the double mutation N24Hβ and G51Vβ provide further evidence for the separation of vSAG and bSAG recognition by one TCR. In contrast to the wild-type D10 TCR, which responds to many bSAGs including SEB, D10αG51Vβ does not respond to these bSAGs. On the other hand, T cells expressing the double mutation D10αN24H,G51Vβ gain a response to the vSAG Mtv-7, without gaining response to SEB. These data are in agreement with the data of Patten et al. (31) and Bellio et al. (32) regarding bSAG recognition, and with the data of Pullen et al. (2) and Cazenave et al. (4) regarding vSAG recognition. In addition, Thibodeau et al. (35) showed that binding sites for vSAGs and bSAGs are different in MHC class II molecules, suggesting two independent SAG recogntion sites on the TCR.

There are many reports that show that different bSAGs (e.g., TSST-1, SEA, SEB) bind to different regions of the MHC class II molecule (36–39). Since all of these SAGs are recognized by various TCR Vβ domains, they are likely to be recognized in many different TCR–MHC orientations. Thus, it is not reasonable to expect that a general orientation of the TCR to its MHC–peptide ligand can be deduced from responses to bSAGs. It has also been shown that different mutant MHC molecules affect antigen presentation and presentation of the bSAG, SEA, to T cells differently (40).

Recently, Hamad et al. (41) showed that SEB presented by a plate-bound anti-SEB mAb can stimulate T cells in the absence of MHC class II molecules. In addition, a Bia-

core® binding study by Seth et al. (42) suggests direct TCR–SEB interaction. Some bSAGs, such as SEC and SEE, can stimulate T cells using MHC class II–negative APCs (43). All these data suggest that bSAG can directly stimulate the T cell without need of a direct interaction of the TCR with MHC class II molecules. By contrast, no comparable observations on binding of Mtv-7 have been obtained (44).

In conclusion, our studies show that residue 24 of the TCR β chain accounts for a contact with the vSAG encoded by Mtv-7. In addition, we find that mutations in CDR2 of Vβ, especially that at residue 51, disrupt SEB recognition without affecting responses to the vSAG Mtv-7 or alloantigen recognition. It is perhaps most surprising that the double mutant of Vβ, G51V,G53D, responds to SEB and to high doses of SEC1, but still lacks a response to SEC2 and SEC3. In addition, a transfectant expressing a single amino acid mutation in Vβ, G53D, responds better than wild-type to SEB and SEC1, and responds normally to SEC2 and SEC3 (Fig. 5). Thus, whereas we can make a model for bSAG interaction with this TCR, it is clear that this model will have to be complex.

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