International Immunology, Vol. 11, No. 6, pp. 967–977

Characterization of the interaction of a TCR α chain variable domain with MHC II I-A molecules

Ayub Qadri[1,2], Jayant Thatte[1,3], Caius G. Radu[1], Bertram Ober[1,4] and E. Sally Ward[1]

[1]Center for Immunology and Cancer Immunobiology Center, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235-8576, USA

[2]Present address: National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi 110067, India
[3]Present address: Department of Pathology, University of Virginia, Charlottesville, VA 22908, USA
[4]Present address: Gwen Knapp Center for Lupus and Immunology Research, University of Chicago, 924 East 57th Street, 4th Floor, Chicago, IL 60637-5420, USA

Keywords: fourth hypervariable region, immunosuppression, MHC class II, TCR, V_α domain

Abstract

The αβ TCR recognizes peptides bound to MHC molecules. In the present study, we analyzed the interaction of a soluble TCR α chain variable domain (V_α4.2–J_α40; abbreviated to V_α4.2) with the MHC class II molecule I-A^u. V_α4.2 bound specifically to I-A^u expressed on the surface of a transfected thymoma cell line. Modifications in the amino acid residues located within the three complementarity-determining regions (CDRs) of the V_α domain did not markedly affect this interaction. However, mutation of glutamic acid to alanine at position 69 of the fourth hypervariable region (HV4α) significantly increased the binding. Antibody inhibition studies suggested that the binding site was partly contributed by a region of the β chain of I-A^u. Furthermore, the binding of V_α4.2 to the MHC molecule was dependent on the nature of the peptide bound in the groove. Soluble V_α4.2 specifically inhibited the activation of TCR transfectants by I-A^u-expressing cells pulsed with an N-terminal peptide of myelin basic protein. V_α4.2 also bound to MHC class II-expressing spleen cell populations from mice of the H-2^u and H-2^d haplotypes. The binding of V_α4.2 to I-A molecules might explain the immunoregulatory effects reported previously for TCR α chains. This V_α4.2 interaction may also be relevant to models of antigen presentation involving the binding of intact proteins to MHC class II molecules followed by their processing to generate epitopes suitable for T cell recognition.

Introduction

The structure of a TCR resembles that of an Ig (1). However, unlike Ig, the TCR recognizes its ligand as a small peptide fragment bound to the groove of an MHC molecule (2,3). The interaction between antibodies and cognate antigens has been extensively studied by X-ray crystallography (4,5). These studies have demonstrated that the recognition of an antigen by an antibody molecule is primarily mediated by the hypervariable regions referred to as complementarity-determining regions (CDRs). In contrast to antibodies, the structural basis of peptide–MHC recognition by the TCR has not been analyzed in such extensive detail, primarily because until recently it was not possible to produce soluble and functional TCR molecules in sufficient amounts for crystallographic studies. The crystal structures of MHC class I and class II with peptides bound to the groove have provided structural information concerning the TCR ligand (6–12). More recently, crystal structures of a TCR V_α domain (13), a TCR β chain (14), a TCRβ–staphylococcal enterotoxin B (SEB) complex (15), several TCRαβ–peptide–MHC class I complexes (16–19) and TCR αβ heterodimers (20,21) have been reported. The 3-D structures of the ternary complexes confirmed the involvement of Ig-like CDRs in ligand binding. Consistent with the structures, the CDR1 and CDR2 of the TCR α chain have also been shown to control MHC restriction (22). The high-resolution crystal structure of a human TCR–MHC class I–peptide complex demonstrated, unexpectedly, that β chain CDR 1

Correspondence to: E. S. Ward

Transmitting editor: C. Martinez-A

Received 13 December 1998, *accepted* 2 March 1999

and 2 have limited, if any, contact with peptide–MHC ligand (17), but this appears not to be the case for other tripartite complexes (18,19). Although the 3-D structures of these trimolecular complexes have provided a view of the TCR–peptide–MHC class I interaction which is most likely general for class I-restricted TCRs, whether this configuration also holds for class II-restricted TCRs awaits the solution of structure(s) of TCR–peptide–MHC class II complex(es). However, experimental support for a similar orientation for the latter has been obtained by the detailed analysis of antigen recognition by an I-A^k-restricted TCR and arguments for the generality of this configuration have been presented (23,24).

In this communication we describe the interaction between a soluble TCR V_α domain (V_α4.2–J_α40; abbreviated to V_α4.2) and the MHC class II molecules I-A^u and I-A^d. V_α4.2 was isolated from the 1934.4 T cell hybridoma (25) that recognizes the N-terminal nonapeptide (or 11mer) of myelin basic protein (MBP) complexed with I-A^u and the X-ray structure of this V_α domain is known (13). The data demonstrate specific binding of V_α4.2 to I-A^u and I-A^d molecules, but not to I-A^q. Unexpectedly, CDR1, 2 and 3 residues of V_α4.2 do not play a significant role in binding to I-A^u, in contrast to a central residue of the fourth hypervariable loop. Thus, the mode of binding is distinct from that expected for a V_α domain in the context of a TCR $\alpha\beta$ heterodimer. The binding of soluble V_α4.2 to I-A^u may have relevance to the reported immunosuppressive functions of TCR α chains (26–28). This interaction may also be representative of an antigen-presenting pathway in which intact, folded protein antigens act as ligands for the initial interaction with recycling, cell surface MHC class II molecules (29,30).

Methods

Cell lines, antibodies and peptides

The I-A^u-expressing B cell line, PL-8 (31), and I-A^u -restricted T cell hybridoma, 1934.4 (25), were generously provided by Dr David Wraith (University of Bristol, UK). The TCR^- thymoma cell line BW5147 and its I-A^u-transfected derivatives, Utm6.15, Utm34a.1 and U400 (32,33), were kindly made available by Dr Harden McConnell (Stanford University, Palo Alto, CA). T cell lines expressing the 1934.4 TCR were made by transfecting the TCR genes into a $CD4^-$, TCR^- cell line $58\alpha^-\beta^-$ (34), kindly provided by Dr Stephen Hedrick (San Diego, CA) with permission from Dr Bernard Malissen (INSERM-CNRS, Marseille-Luminy, France), using the α and β shuttle vectors (35) kindly provided by Dr Mark Davis (Stanford University School of Medicine, Palo Alto, CA). T cell transfectants were made as described elsewhere (36). TCR expression was analyzed by indirect immunofluorescence using the anti-V_β8 mAb F23.1 (37), a generous gift from Drs John Kappler and Philippa Marrack (University of Colorado Health Science Center, Denver, CO).

Hybridomas HB183 and TIB93 secreting anti-I-A mAb Y3P (38) and 10.2.16 (39), respectively were obtained from ATCC (Rockville, MD). mAb 10.3.6 (39) was kindly provided by Dr David Wraith. Anti-polyhistidine mAb was obtained from Sigma (St Louis, MO). FITC-labeled anti-mouse Ig and FITC–streptavidin were from Cappel. The N-terminal peptide (Ac1-11; acetylated at position 1) of rat MBP and an analog in which the wild-type lysine at position 4 is substituted by tyrosine (Ac1-11[4Y]) were synthesized at the Peptide Synthesis Unit of the Howard Hughes Medical Institute, UT Southwestern Medical Center, Dallas, TX.

Expression of TCR V_α4.2 and its mutants

TCR V_α4.2 was produced in a bacterial expression system as described previously (40) and V_α4.2 purified by affinity chromatography on Ni^{2+}-NTA agarose (Qiagen, Germany) as described (40). V_α4.2 produced using this approach was used previously for X-ray crystallography (13) and is therefore correctly folded.

Mutagenesis of residues encompassing CDR1, 2 and 3, and the fourth hypervariable region (HV4) were carried out as described by Kunkel (41). Four different derivatives of V_α4.2 were produced: one in which CDR1 residues were mutated, the second in which CDR2 residues were mutated and the third with amino acid residues in all three CDRs mutated. A fourth derivative with glutamic acid at position 69 of HV4 mutated to alanine was also produced by the same strategy. The oligonucleotides (synthesized on an Applied Biosystems DNA synthesizer) used were: 5′-CCA GAA CAG AGC TCC GGC CGC TCC GGC TGA GTA GTT GCA-3′ for mutating CDR1 residues at positions 27, 28, 29 and 30 [Kabat numbering (42)]; 5′-GCT GCT TCC TTT CGA TGC GCC CGC TGA GGC TCT AAA-3′ for mutating CDR2 residues at positions 51, 52, 53 and 54; 5′-AGT TAT TTT CTC AGA TGC ACC GGC TTC ACT CAG AGC-3′ for mutating CDR3 residues at positions 98, 99, 100 and 101; and 5′-AGG TGG CTG CTT TAT TG-3′ for altering HV4 residue 69. The mutations were confirmed by determining the nucleotide sequence using Sequenase version 2.0 kits (USB, Cleveland, Ohio). The mutated derivatives of V_α4.2 were expressed and purified as described above for the wild-type V_α4.2.

Flow cytometric analysis of V_α4.2 binding

The interaction of V_α4.2 with I-A-expressing cells (*in vitro* cell lines, splenocytes or thymocytes) was analyzed by flow cytometry. Briefly, cells (3×10^5 cells/well in a 200 µl volume) were incubated with varying concentrations of V_α4.2 at 37°C for 1 h. After washing with PBS containing 1% BSA, cells were incubated for 1 h at 4°C with anti-polyhistidine mAb (to detect the V_α domain with a C-terminal polyhistidine tag) diluted in PBS/BSA. Subsequently cells were washed and incubated with FITC-labeled anti-mouse Ig for 1 h at 4°C. Controls were incubated only with anti-polyhistidine antibody and FITC–anti-mouse Ig. Cells were washed with PBS/BSA, suspended in PBS/BSA and analyzed in a flow cytometer (Becton Dickinson, Mountain View, CA). In some assays, biotinylated V_α4.2 was employed for binding studies [V_α was biotinylated as described by Meier *et al.* (43)] and FITC-labeled streptavidin was used to detect binding. Binding studies were also carried out in the presence of anti-I-A antibodies or I-A^u-binding peptides. For these experiments, V_α was co-incubated with the cells and the antibodies or the peptides. The remainder of the procedure was as described above.

In vitro T cell stimulation assays

The effect of V_α4.2 binding to I-A^u on peptide–MHC recognition by specific T cells was analyzed *in vitro*. T cell transfectants expressing the 1934.4 TCR were stimulated *in vitro* with MBP peptide Ac1-11 in the presence of varying concentrations of V_α4.2 or, as a control, a V_α domain (V_α11–J_α17; abbreviated to V_α11) derived from an irrelevant TCR designated qcII85.33 (44). Utm6.15, an I-A^u-transfected BW5147 thymoma cell line (32,33), or PL-8, a B cell lymphoblastoid line expressing I-A^u (31), were employed as antigen-presenting cells (APC). At 24 h post-stimulation, culture supernatants were collected and analyzed for IL-2 levels using an IL-2-dependent cell line CTLL-2 as described (45). Data shown for T cell stimulation assays represents the means of triplicate measurements with errors ranging from 5 to 10%.

Surface plasmon resonance (SPR) experiments

SPR experiments were carried out using a BIAcore 2000. Flow cells of CM5 chips were coupled with either V_α4.2 or, as a reference cell, D1.3 antibody (46) using amine coupling chemistry. Densities of coupling were 1112 and 1773 RU (two flow cells for V_α4.2) and 2752 RU (D1.3 antibody). Ac1-11 or its analog, Ac1-11[4Y], were injected over the flow cells using programmed methods and the BIAcore control software. Flow rates of 5 or 80 μl/min were used at a temperature of 25°C. Ac1-11 or Ac1-11[4Y] were injected at a concentration of 200 μg/ml in PBS, pH 7.2, plus 0.01% Tween 20. All injections were carried out in triplicates to ensure reproducibility. For data processing, the BIAevaluation 2.1 software was used. Data was zero adjusted and reference (D1.3 antibody coupled) flow cell data subtracted from V_α4.2 flow cell data. Similar results were obtained when a 'blank' flow cell (i.e. treated with coupling buffer only during coupling cycle) was used. In addition, the presence of immobilized V_α4.2 was verified by injecting a 1:1000 dilution of anti-polyhistidine antibody (Sigma) over the sensor chip surface.

Results and discussion

The TCR α chain variable domain (V_α4.2) binds to cell surface MHC class II molecules

In the present study, we analyzed the interaction of a TCR α chain with MHC class II by examining the binding of a soluble TCR α chain variable domain (V_α4.2) to I-A^u. TCR V_α4.2 is one of the predominant α chains expressed by the TCR of encephalitogenic T cells isolated from PL/J mice (H-2^u) following induction of experimental autoimmune encephalomyelitis (47). These autoreactive T cells recognize the immunodominant epitope Ac1-11 of MBP (48). To study the binding of V_α4.2 to I-A^u, two different types of I-A^u -expressing cell lines were employed: PL-8 [a B cell line, derived by fusing LPS-activated splenocytes from H-2^u mice with the M-12.C3 B lymphoblast line (31)] and BW5147 thymoma derived cell lines transfected with I-A^u (Utm6.15, Utm34a.1 and U400). Utm6.15 and Utm34a.1 express membrane-bound I-A^u, and U400 has GPI-linked I-A^u (33). V_α4.2 was expressed in *Escherichia coli* in a form previously used for X-ray crystallography (13) and the binding analyzed by indirect immunofluorescence using flow cytometry. In the absence of an antibody against TCR V_α4.2, a mAb directed against the polyhistidine tag present at the C-terminus of the V_α domain was employed to detect binding. V_α4.2 showed a dose-dependent binding at 37°C to Utm6.15 which expresses high levels of I-A^u (Fig. 1a). Untransfected BW5147 cells did not show any significant binding, demonstrating the specificity of interaction with I-A^u expressed by the transfectants (Fig. 1b). Moreover, BW5147 transfectants Utm34a.1 and U400, expressing lower levels of surface I-A^u (assessed by anti-I-A antibody, Y3P, binding), showed reduced binding of V_α4.2 (Fig. 1c and d). The specificity of this interaction was also demonstrated by the almost undetectable binding of soluble V_α11–J_α17 [V_α11; derived from the qcII85.33 hybridoma (44)] to Utm6.15 (Fig. 1e). The secondary structure of V_α4.2 after incubation at 37°C was similar to the native molecule (40) as revealed by CD spectroscopy (data not shown). The V_α4.2–I-A^u interaction therefore appears to be distinct from the association of partially folded polypeptides with MHC class II molecules that has been reported for both invariant chain -positive (49–53) and -negative cell lines (54). However, it cannot be excluded that following initial binding of V_α4.2 to I-A^u, some unfolding that is favored by V_α4.2–I-A^u interaction occurs.

The strict correlation between the levels of surface I-A^u and the extent of binding seen with BW5147 transfectants did not, however, hold true for the B cell line PL-8. V_α4.2 bound at much lower levels to PL-8 relative to Utm6.15 although both cell lines expressed comparable levels of surface I-A^u (Fig. 2a and b). This was not due to increased internalization of I-A^u by PL-8 cells as similar results were obtained with paraformaldehyde-fixed cells (data not shown). The levels of surface I-A^u were determined by flow cytometry with three different anti-I-A mAb, Y3P, 10.2.16 and 10.3.6. There was no significant difference between PL-8 and Utm6.15 in binding to the three mAb (data not shown), suggesting that there were no major conformational differences between I-A^u molecules present on the two cell lines although this does not exclude the presence of subtle differences. Utm6.15 was also more effective at activating peptide (MBP-derived N-terminal peptide, Ac1-11)-specific 1934.4 hybridoma cells (Fig. 2c) although it did not differ significantly from PL-8 in its ability to bind a biotinylated high-affinity analog of Ac1-11 (Ac1-11[4Y]; data not shown), which due to replacement of the position 4 lysine of wild-type Ac1-11 by tyrosine has higher affinity for binding to I-A^u (55). However, for these peptide binding studies with the PL-8 line the degree of MHC class II-specific binding of Ac1-11[4Y] cannot be determined with certainty since I-A^u -negative PL-8 cells are not available. This is clearly not the case for Utm6.15 as untransfected BW5147 cells serve as a negative control.

The differences in binding of PL-8 and BW5147 transfectants to V_α4.2 and in activating specific T cells are possibly due to differences in the peptide repertoires loaded on the I-A^u molecules from the two cell types. A difference in the peptide repertoires of the two cell lines was also suggested by the inability of Utm6.15 to stimulate an alloreactive T cell hybridoma [qcII85.33 (44)] which PL-8 activated very efficiently (data not shown). Furthermore, Utm6.15 cells were less potent than PL-8 in mediating SEB-induced T cell activation (Fig. 2d) and antigenic peptides have been shown to

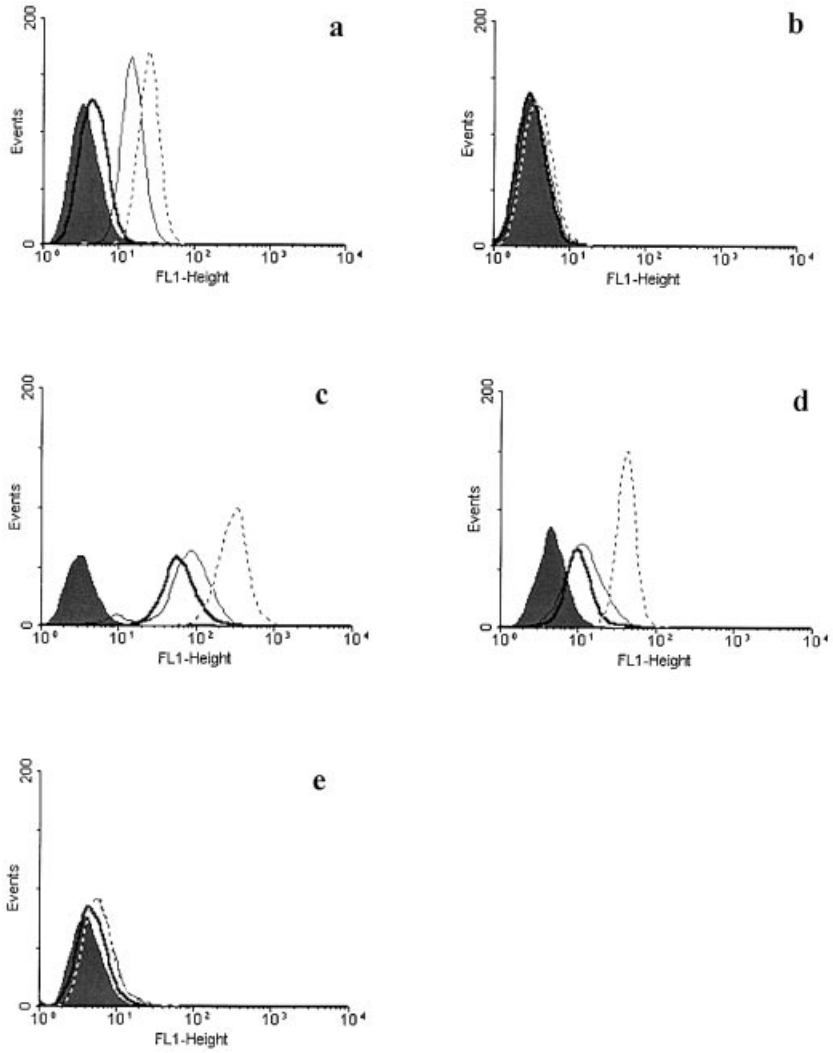

Fig. 1. Binding of V_α4.2 to (a) Utm6.15 and (b) the parent I-A^u negative cell line. Cells were incubated at 37°C for 1 h with varying concentrations of V_α4.2, followed by anti-polyhistidine antibodies and FITC–anti-mouse Ig. Controls (shaded curve) were treated identically except that V_α4.2 was replaced with 1% BSA. Concentrations of V_α4.2 were 1.15 (thick line), 11.5 (thin line) and 23 (dashed line) μg/ml. (c and d) Analysis of effect of I-A^u levels on V_α4.2 binding to I-A^u. BW5147 (shaded) and the transfectants expressing I-A^u Utm34a.1 (thick line), U400 (thin line) and Utm6.15 (dashed line) were incubated with (c) anti-class II antibody Y3P (100 ng/ml) followed by FITC-anti-mouse Ig or (d) V_α4.2 (57.5 μg/ml) for 1 h followed by anti-polyhistidine and FITC-labeled anti-mouse Ig. (e) Binding of V_α11 to Utm6.15 carried out as outlined in (a). Shaded curve represents the control. Concentrations of V_α11 were 1.15 (thick line), 11.5 (thin line) and 23 (dashed line) μg/ml.

affect the efficiency of presentation of bacterial superantigens (56–58).

The binding of V_α4.2 to I-A^u on the surface of splenocytes from PL/J mice was also investigated. Significant levels of binding to I-A^u-positive splenocytes relative to H-2^u thymocytes was observed (Fig. 3a). This demonstrates that the interaction on PL-8 and Utm6.15 is not an artifact of using *in vitro* cell lines. Binding was also observed with *ex vivo* splenocytes but not thymocytes derived from Swiss or BALB/c mice (both H-2^d haplotype; data shown only for Swiss mice, Fig. 3b). V_α4.2 did not, however, interact with a B cell-derived transfectant expressing I-A^q (data not shown) nor did it show significant MHC class II-specific binding to splenocytes from DBA1/Lac.J mice (H-2^q haplotype; Fig. 3c). The interaction therefore shows specificity for certain I-A haplotypes.

The putative V_α4.2 interaction site involves the β chain of I-A^u

The demonstration of V_α4.2 binding to I-A^u offers a system to investigate which MHC class II chain the V_α domain interacts with. This was carried out by analyzing binding of V_α4.2 to I-A^u in the presence of antibodies to MHC class II. Two of these, 10.2.16 and 10.3.6, are directed against the β chain while the third (Y3P) recognizes an α chain-dependent determinant on the MHC class II heterodimer (59). 10.2.16 partially inhibited the binding of V_α to I-A^u (Fig. 4a) suggesting that the interaction was at least partly mediated through residues on the β^u chain. The other two mAb had no significant effect (data shown only for Y3P, binding in presence of 10.3.6 was overlapping with V_α4.2-binding in the absence of any antibody). The three antibodies showed comparable binding to I-A^u on Utm6.15 (data not shown). Our results suggest that

this inhibition may be a result of blockade of the interaction between the β chain of I-A^u and V_α4.2. The data therefore indicate a role for V_α4.2–I-A^u β chain contacts in the interaction and this is supported by our observation that the binding was not affected by another MHC class II ligand, SEB (data not shown), which is believed to interact with the α chain of I-A molecules (60). However, these data do not exclude the possibility of an interaction of V_α4.2 with other regions of the I-A^u molecule distinct from the recognition sites for 10.3.6, Y3P or SEB. Interestingly, the importance of the 10.2.16-binding site in the 1934.4 TCR αβ–peptide–MHC interaction was also indicated by more efficient blockade of 1934.4 T cell recognition of Ac1-11–I-A^u (on both PL-8 and Utm6.15 cells) by this antibody relative to Y3P (Fig. 4b), although both antibodies bound equally well to I-A^u on PL-8 and Utm6.15 cells. 10.2.16 does not recognize I-A^q and this might be one of the reasons for the lack of V_α4.2 binding to this MHC class II haplotype.

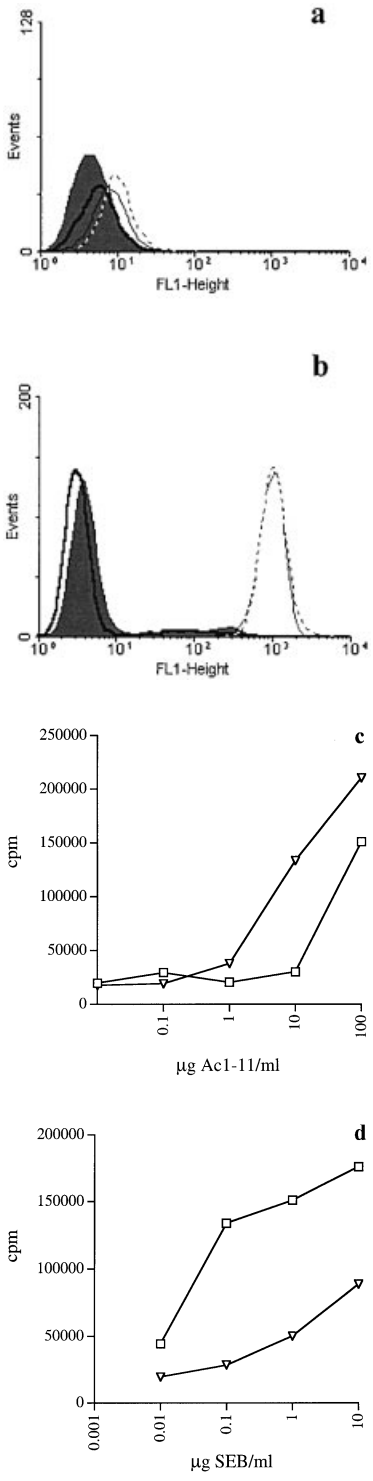

Fig. 2. (a) Interaction of V_α4.2 with I-A^u-expressing B cell line PL-8. The assay was carried out as described in Fig. 1(a). Concentrations of V_α4.2 were 11.5 (thick line), 23 (thin line) and 57.5 (dashed line) μg/ml. (b) I-A^u levels on PL-8 (solid line) and Utm6.15 (dashed line) determined by Y3P binding (assay described in Fig. 1). Shaded curve and bold line represent binding of PL-8 and Utm6.15 respectively with isotype-matched control antibody. (c) Activation of Ac1-11-specific T cell hybridoma 1934.4 by the two cell lines PL-8 and Utm6.15. 1934.4 cells were incubated with varying concentrations of Ac1-11 in the presence of PL-8 or Utm6.15 as APC and after 24 h, culture supernatants were analyzed for the levels of IL-2 by measuring proliferation of an IL-2-dependent cell line CTLL-2. □ PL-8; ▽, Utm6.15. (d) presentation of SEB to 1934.4 hybridoma cells by the two cell lines PL-8 and Utm6.15. The assay was carried out as described in (c) with the exception of using SEB instead of Ac1-11. □ PL-8; ▽, Utm6.15. All values are averages of triplicates with errors between 5 and 10%.

Effect of antigenic peptide on the V_α4.2–I-A^u interaction

The interaction site for V_α on I-A^u was further probed by analyzing binding in the presence of a known I-A^u -binding peptide. The N-terminal peptide Ac1-11 of MBP was chosen for this purpose because it is an encephalitogenic immunodominant peptide in H-2^u mice recognized in association with I-A^u by T cells which predominantly express V_α4 or V_α2 (47). Perhaps unexpectedly, there was a dose-dependent reduction in the binding of V_α4.2 to I-A^u in the presence of Ac1-11 (Fig. 5a). The effect was more pronounced when a high affinity derivative of this peptide, Ac1-11[4Y], was co-incubated with V_α4.2 (Fig. 5b). An unrelated peptide (derived from bovine type II collagen) that does not bind to I-A^u did not have any effect (Fig. 5c). To exclude the possibility that the inhibitory effects of Ac1-11 or Ac1-11[4Y] were due to binding of V_α4.2 to these peptides, SPR experiments were carried out. These analyses demonstrated that binding of Ac1-11 or Ac1-11[4Y] at a concentration of 200 μg/ml to immobilized V_α4.2 was not detectable under the conditions of the SPR experiments (Fig. 6).

The peptide inhibition data suggest that the I-A^u residues involved in binding to soluble V_α4.2 are located close to or overlapping with the peptide-binding groove of I-A^u. However, rather than competition between V_α4.2 and the peptides for binding to the empty groove, we speculate that it is the differential ability of V_α4.2 to displace peptides of different affinities from the groove that results in the affinity-dependent modulation of V_α4.2 binding to I-A^u by the MBP peptides. Studies carried out previously with I-A^u molecules purified from the same BW5147 transfectants used in the current

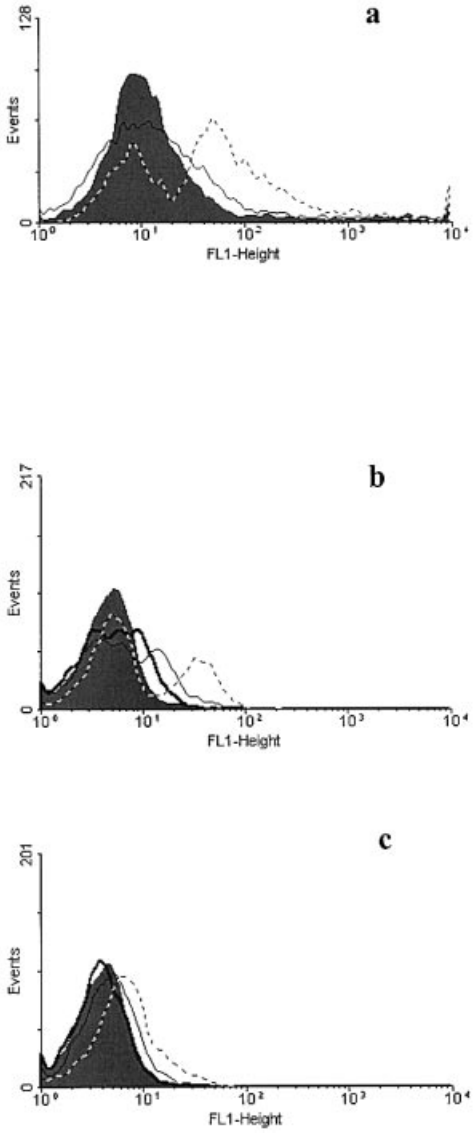

Fig. 3. Binding of V_α4.2 to spleen cells and thymocytes isolated from (a) PL/J (H-2^u) mice, (b) Swiss (H-2^d) mice or (c) DBA/LacJ (H-2^q) mice. Spleen cells were treated with 0.9% ammonium chloride solution to lyse red blood cells and incubated at 37°C for 1 h in some experiments to remove the adherent cell population. Cells were incubated with varying concentrations of biotinylated V_α4.2 at 37°C for 1 h followed by FITC-labeled streptavidin. Shaded curve represents binding of V_α4.2 (100 μg/ml) to thymocytes, and for (a) binding of V_α4.2 at 25 (solid line) and 75 (dashed line) μg/ml, and (b and c) binding of V_α4.2 at 10 (thick line), 25 (thin line) and 100 (dashed line) μg/ml to spleen cells are shown. Binding to BALB/c splenocytes was the same as to Swiss splenocytes.

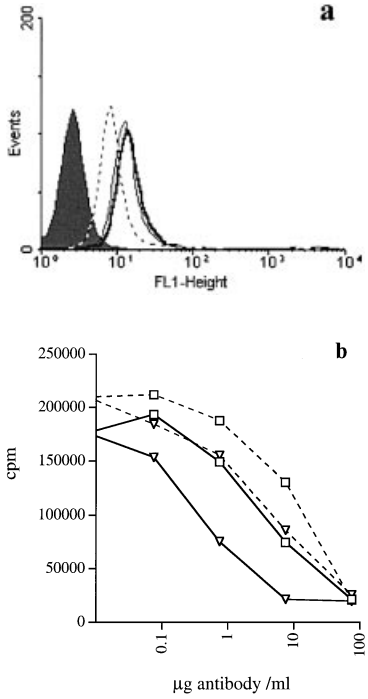

Fig. 4. (a) Localization of putative binding site for V_α4.2 on I-A^u. Utm6.15 cells were incubated with biotinylated V_α4.2 (17.5 μg/ml) in the presence of different anti-I-A mAb (20 μg/ml) followed by FITC-labeled streptavidin. Shaded curve represents staining of cells incubated only with streptavidin–FITC. Staining with biotinylated V_α4.2 (thick line), staining in the presence of Y3P (thin line) and 10.2.16 (dashed line) are shown. (b) Effect of anti-MHC class II antibodies on Ac1-11 presentation to 1934.4 T cell hybridoma cells by the two cell lines PL-8 (solid line) and Utm6.15 (dashed line). The assay was carried out as described in Fig. 2(c) in the presence of varying concentrations of the antibodies Y3P (□) and 10.2.16 (▽). The c.p.m. values in the absence of any antibody were 176,212 and 196,976 respectively, for PL-8 and Utm6.15 as APC. All values are averages of triplicates with errors between 5 and 10%.

analyses (32,33) suggest that the large majority of these molecules are occupied by peptide. Therefore, the interaction reported here is unlikely to be due to binding of V_α4.2 to empty I-A^u molecules on Utm6.15. A major role for empty molecules in the V_α4.2–I-A interaction is also excluded by the ability of this V_α domain to bind to *ex vivo* spleen cells from H-2^d and H-2^u mice (Fig. 3). In our model for V_α–I-A interaction, the initial low-affinity binding might resemble the V_α component of the TCRαβ–peptide–MHC interaction and subsequently this V_α would displace peptide from the groove to form a high-affinity, stable complex that is long lived enough to be detectable by flow cytometry. By analogy with the affinities of TCRαβ–peptide–MHC interactions reported (61–65) the affinity of the initial V_α–I-A complex would be expected to be very low and would not be sufficiently stable to be detected under the conditions used in our binding assays. Irrespective of the mechanism of binding, it is clear that the interaction between V_α4.2 and I-A^u/I-A^d is specific and the binding site on I-A^u overlaps with the 10.2.16 interaction site. The interaction is also of sufficiently high affinity to be detected by flow cytometry and V_α4.2 competes with antigenic peptide for binding to the I-A^u groove. These unexpected features prompted us to probe further the molecular nature of the interaction by carrying out mutagenesis of the hypervariable loops of the V_α4.2 domain.

The interaction is not significantly modulated by mutations in the CDR residues; mutation of HV4 residue 69 enhances binding

The role of CDR residues which are known in a number of systems to be involved in the recognition of peptide–MHC

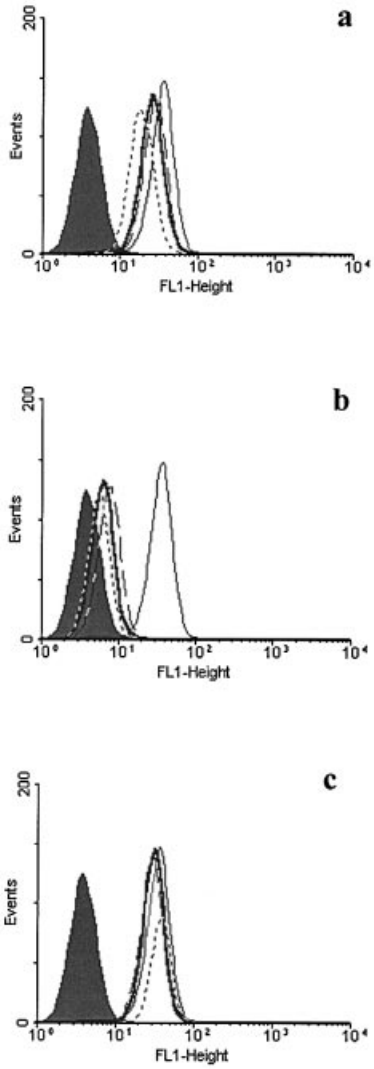

Fig. 5. Influence of peptide bound to the MHC class II groove on V_α4.2-binding to I-A^u. Cells were incubated with V_α4.2 (25 μg/ml) in the presence of varying concentrations of (a) wild-type Ac1-11, (b) a high-affinity analog of the wild-type peptide, Ac1-11[4Y] and (c) an unrelated peptide derived from bovine type II collagen. Binding of V_α4.2 was detected as described in Fig. 1(a). Shaded curve represents the control (no V_α added). Binding of V_α4.2 in the absence (thin line) and presence of 40 μg/ml (long dashed line), 200 μg/ml (thick line) or 1 mg/ml peptide (short dashed line) is shown.

complexes by TCR (16–19,24,66–68) was analyzed for the V_α4.2–I-A^u interaction. For this analysis, the central amino acid residues of CDR1, 2 and 3, identified from the X-ray structure (13), were mutated. Three different derivatives of V_α4.2 were produced: a CDR1 mutant in which serine, glycine, tyrosine and proline at positions 27, 28, 29 and 30 [Kabat numbering (42)] were mutated to glycine, alanine, alanine and glycine respectively; a CDR2 mutant in which arginine, aspartic acid, lysine and glutamic acid at positions 51, 52, 53 and 54 were mutated to alanine, glycine, alanine and serine respectively; and a mutant in which residues in all three CDRs were altered, with CDR3 residues asparagine, tyrosine, glycine and asparagine at positions 98, 99, 100 and 101 modified to alanine, glycine, alanine and serine respectively. A fourth analog was also produced in which glutamic acid at position 69 [Kabat numbering (42)] in HV4 of V_α4.2 (HV4α) was mutated to alanine. When analyzed for binding to I-A^u by flow cytometry, the three V_α4.2 mutants with alterations of CDR residues did not differ markedly from the wild-type V_α4.2 in the extent of interaction with I-A^u. CDR2 and CDR1, 2 and 3 mutants showed slight reductions in binding, indicating a minor role for CDR2 residues in interacting with I-A^u (Fig. 7a). Interestingly, however, modification of glutamic acid to alanine at position 69 (designated E69A) in HV4α significantly increased the binding to I-A^u (Fig. 7b), suggesting an important role for this region of the soluble V_α4.2 domain in the V_α4.2–I-A^u interaction.

The data for the V_α4.2 mutants indicate that the mode of binding observed here is unconventional insofar as a critical role for CDR residues is not observed. The nature of the interaction also appears to be distinct from that described for peptides/proteins which interact with MHC molecules at sites that do not encompass the peptide-binding groove (69,70). Analysis of the HV4α mutant E69A indicates that, in contrast to CDR residues, glutamic acid at position 69 plays a significant role in the binding of soluble V_α4.2 to I-A^u. Interestingly, in a recent study in which V_α4.2 with the E69A mutation was expressed as a TCR αβ heterodimer in transfectants, a reduction in antigen responsiveness was observed (36). Thus, whilst both these analyses indicate involvement of residue 69 of HV4 in recognition of I-A^u, alteration of glutamic acid to alanine appears to result in different consequences depending upon whether the V_α is expressed as a single domain or TCR αβ heterodimer, with the former resulting in enhancement in the binding of soluble V_α to I-A^u and the latter resulting in a TCR with reduced affinity for cognate peptide–I-A^u complexes. This in turn underscores the differences between the two interactions. In addition, V_α4.2 exists as a homodimer in solution (13) and it is currently an open question as to whether both monomers in a dimer are involved in binding to a single I-A^u molecule.

V_α4.2 binding to I-A^u modulates recognition of the peptide–MHC complex by the 1934.4 TCR

The TCR α chain–MHC class II interaction was further studied by analyzing the effect of V_α4.2 binding to I-A^u on Ac1-11–I-A^u recognition by a specific TCR expressed by T cell transfectants (TCR$^-$ 58$\alpha^-\beta^-$ cells transfected with the 1934.4 α and β chain genes containing V_α4.2–J_α40 and V_β8.2–J_β2.3). Activation of peptide-specific T cells (1934.4 TCR transfectants) was investigated in the presence of V_α4.2. This V_α inhibited the recognition of the Ac1-11–I-A^u complex by the 1934.4 TCR transfectants in a dose-dependent fashion (Fig. 8a). The inhibition was seen only for the wild-type peptide and not for the higher affinity derivative, Ac1-11[4Y] (data not shown). The mutated derivative of V_α4.2, E69A, was more effective in blocking Ac1-11–I-A^u recognition by the T cells (Fig. 8b), consistent with the higher affinity of this mutant for I-A^u. In contrast to V_α4.2 and the E69A mutant, addition of soluble V_α11–J_α17 [derived from the qcII85.33 hybridoma (44)] at a concentration of 100 μg/ml did not have a significant effect on the stimulation of 1934.4 T cell transfectants (data not shown), demonstrating the specificity of the inhibitory

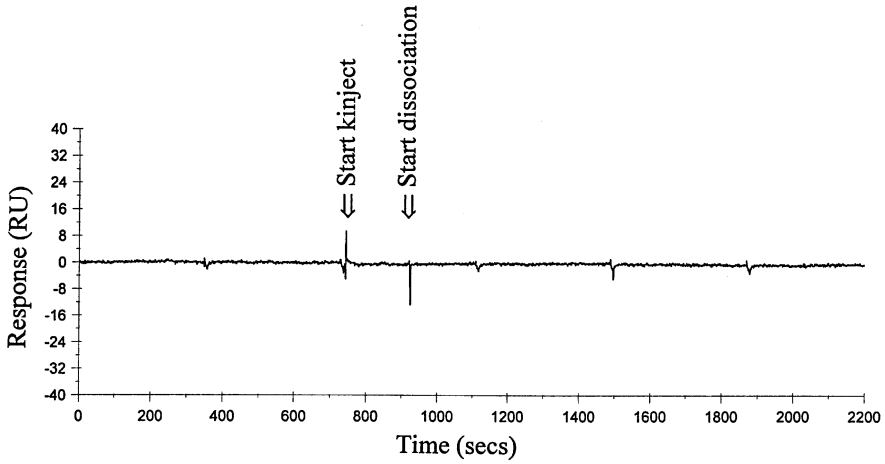

Fig. 6. Analysis of binding of Ac1-11[4Y] to V_α4.2 using SPR. Ac1-11[4Y] (200 μg/ml) was injected (using the kinject command) over flow cells coupled with either V_α4.2 or D1.3 antibody [(46) used as reference surface] as described in Methods. The sensorgram shown was obtained from raw data by zero adjustment and subtraction of the D1.3 antibody flow cell data (i.e. reference flow cell) from the V_α4.2 flow cell data. Arrows indicate the start of the injection and dissociation phase. The data is a representative sensorgram of triplicates. Similar results were obtained for the analysis of the interaction of Ac1-11 with immobilized V_α4.2 (data not shown).

effects. The data suggest that V_α4.2 can specifically modulate the recognition of peptide–MHC ligand by the TCR, the effect being dependent on the affinity of the peptide for MHC. Whether the V_α mediates the inhibitory effects through displacement of Ac1-11 from I-A^u or blockade of the interaction of Ac1-11–I-A^u by the TCR, or a combination of both, cannot be determined by the current experiments. The modulation of T cell recognition was seen only with Utm6.15 and not with PL-8 as the APC, which is consistent with the direct binding data (Fig. 1). It is, however, possible that at higher concentrations V_α4.2 may also modulate recognition of PL-8-mediated peptide presentation.

The affinity-dependent modulation of T cell recognition by soluble V_α demonstrated in the present study might explain the immunoregulatory effects reported for soluble TCR α chains in a number of systems *in vitro* and *in vivo* (26–28). The influence of the nature/affinity of the peptide bound in the groove of the MHC molecule on V_α binding suggests that the phenomenon will not lead to generalized non-specific inhibition of peptide–MHC recognition by T cells. The affinity dependence would impart some kind of specificity similar to that observed previously (27). The efficiency with which a soluble V_α can bring about modulation of the T cell response would also depend on the relative affinity of a particular α chain for a given MHC haplotype and therefore different V_αs might have differential effects. The present study does not, however, exclude other mechanisms which have been proposed for immunoregulation by soluble TCR α chains (28).

In summary, we have demonstrated specific binding of a correctly folded TCR V_α domain to MHC class II I-A molecules on the surface of both *in vitro* cell lines and *ex vivo* splenocytes. In addition, V_α4.2 can bind to I-A^u in a manner that is influenced by the affinity of the peptide present in the groove. Thus, the interaction appears to be unconventional and in support of this, CDR mutations do not have significant effects whereas mutation of E69 in HV4 enhances binding. Further studies will be required to evaluate the generality of this type of interaction and assess the role of peptide repertoire/affinity in V_α binding. The binding of soluble TCR α chain to MHC class II may be of relevance in certain physiological situations such as in mediating suppressor activity of α chains (26–28)

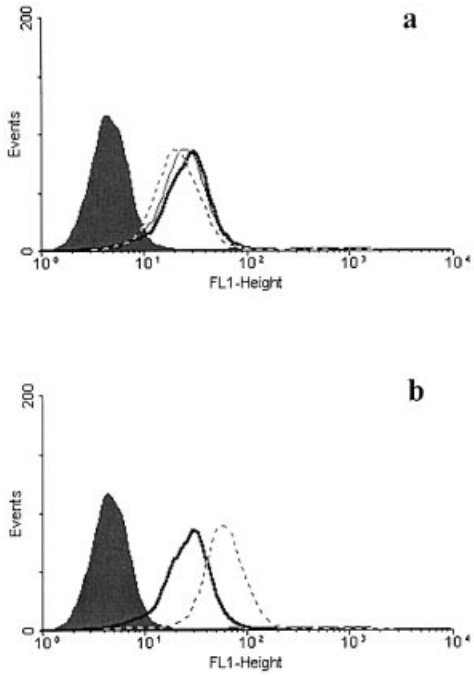

Fig. 7. Analysis of binding by wild-type and mutated derivatives of V_α4.2. Utm6.15 cells were incubated with V_α4.2 and its mutant derivatives at a concentration of 25 μg/ml. The assay was as described in Fig. 1(a). (a) shaded curve represents control (no V_α added). Binding by wild-type V_α4.2 (thick line), binding by CDR1 mutant (thin line) and binding by CDR2 mutant (dashed line) are shown. Binding by CDR1,2,3 mutant overlapped with CDR2 mutant. (b) Binding by HV4α mutant E69A (dashed line) compared with binding by wild-type V_α4.2 (thick line).

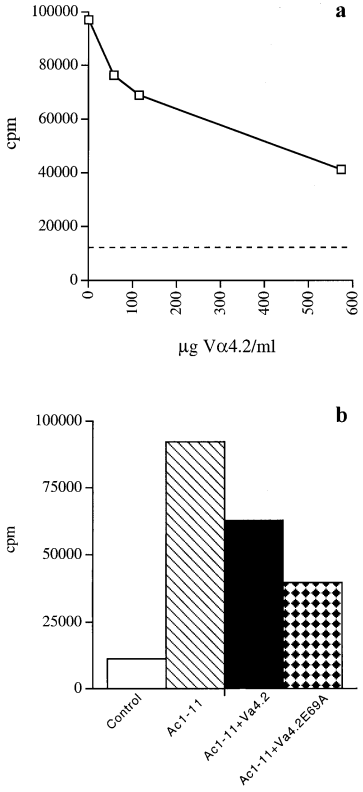

Fig. 8. Effect of V_α4.2 binding to I-A^u on peptide–MHC recognition by the 1934.4 T cell transfectants. (a) Ac1-11-specific T cells were incubated with Ac1-11 (5 μg/ml) in the presence of varying concentrations of wild-type V_α4.2. Utm6.15 were used as APC. Dashed line represents IL-2 production by cells in the absence of Ac1-11. (b) Effect of wild-type V_α4.2 and its HV4α analog (E69A: Glu69 to Ala69) on the recognition of the Ac1-11–I-A^u complex (Utm6.15 cells as APC) by 1934.4 T cell transfectants. The concentration of Ac1-11 was 5 μg/ml and wild-type V_α4.2 or the HV4α analog were added to a final concentration of 100 μg/ml. The control was 1934.4 T cell transfectants and Utm6.15 without any peptide. IL-2 levels were quantitated using the IL-2-dependent cell line CTLL-2. Addition of V_α4.2 did not have any significant effect on the activation of 1934.4 T cell transfectants by Utm6.15 in the presence of the high-affinity analog of Ac1-11, Ac1-11[4Y], nor did it affect activation of an alloreactive T cell hybridoma qcII85.33 (44) by PL-8 cells (not shown). All values are averages of triplicates with errors between 5 and 10%.

for which a mechanistic basis is currently unknown. This hypothesis is supported by the observation that V_α4.2 binds to *ex vivo* splenocytes of the I-A^u/I-A^d haplotypes and blocks T cell stimulation by peptide–I-A^u complexes *in vitro*. Finally, V_α binding to I-A molecules may be an initial step in an antigen-presentation pathway reminiscent of that described by others, whereby intact proteins bind to surface MHC class II molecules prior to endocytic uptake and recycling (29,30,50). In this respect, it will be of interest to investigate whether the region of V_α4.2 encompassing HV4α is immunodominant, consistent with determinant capture models of epitope generation (52).

Acknowledgements

We are indebted to Dr Mark Mummert for providing valuable advice concerning CD analyses. We would also like to thank Drs Harden McConnell, John Kappler, Philippa Marrack, Mark Davis, Stephen Hedrick and David Wraith for providing essential reagents. This study was supported by grants from the National Institutes of Health (AI31592, AI/NS42949), National Multiple Sclerosis Society (RG-2411) and the Yellow Rose Gala. E. S. W holds an Established Investigator Award from the American Heart Association (9640277N).

Abbreviations

APC	antigen-presenting cell
CDR	complementarity-determining region
HV4	fourth hypervariable region
MBP	myelin basic protein
SEB	staphylococcal enterotoxin B
SPR	surface plasmon resonance

References

1 Fields, B. A. and Mariuzza, R. A. 1996. Structure and function of the T-cell receptor: insights from X-ray crystallography. *Immunol. Today* 17:330.
2 Germain, R. N. 1994. MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. *Cell* 76:287.
3 York, I. A. and Rock, K. L. 1996. Antigen processing and presentation by the class I major histocompatibility complex. *Annu. Rev. Immunol.* 14:369.
4 Alzari, P. M., Lascombe, M. B. and Poljak, R. J. 1988. Three-dimensional structure of antibodies. *Annu. Rev. Immunol.* 6:555.
5 Davies, D. R., Padlan, E. A. and Sheriff, S. 1990. Antibody–antigen complexes. *Annu. Rev. Biochem.* 59:439.
6 Bjorkman, P. J., Saper, M. A., Samraoui, B., Bennett, W. S., Strominger, J. L. and Wiley, D. C. 1987. Structure of the human class I histocompatibility antigen, HLA-A2. *Nature* 329:506.
7 Brown, J. H., Jardetzky, T. S., Gorga, J. C., Stern, L. J., Urban, R. G., Strominger, J. L. and Wiley, D. C. 1993. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature* 364:33.
8 Stern, L. J., Brown, J. H., Jardetzky, T. S., Gorga, J. C., Urban, R. G., Strominger, J. L. and Wiley, D. C. 1994. Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide. *Nature* 368:215.
9 Fremont, D. H., Hendrickson, W. A., Marrack, P. and Kappler, J. 1996. Structures of an MHC class II molecule with covalently bound single peptides. *Science* 272:1001.
10 Smith, K. J., Reid, S. W., Stuart, D. I., McMichael, A. J., Jones, E. Y. and Bell, J. I. 1996. An altered position of the α 2 helix of MHC class I is revealed by the crystal structure of HLA-B*3501. *Immunity* 4:203.
11 Fremont, D. H., Monnaie, D., Nelson, C. A., Hendrickson, W. A. and Unanue, E. R. 1998. Crystal structure of I-A^k in complex with a dominant epitope of lysozyme. *Immunity* 8:305.
12 Scott, C. A., Peterson, P. A., Teyton, L. and Wilson, I. A. 1998. Crystal structures of two I-A^d–peptide complexes reveal that high affinity can be achieved without large anchor residues. *Immunity* 8:319.
13 Fields, B. A., Ober, B., Malchiodi, E. L., Lebedeva, M. I., Braden, B. C., Ysern, X., Kim, J. K., Shao, X., Ward, E. S. and Mariuzza, R. A. 1995. Crystal structure of the V_α domain of a T cell antigen receptor. *Science* 270:1821.
14 Bentley, G. A., Boulot, G., Karjalainen, K. and Mariuzza, R. A. 1995. Crystal structure of the β chain of a T cell antigen receptor. *Science* 267:1984.
15 Fields, B. A., Malchiodi, E. L., Li, H., Ysern, X., Stauffacher, C. V., Schlievert, P. M., Karjalainen, K. and Mariuzza, R. A. 1996. Crystal structure of a T-cell receptor β-chain complexed with a superantigen. *Nature* 384:188.
16 Garcia, K. C., Degano, M., Stanfield, R. L., Brunmark, A., Jackson, M. R., Peterson, P. A., Teyton, L. and Wilson, I. A. 1996. An αβ T cell receptor structure at 2.5 Å and its orientation in the TCR-MHC complex. *Science* 274:209.

17 Garboczi, D. N., Ghosh, P., Utz, U., Fan, Q. R., Biddison, W. E. and Wiley, D. C. 1996. Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. *Nature* 384:134.

18 Ding, Y. H., Smith, K. J., Garboczi, D. N., Utz, U., Biddison, W. E. and Wiley, D. C. 1998. Two human T cell receptors bind in a similar diagonal mode to the HLA-A2/Tax peptide complex using different TCR amino acids. *Immunity* 8:403.

19 Garcia, K. C., Degano, M., Pease, L. R., Huang, M., Peterson, P. A., Teyton, L. and Wilson, I. A. 1998. Structural basis of plasticity in T cell receptor recognition of a self peptide–MHC antigen. *Science* 279:1166.

20 Wang, J., Lim, K., Smolyar, A., Teng, M., Liu, J., Tse, A. G., Hussey, R. E., Chishti, Y., Thomson, C. T., Sweet, R. M., Nathenson, S. G., Chang, H. C., Sacchettini, J. C. and Reinherz, E. L. 1998. Atomic structure of an αβ T cell receptor (TCR) heterodimer in complex with an anti-TCR Fab fragment derived from a mitogenic antibody. *EMBO J.* 17:10.

21 Housset, D., Mazza, G., Gregoire, C., Piras, C., Malissen, B. and Fontecilla-Camps, J. C. 1997. The three-dimensional structure of a T-cell antigen receptor $V_\alpha V_\beta$ heterodimer reveals a novel arrangement of the V_β domain. *EMBO J.* 16:4205.

22 Sim, B. C., Zerva, L., Greene, M. I. and Gascoigne, N. R. J. 1996. Control of MHC restriction by TCR V_α CDR1 and CDR2. *Science* 273:963.

23 Sant'Angelo, D. B., Waterbury, G., Preston-Hurlburt, P., Yoon, S. T., Medzhitov, R., Hong, S. C. and Janeway, C. A. J. 1996. The specificity and orientation of a TCR to its peptide–MHC class II ligands. *Immunity.* 4:367.

24 Hong, S. C., Sant'Angelo, D. B., Dittel, B. N., Medzhitov, R., Yoon, S. T., Waterbury, P. G. and Janeway, C. A. J. 1997. The orientation of a T cell receptor to its MHC class II:peptide ligands. *J. Immunol.* 159:4395.

25 Wraith, D. C., Smilek, D. E., Mitchell, D. J., Steinman, L. and McDevitt, H. O. 1989. Antigen recognition in autoimmune encephalomyelitis and the potential for peptide-mediated immunotherapy. *Cell* 59:247.

26 Kuchroo, V. K., Byrne, M. C., Atsumi, Y., Greenfield, E., Connolly, J. B., Whitters, M. J., O'Hara, R. M. J., Collins, M. and Dorf, M. E. 1991. T-cell receptor alpha chain plays a critical role in antigen-specific suppressor cell function. *Proc. Natl Acad. Sci. USA* 88:8700.

27 O'Hara, R. M. J., Byrne, M. C., Kuchroo, V. K., Nagelin, A., Whitters, M. J., Jayaraman, S., Henderson, S. L., Dorf, M. E. and Collins, M. 1995. T cell receptor alpha-chain defines the antigen specificity of antigen-specific suppressor factor but does not impart genetic restriction. *J. Immunol.* 154:2075.

28 Ishii, Y., Nakano, T. and Ishizaka, K. 1996. Cellular mechanisms for the formation of a soluble form derivative of T-cell receptor alpha chain by suppressor T cells. *Proc. Natl Acad. Sci. USA* 93:7207.

29 Pinet, V., Malnati, M. S. and Long, E. O. 1994. Two processing pathways for the MHC class II-restricted presentation of exogenous influenza virus antigen. *J. Immunol.* 152:4852.

30 Pinet, V., Vergelli, M., Martin, R., Bakke, O. and Long, E. O. 1995. Antigen presentation mediated by recycling of surface HLA-DR molecules. *Nature* 375:603.

31 Wraith, D. C., Smilek, D. E. and Webb, S. 1992. MHC-binding peptides for immunotherapy of experimental autoimmune disease. *J. Autoimmun.* 5 (Suppl. A):103.

32 Mason, K., Denney, D. W. J. and McConnell, H. M. 1995. Myelin basic protein peptide complexes with the class II MHC molecules $I\text{-}A^u$ and $I\text{-}A^k$ form and dissociate rapidly at neutral pH. *J. Immunol.* 154:5216.

33 Mason, K., Denney, D. W. J. and McConnell, H. M. 1995. Kinetics of the reaction of a myelin basic protein peptide with soluble IA^u. *Biochemistry* 34:14874.

34 Letourneur, F. and Malissen, B. 1989. Derivation of a T cell hybridoma variant deprived of functional T cell receptor alpha and beta chain transcripts reveals a nonfunctional alpha-mRNA of BW5147 origin. *Eur. J. Immunol.* 19:2269.

35 Patten, P. A., Rock, E. P., Sonoda, T., Fazekas de St Groth, B., Jorgensen, J. L. and Davis, M. M. 1993. Transfer of putative complementarity-determining region loops of T cell receptor V domains confers toxin reactivity but not peptide/MHC specificity. *J. Immunol.* 150:2281.

36 Thatte J., Qadri A., Radu C. G. and Ward E. S. 1999. Molecular requirements for T cell recognition by MHC class II restricted T cell receptor: the involvement of the fourth hypervariable loop of the V_α domain. *J. Exp. Med.* 189:509.

37 Staerz, U. D., Rammensee, H. G., Benedetto, J. D. and Bevan, M. J. 1985. Characterization of a murine monoclonal antibody specific for an allotypic determinant on T cell antigen receptor. *J. Immunol.* 134:3994.

38 Janeway, C. A. J., Conrad, P. J., Lerner, E. A., Babich, J., Wettstein, P. and Murphy, D. B. 1984. Monoclonal antibodies specific for Ia glycoproteins raised by immunization with activated T cells: possible role of T cell bound Ia antigens as targets of immunoregulatory T cells. *J. Immunol.* 132:662.

39 Oi, V. T., Jones, P. P., Goding, J. W. and Herzenberg, L. A. 1978. Properties of monoclonal antibodies to mouse Ig allotypes, H-2, and Ia antigens. *Curr. Top. Microbiol. Immunol.* 81:115.

40 Ward, E. S. 1992. Secretion of T cell receptor fragments from recombinant *Escherichia coli* cells. *J. Mol. Biol.* 224:885.

41 Kunkel, T. A., Roberts, J. D. and Zakour, R. A. 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Methods Enzymol.* 154:367.

42 Kabat E. A., Wu T. T., Perry H. M., Gottesman K. S. and Foeller C. 1991. *Sequences of Proteins of Immunological Interest*, vol. 1, 5th edn. US Department of Health and Human Services, Washington DC.

43 Meier, T., Arni, S., Malarkannan, S., Poincelet, M. and Hoessli, D. 1992. Immunodetection of biotinylated lymphocyte-surface proteins by enhanced chemiluminescence: a nonradioactive method for cell-surface protein analysis. *Anal. Biochem.* 204:220.

44 Rosloniec, E. F., Brand, D. D., Whittington, K. B., Stuart, J. M., Ciubotaru, M. and Ward, E. S. 1995. Vaccination with a recombinant V_α domain of a TCR prevents the development of collagen-induced arthritis. *J. Immunol.* 155:4504.

45 Lipsky P. E. and Bottomly K. 1996. Measurement of human and murine interleukin 2 and interleukin 4. In Coligan J. E., ed., *Current Protocols in Immunology*, p. 6.3.1. John Wiley, New York.

46 Amit, A. G., Mariuzza, R. A., Phillips, S. E. V. and Poljak, R. J. 1986. Three-dimensional structure of an antigen–antibody complex at 2.8 Å Resolution. *Science* 233:747.

47 Acha-Orbea, H., Mitchell, D. J., Timmermann, L., Wraith, D. C., Tausch, G. S., Waldor, M. K., Zamvil, S. S., McDevitt, H. O. and Steinman, L. 1988. Limited heterogeneity of T cell receptors from lymphocytes mediating autoimmune encephalomyelitis allows specific immune intervention. *Cell* 54:263.

48 Zamvil, S. S., Mitchell, D. J., Moore, A. C., Kitamura, K., Steinman, L. and Rothbard, J. B. 1986. T-cell epitope of the autoantigen myelin basic protein that induces encephalomyelitis. *Nature* 324:258.

49 Sette, A., Adorini, L., Colon, S. M., Buus, S. and Grey, H. M. 1989. Capacity of intact proteins to bind to MHC class II molecules. *J. Immunol.* 143:1265.

50 Lindner, R. and Unanue, E. R. 1996. Distinct antigen MHC class II complexes generated by separate processing pathways. *EMBO J.* 15:6910.

51 Jensen, P. E. 1993. Acidification and disulfide reduction can be sufficient to allow intact proteins to bind class II MHC. *J. Immunol.* 150:3347.

52 Deng, H., Apple, R., Clare-Salzler, M., Trembleau, S., Mathis, D., Adorini, L. and Sercarz, E. 1993. Determinant capture as a possible mechanism of protection afforded by major histocompatibility complex class II molecules in autoimmune disease. *J. Exp. Med.* 178:1675.

53 Castellino, F., Zappacosta, F., Coligan, J. E. and Germain, R. N. 1998. Large protein fragments as substrates for endocytic antigen capture by MHC class II molecules. *J. Immunol.* 161:4048.

54 Busch, R., Cloutier, I., Sekaly, R. P. and Hammerling, G. J. 1996. Invariant chain protects class II histocompatibility antigens from binding intact polypeptides in the endoplasmic reticulum. *EMBO J.* 15:418.

55 Fairchild, P. J., Wildgoose, R., Atherton, E., Webb, S. and Wraith, D. C. 1993. An autoantigenic T cell epitope forms unstable complexes with class II MHC: a novel route for escape from tolerance induction. *Int. Immunol.* 5:1151.
56 Wen, R., Cole, G. A., Surman, S., Blackman, M. A. and Woodland, D. L. 1996. Major histocompatibility complex class II-associated peptides control the presentation of bacterial superantigens to T cells. *J. Exp. Med.* 183:1083.
57 Thibodeau, J., Cloutier, I., Lavoie, P. M., Labrecque, N., Mourad, W., Jardetzky, T. and Sekaly, R. P. 1994. Subsets of HLA-DR1 molecules defined by SEB and TSST-1 binding. *Science* 266:1874.
58 von Bonin, A., Ehrlich, S., Malcherek, G. and Fleischer, B. 1995. Major histocompatibility complex class II-associated peptides determine the binding of the superantigen toxic shock syndrome toxin-1. *Eur. J. Immunol.* 25:2894.
59 Romagnoli, P. and Germain, R. N. 1994. The CLIP region of invariant chain plays a critical role in regulating major histocompatibility complex class II folding, transport, and peptide occupancy. *J. Exp. Med.* 180:1107.
60 Jardetzky, T. S., Brown, J. H., Gorga, J. C., Stern, L. J., Urban, R. G., Chi, Y. I., Stauffacher, C., Strominger, J. L. and Wiley, D. C. 1994. Three-dimensional structure of a human class II histocompatibility molecule complexed with superantigen. *Nature* 368:711.
61 Matsui, K., Boniface, J. J., Reay, P. A., Schild, H., Fazekas de St Groth, B. and Davis, M. M. 1991. Low affinity interaction of peptide–MHC complexes with T cell receptors. *Science* 254:1788.
62 Weber, S., Traunecker, A., Oliveri, F., Gerhard, W. and Karjalainen, K. 1992. Specific low-affinity recognition of major histocompatibility complex plus peptide by soluble T-cell receptor. *Nature* 356:793.
63 Corr, M., Slanetz, A. E., Boyd, L. F., Jelonek, M. T., Khilko, S., al-Ramadi, B. K., Kim, Y. S., Maher, S. E., Bothwell, A. L. and Margulies, D. H. 1994. T cell receptor–MHC class I peptide interactions: affinity, kinetics, and specificity. *Science* 265:946.
64 Alam, S. M., Travers, P. J., Wung, J. L., Nasholds, W., Redpath, S., Jameson, S. C. and Gascoigne, N. R. 1996. T-cell-receptor affinity and thymocyte positive selection. *Nature* 381:616.
65 Lyons, D. S., Lieberman, S. A., Hampl, J., Boniface, J. J., Chien, Y., Berg, L. J. and Davis, M. M. 1996. A TCR binds to antagonist ligands with lower affinities and faster dissociation rates than to agonists. *Immunity* 5:53.
66 Hong, S. C., Chelouche, A., Lin, R. H., Shaywitz, D., Braunstein, N. S., Glimcher, L. and Janeway, C. A. J. 1992. An MHC interaction site maps to the amino-terminal half of the T cell receptor alpha chain variable domain. *Cell* 69:999.
67 Jorgensen, J. L., Esser, U., Fazekas de St Groth, B., Reay, P. A. and Davis, M. M. 1992. Mapping T-cell receptor–peptide contacts by variant peptide immunization of single-chain transgenics. *Nature* 355:224.
68 Brawley, J. V. and Concannon, P. 1996. Modulation of promiscuous T cell receptor recognition by mutagenesis of CDR2 residues. *J. Exp. Med.* 183:2043.
69 Wilson, N. A., Wolf, P., Ploegh, H., Ignatowicz, L., Kappler, J. and Marrack, P. 1998. Invariant chain can bind MHC class II at a site other than the peptide binding groove. *J. Immunol.* 161:4777.
70 Tompkins, S. M., Moore, J. C. and Jensen, P. E. 1996. An insulin peptide that binds an alternative site in class II major histocompatibility complex. *J. Exp. Med.* 183:857.