A Role for the Region Encompassing the c'" Strand of a TCR V\alpha Domain in T Cell Activation Events

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The distinct strand topology of TCR V\alpha domains results in a flatter surface in the region encompassing the c'" strand than the corresponding region in Ig V domains. In the current study a possible role for this region in T cell activation has been investigated by inserting a potential glycosylation site at V\alpha residue 82. This residue is in proximity to the c'" strand and distal to the putative interaction site for cognate peptide:MHC ligand. An additional N-linked carbohydrate at this position would create a protrusion on the V\alpha domain surface, and this may interfere with TCR aggregation and/or recruitment of signaling molecules. The modified TCR has been expressed in transfected T cells, and the phenotype following stimulation has been compared with that of cells expressing the wild-type TCR. The mutation has significant effects on activation-induced cell death and TCR internalization, but, unexpectedly, does not affect IL-2 secretion. Furthermore, analyses with tetrameric, peptide:MHC class II complexes suggest that the mutation decreases the ability of the TCR to aggregate into a configuration compatible with avid binding by these multivalent ligands. The Journal of Immunology, 2000, 165: 820–829.

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also results in a reorientation of the Vα CDR2 by about 90° to produce a more compact Ag interaction site (reviewed in Ref. 43). This unusual strand topology is invariably observed for Vα in other more recently solved TCR αβ structures (37, 42; reviewed in Ref. 43). In Vβ domains the position of the c strand shows greater variation (44), but in one Vβ domain analyzed to date a switch similar to that present in the Vα domain was observed (40). Furthermore, analysis of Vα domain sequences indicate that there is an exclusion of potential glycosylation sites in the vicinity of the Vα c strand (34), in contrast to the corresponding region of Vβ domains.

The distinctive structural features of Vα domains prompted us to analyze a possible role for the region encompassing the Vα c strand in events subsequent to ligand recognition by the TCR. To do this, we have mutated a serine to asparagine to insert a potential glycosylation site on this surface of the Vα domain. Our rationale was that N-linked glycosylation at this asparagine would insert a protrusion in this region of the TCR that might affect receptor aggregation and/or association with other signaling molecules. Mutated α-chains have been expressed in association with the wild-type (WT) β-chain in a TCRαβ thymoma cell line, and the characteristics of the resulting T cell transfectants have been compared with those of similarly generated WT TCR transfectants. The results indicate that the additional N-linked carbohydrate differentially affects IL-2 secretion, apoptosis, and TCR internalization. In addition, analysis of transfectants with fluorescent tetrameric, cognate peptide:MHC class II complexes indicates that there are differences in the ability of the mutant and WT TCR to form a configuration that allows tetrameric ligand binding.

Materials and Methods

Cell lines, Abs, and reagents

The I-Aa-expressing B cell line, PL-8 (45) was provided by Dr. David Wraith (University of Bristol, Bristol, U.K.). The I-Aa-transfected derivative of BW4157, Utm6.15 (46, 47) was made available by Dr. Harden McConnell (Stanford University, Stanford, CA). FITC-labeled H57-597 was provided by Dr. Bernard Malissen (Kearse (East Carolina University, Greenville, NC)). The Vα8.2Vβ7.2 cell line, TCR-negative cell transfectants of the peptide Ac1–11[4Y] in the presence of an I-Aa-transfected thymoma line, Utm6.15, or B cell line, PL-8, at 37°C in a humidified CO2 incubator. Control wells contained Utm6.15 and T cells but no peptide. The transfectants were also activated using PMA and calcium ionophore A23187 (Sigma, St. Louis, MO) or with plate-bound mAbs F23.1 (anti-Vβ8), 145-2C11 (anti-CD3ε), and recombinant MBP1–11[4Y]:I-Aα complexes have been described previously (54). Abs and the recombinant molecules were coated onto 96-well plates for 2 h at 37°C (54). Twenty to 24 h later the supernatants were collected, and IL-2 levels were determined using an IL-2-dependent cell line, CTLL-2, as previously described (55) or by cytokine ELISA using JES6-1A12 (rat anti-mouse IL-2, Pharmingen) as a capture Ab and biotinylated JES6-5H4 (rat anti-mouse IL-2, Pharmingen) followed by Extravidin-HRP (Sigma) for detection.

Analysis of apoptosis

Following activation with the appropriate stimuli (as above), cells were washed once with 1% BSA/PBS. Propidium iodide (PI) was added to a final concentration of 10 μg/ml, and cells were analyzed by flow cytometry (56). To confirm that the death was apoptotic, in some experiments cells were stimulated for 6 h at 37°C with 1 μg/ml of recombinant MBP1–11[4Y]:I-Aα, stained with both annexin V-FITC (2.5 μg/ml) and PI (10 μg/ml), and analyzed by flow cytometry. For annexin V staining the buffer comprised 10 mM HEPES/NaOH (pH 7.4), 150 mM NaCl, 1 mM MgCl2, 5 mM KCl, and 1.8 mM CaCl2. TCR down-regulation

For TCR down-regulation experiments the T cell transfectants were stimulated with 1 μg/ml of recombinant MBP1–11[4Y]:I-Aα complexes or PMA and calcium ionophore A23187. After activation, TCR levels were determined by flow cytometric staining using an FITC-labeled anti-βAb (H57-597) or an anti-Vβ mAb, F23.1 followed by FITC-labeled anti-mouse IgG.
Anti-phosphotyrosine immunoblotting

T cells (2 × 10^6) were stimulated at 37°C using 100 μg/ml of Ac1–11[4Y] and 10° U/ml 145-2C11. After 5 min cells were resuspended in 1 ml of lysis buffer (250 mM NaCl, 50 mM Tris-Cl, 0.5% Triton X-100, 1 μg/ml peptatin, 1 mM Pefabloc, 10 μg/ml leupeptin, 2 μg/ml aprotinin, 1 mM activated sodium orthovanadate, and 5 mM NaF) for 2 h at 4°C. The TCR/CD3 complex was immunoprecipitated using F23.1 cross-linked to protein A-Sepharose beads. Following washes with lysis buffer and PBS, the beads were boiled for 5 min in SDS-PAGE loading buffer. 2-ME was added to the supernatants at a final concentration of 2%; the samples were electrophoresed using 10% SDS-PAGE and transferred to an Immobilon-P membrane. The membrane was dried for 12 h at room temperature and then incubated with the anti-phosphotyrosine Ab, 4G10, followed by HRP-conjugated anti-mouse IgG. The blot was developed using the ECL reagent.

Flow cytometric analyses using MBP1–11[4Y]:I-A^u tetramers

MBP1–11[4Y]:I-A^u tetramers were prepared as described elsewhere.9 T cells were incubated with the MHC class II tetramers labeled with PE or, as a control, with streptavidin-PE, in the presence of the anti-CD3 Ab, 4G10, followed by HRP-conjugated anti-mouse IgG. The blot was developed using the ECL reagent.

Results

TCR expression by the transfectants

Mycophenolic acid-resistant TCR-positive transfectants were generated by cotransfection of either the 1934.4 WT or αS82N mutant α-chain construct together with the β-chain shuffle plasmid into 58αβ/CD4^- cells. The location of the mutation site on the Vα4.2 structure (34) is shown in Fig. 1. The transfectants expressed different levels of TCR as determined by reactivity with the anti-Vβ8 mAb, F23.1 (Fig. 2A). Mutation of serine 82 to asparagine in the TCR α-chain does not have a significant effect on the surface expression of the TCR in transfectants (Fig. 2A, MUT-1 and MUT-2). However, despite analyzing about 20–30 mutant transfectants, expression levels as high as those seen for WT transfectants such as WT-1 were not observed. This suggests that the mutation may have a minor effect on the stability of the α-chain and/or the efficiency of assembly of the corresponding TCR. Immunoprecipitation of the expressed TCRs using F23.1 indicated that the α-chain containing the αS82N mutation had a higher m.w. than the WT α-chain (size difference of ~2–3 kDa using SDS-PAGE), and this size difference was eliminated following digestion with N-glycosidase F (Fig. 2B). The additional glycosylation site is therefore used in the αS82N mutant.

IL-2 secretion by the T cell transfectants

The 1934.4 TCR recognizes the N-terminal 11 residues (or nonamer) of MBP in association with the MHC class II molecule, I-A^u. For recognition, the antigenic peptide requires acetylation at residue 1 to block the N-terminal charge (57), and position 4 analogues of this peptide that bind with higher affinity to I-A^u than the WT peptide stimulate T cells more efficiently (46, 58). For example, replacement of position 4 lysine with tyrosine results in a peptide that gives >100-fold shifts in dose-response curves (58), and this is also observed for the transfectants (59). For this reason, the higher affinity analogue (Ac1–11[4Y]) has been used in the current study.

Initially, transfectants were stimulated with plate-bound anti-Vβ8 mAb, F23.1, and anti-CD3ε Ab, 145-2C11. The responsiveness of the transfectants, assessed by quantitating IL-2 levels, was dependent on the levels of surface TCR (data not shown). This dependence on expression levels is consistent with the findings of other studies using TCR transfectants (49, 59). For this reason two transfectants (WT-3 and MUT-2), which showed only minor differences (~2-fold; Fig. 2A) in surface TCR levels, were used for all subsequent studies. Similar levels of responsiveness were seen when the T cells were stimulated by cross-linking the TCR with F23.1 or the anti-CD3-ε Ab 145-2C11 (Fig. 3, A and B). Thus, the αS82N mutation does not have a significant effect on IL-2 production in response to Ab-mediated cross-linking.

The transfectants were also activated with PMA plus the calcium ionophore A23187 and, to investigate IL-2 secretion in response to antigenic stimulation, with peptide-pulsed, I-A^u-expressing transfectants. Again, no significant differences were seen with the WT-3 and MUT-2 transfectants (Fig. 3, C and D).

Apoptosis induction in the TCR transfectants

To evaluate activation-induced cell death (AICD), recombinant MBP1–11[4Y]:I-A^u complexes adsorbed onto the wells of microtiter plates were used to stimulate the cells (Fig. 4). The MBP1–11[4Y]:I-A^u complexes were functionally expressed by insertion

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9 C. G. Radu, S. M. Anderton, M. Firan, D. C. Wraith, and E. S. Ward. Quantitative analysis of T helper cells specific for an immunodominant epitope of myelin basic protein. Submitted for publication.
of the N-terminal epitope of MBP between codons 2 and 3 of the gene encoding the mature β polypeptide to result in antigenic peptide covalently tethered to the I-A<sup>m</sup> molecule (54). These molecules are potent and specific stimulators of T cell transfectants/hybridomas expressing the WT 1934.4 TCR (54). They are also efficient inducers of AICD, and these were used in apoptosis studies in preference to Ag-pulsed APCs because this simplified the flow cytometric analyses.

When stimulated with recombinant, plate-bound MBP<sub>1–11</sub>[4Y]:I-A<sup>m</sup> complexes, the WT-3 cells consistently produced slightly higher levels of IL-2 than MUT-2 cells at low Ag concentrations (Fig. 4A). In contrast to the minor difference in IL-2 secretion levels following treatment with plate-bound MBP<sub>1–11</sub>[4Y]:I-A<sup>m</sup> complexes, there was a marked difference between the WT-3 and MUT-2 transfectants in AICD, as assessed by PI staining. The MUT-2 cells were markedly more resistant to AICD (Fig. 4A). This could not be explained by differences in IL-2 levels, since Ag doses that induced similar levels of IL-2 production by the two transfectants resulted in significant differences in sensitivity to apoptotic death (Fig. 4A). Importantly, for these experiments IL-2 levels were determined by ELISA, and under the conditions of the assay, OD values corresponded in direct proportion to IL-2 concentrations. Annexin V staining was used to confirm that the death was apoptotic and showed the same difference in induction of programmed cell death for the WT and mutant transfectants as PI staining (Fig. 4B). Significantly, stimulation by PMA and calcium ionophore A23187 resulted in similar levels of IL-2 secretion and apoptotic death for both transfectants (Fig. 3C and 4C). Thus, the resistance to cell death by MUT-2 cells was only observed following antigenic stimulation and can be bypassed by activation of pathways distal to TCR triggering. Importantly, qualitatively similar results were observed for other WT and α82N transfectants (data not shown), indicating that the resistance to AICD was not a peculiarity of MUT-2 cells.

**TCR down-regulation**

To analyze another important marker of T cell activation, TCR down-regulation, the surface levels of TCR on the transfectants following stimulation were analyzed by flow cytometry using the anti-V<sub>β</sub> Ab F23.1 or the anti-C<sub>β</sub> Ab H57-597. Following activation by the recombinant MBP<sub>1–11</sub>[4Y]:I-A<sup>m</sup> complexes, mutant transfectants showed markedly reduced internalization of TCR compared with WT transfectants (Fig. 5A). The relatively low levels of down-regulation observed with the WT transfectants are
consistent with the observations of others in studies in which immortalized T cells were compared with T cell clones (60).

To exclude the possibility that the lower level of TCR down-regulation for mutant cells relative to WT cells is due to the difference in expression levels, TCR internalization in response to phorbol ester (PMA)-mediated stimulation was also assessed. TCR down-regulation in response to PMA was almost abrogated in transfectants expressing the mutated TCR α-chain (Fig. 5B), whereas down-regulation was observed for WT transfectants. Similar results were observed for the other transfectants shown in Fig. 2 (data not shown).

**Anti-phosphotyrosine immunoblotting**

The observed differences between the WT and mutant transfectants prompted us to investigate the phosphorylation of TCR-associated proteins following Ag-specific stimulation, i.e., events proximal to TCR-mediated activation. The transfectants were activated for 5 min at 37°C with Ac1–11[4Y]-pulsed APCs, and the TCR/CD3 complexes were immunoprecipitated using F23.1 cross-linked to protein A-Sepharose beads. Immunoblotting of the F23.1-captured immunoprecipitates with the anti-phosphotyrosine Ab 4G10 demonstrated that the same TCR-associated phosphoproteins were present in the WT-3 and MUT-2 cells following stimulation with Ac1–11[4Y]-pulsed I-Au-expressing cells (Fig. 6). Consistent with the F23.1 staining data (Fig. 2), the amount of immunoprecipitated phosphoproteins was lower for mutant cells than for WT cells. Importantly, for both transfectants the ratio of pp23 to pp21 forms of TCR-ζ was similar (Fig. 6). Taken together, the data indicate that the αS82N mutation and the resultant additional N-linked carbohydrate do not qualitatively affect the TCR-proximal phosphorylation events following antigenic stimulation.

**Analyses of the transfectants using MBP1–11[4Y]:I-Au tetramers**

To further investigate the characteristics of the mutated TCR, flow cytometric analyses were conducted with fluorescently labeled MBP1–11[4Y]:I-Au tetramers. These tetramers specifically stain MBP1–11[4Y]:I-Au-responsive T cells and activate cognate T cells to secrete IL-2 when used in T cell stimulation assays.9 Levels of staining were increased when the cells were coincubated with the anti-CD3ε Ab, 145-2C11 (see Footnote 9). This enhanced staining is most likely due to the ability of this Ab to cross-link TCRs and is consistent with the observations for tetramer staining of T cell hybridomas (61). This Ab was therefore used in all flow cytometry experiments with tetramers. The tetramers stained MUT-2 poorly relative to WT-3 cells, whereas staining of the transfectants with the anti-Cβ Ab H57-597 showed only minor differences that are consistent with the F23.1 staining data (Fig. 2). There are two possible explanations for the poor tetramer staining of MUT-2 cells. First, from studies in other peptide:MHC class II systems (62, 63) tetramer staining has been shown to correlate with TCR:ligand affinity, and the mutation may therefore result in a reduced affinity of the TCR for cognate ligand. However, the similarity of MUT-2 and WT-3 in dose responses to recombinant MBP1–11[4Y]:I-Au complexes (Figs. 3 and 4) would make this seem improbable. Second, the mutation may prevent the aggregation of the αS82N TCR into a configuration that allows multivalent binding of the tetrameric complexes. The former possibility was investigated further by analyzing transfectants bearing a mutated derivative of the 1934.4 TCR in which mutation of glutamic acid 69 to alanine (E69A) results in a significant reduction in Ag responsiveness (59).
anti-Cβ (H57-597) or tetramers. IL-2 levels produced by the transfectants in response to plate-bound MBP1–11[4Y]:I-A<sup>α</sup> complexes were also analyzed (Fig. 7C) and for the WT-3 and MUT-2 cells are reminiscent of the data shown in Fig. 4. Similar results were obtained using biotinylated MBP1–11[4Y]:I-A<sup>α</sup> immobilized on streptavidin-coated plates (not shown). As expected from our

FIGURE 4. Analysis of IL-2 secretion and AICD in the T cell transfectants after stimulation with recombinant MBP1–11[4Y]:I-A<sup>α</sup> molecules. A, Cells (5 × 10<sup>4</sup>/well) were incubated for 24 h with different concentrations of the recombinant molecules coated onto 96-well plates. Supernatants were analyzed for IL-2 levels using an IL-2 ELISA for WT-3 (△) and MUT-2 cells (●). AICD was analyzed by staining the cells with PI and was expressed as the percentage of dead cells (□, WT-3 cells; ■, MUT-2 cells). Values for IL-2 levels are the means of triplicates. B, Annexin V-FITC staining of WT-3 and MUT-2 cells after 6 h of incubation with 1 μg/ml of plate-bound MBP1–11[4Y]:I-A<sup>α</sup> complexes. Cells double positive for PI and annexin V-FITC (15% for WT-3 and 5% for MUT-2) were gated out. Annexin V staining of stimulated (thick lines) and unstimulated (thin lines) cells is shown. C, Analysis of AICD following 24-h incubation with 10 ng/ml PMA and 500 ng/ml of the calcium ionophore A23187. The percentage of PI-positive cells in the absence of stimulation was 13% for WT-3 cells and 22% for MUT-2 cells. Data shown in each panel are representative of at least two independent experiments.

FIGURE 5. TCR down-regulation following stimulation of WT-3 and MUT-2 cells. Cells were treated with 1 μg/ml plate-bound MBP1–11[4Y]:I-A<sup>α</sup> complexes (A) or 10 ng/ml PMA plus 500 ng/ml calcium ionophore (A23187; B). After a 2-h incubation, surface TCR levels were assessed by staining with F23.1 (anti-Vβ8) followed by FITC-labeled anti-mouse IgG or with FITC-labeled H57-597 (anti-Cβ). Data are expressed as the percent TCR, with 100% representing the expression levels for cells treated with PBS only. Data are representative of three independent experiments.
The present study involves analysis of the role of the region encompassing the c’ strand (residues 54–60) of the TCR Vα domain in T cell recognition and signaling. Although earlier crystallographic studies suggested a role for this region of the TCR in dimerization, more recent structural data have questioned the validity of this model (37–42, 44). However, the unusual nature of this surface, which is distinct from the corresponding region in the majority of Vβ domains analyzed to date (34; reviewed in Ref. 43), prompted us to investigate a possible role in T cell function. A potential glycosylation site was therefore introduced at residue 82, which is in close proximity to the c’ strand and is distal to the putative Ag binding site (Fig. 1). Glycosylation at this site would be predicted to introduce a protrusion in this region of the α-chain, and biochemical data indicate the presence of additional carbohydrate in the mutated TCR α-chain.

The activity of T cell transfectants carrying the mutated α-chain together with a WT β-chain was compared with the activity of those expressing the WT TCR. The effects on IL-2 secretion, apoptosis, and TCR down-regulation following stimulation were analyzed. The data show that the αS82N mutation has a minor, if any, effect on IL-2 secretion by the T cells in response to either Ab-mediated cross-linking or cognate ligand. Significantly, this demonstrates that modification of the TCR α-chain did not affect the ability of the TCR to recognize cognate peptide-MHC complexes. Thus, insertion of the N-linked carbohydrate in this region of the TCR did not affect the signaling cascade that resulted in IL-2 secretion. However, the mutation significantly affected AICD and TCR down-regulation, and this provides a functional separation between TCR internalization or AICD and IL-2 production. Earlier studies involving analysis of T cells expressing TCR β-chains mutated in the transmembrane region (65, 66) or CD3γ,δ/TCR α-chains with truncations in their cytoplasmic tail (67, 68) resulted in T cells that, although not affected in cytokine secretion, were defective in either apoptosis or down-regulation, respectively. Therefore, the current study is distinct in that it shows functional defects in both AICD and internalization due to an alteration in a membrane-distal region, i.e., the Vα domain, of the TCR. Furthermore, in contrast to the studies involving the mutated TCR β-chain (65), c’-chain phosphorylation following activation appears to be normal in MUT-2 cells.

TCR down-regulation following ligand recognition is believed to be a consequence of serial engagement of multiple TCRs that results in sustained T cell activation (69) and has been shown to play a role in the regulation of T cell responses (70–72). However, analyses of TCRs bearing an α-chain, CD3δ, or CD3γ with cytoplasmic tail truncations indicate that down-regulation is not essential for cytokine production (67, 68), and this is consistent with our observations. The molecular mechanism(s) of internalization is not fully understood, and both tyrosine and serine protein kinases have been implicated in the process of down-regulation (73). Furthermore, because ligation of a TCR with a peptide:MHC complex or with anti-TCR Abs also induces activation of protein kinase C, it was assumed previously that TCR internalization in response to PMA stimulation and TCR triggering might follow similar mechanisms. However, more recent data suggest that this might not be the case (67, 74, 75). For example, phosphorylation of serine 126 in the CD3γ cytoplasmic tail, which is required for PMA-induced down-regulation, does not appear to be necessary for ligand-induced TCR internalization (75). Furthermore, TCRs internalized following PMA down-regulation are recycled, whereas agonist-induced down-regulation results in lysosomal degradation (74, 76).

In the current study insertion of an N-linked carbohydrate in the proximity of the c” strand of the α-chain affected both PMA-induced and peptide:MHC-mediated down-regulation, suggesting that this structural modification affected the recruitment or activation of a common machinery that is used in response to the mechanistically distinct stimuli. For example, the modification may affect the recruitment/activation of Rab5 GTPase and/or of clathrin-coated vesicle adaptor proteins that are known to be involved in TCR internalization (72, 75, 77, 78). In this context, clathrin assembles with membrane-associated adaptor proteins into a polyhedral lattice to bring about receptor internalization (79, 80). It is therefore possible that the additional glycosylation might not prevent the mutated TCR-CD3 complexes to configure into aggregates that are permissive for lattice formation.

A possible explanation for the observed phenotype of cells expressing the αS82N mutant TCR is that the protrusion induced by
glycosylation affects the association and/or activity of two or more distinct cellular components that are exclusively involved in two bifurcating processes (AICD and down-regulation) that do not involve any common intermediates. This is consistent with the effects of PMA and ionomycin on the transfectants; although the difference in down-regulation is still observed following treatment with these stimuli, apoptosis is induced to similar extents in both WT-3 and MUT-2 cells. The marked reduction in tetramer (MBP1–11[4Y]:I-Au) staining by the mutant transfectants indicates that the additional carbohydrate severely limits aggregation of the TCRs into a configuration that allows multivalent binding by the tetramer. Whether this configuration is the same as that required for association of the proteins involved in AICD and/or down-regulation is not clear from the current experiments. However, it is probable that the loss of tetramer binding is a manifestation of the steric effects of the additional N-linked carbohydrate, and these effects may also impact on alternative configurations of the TCR and associated proteins necessary for either down-regulation or programmed cell death. Consistent with a role for the architecture of the TCR and associated proteins in programmed cell death, a recent study has demonstrated that altered peptide ligands that induce focal aggregation/capping of the TCR also induce apoptosis (81). Significantly, capping analyses indicate that the mutated TCR is able to aggregate following Ab-mediated cross-linking, demonstrating that this modified TCR retains the ability to undergo surface redistribution.

Recent studies indicate that there might be a redundancy in the TCR-proximal signaling mechanisms operative during down-regulation of the TCR and AICD. For example, constitutive p56lck activation targets TCRs to lysosomal compartments (82), and this kinase has been suggested to play a critical role during cell death in T cells by up-regulating FasL expression (83, 84). Evidence supports involvement of the transcription factors NF-kB and NF-AT in regulating Fas ligand expression (85–87), but the unaffected IL-2 secretion by MUT-2 cells suggests that these factors are functionally intact. However, it has recently been shown that cytokine secretion and AICD are differentially sensitive to NF-kB deprivation (88). Thus, it is possible that while a signaling competent configuration with respect to IL-2 secretion is induced by cognate ligand in the mutant transfectants, the configuration for...
p56^la^ to optimally phosphorylate substrates to levels necessary for apoptosis and TCR internalization does not form. These substrates may be as yet unidentified and may be distinct for the two processes. This (partial) defect in p56^la^ activity could be due to either sequestration of the putative substrates and/or inappropriate orientation of p56^la^. By analogy, proximity and spatial orientation of the tyrosine kinase ZAP-70 have been shown to be key parameters in T cell signaling (89).

In conclusion, the results indicate that following stimulation, a specific configuration of the TCR complex is needed for AICD and receptor internalization that is distinct from that needed for IL-2 production. The generation of this configuration appears to correlate with tetrmeric ligand binding and involves a region of the TCR V_{alpha} domain that, due to a specific strand rearrangement, has a flatter surface than the corresponding surface in Ig V domains and the majority of TCR V_{beta} domains analyzed to date. The resulting complex may form an optimal module necessary for recruitment of interaction with the molecular machinery critical for TCR down-regulation and AICD. The transfectants described in this study could provide a useful system to dissect mechanisms of T cell apoptosis and TCR internalization, and this may lead to an improved understanding of the requirements for a particular higher order configuration of the TCR and associated polypeptides in the two processes. Finally, the resistance of the transfectants bearing the mutated TCR to programmed cell death suggests that our studies are of relevance to diseases involving dysregulated T cell homeostasis, such as neoplasia and autoimmunity.

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