J. Mol. Biol. (1992) 224, 885–890

COMMUNICATIONS

Secretion of T Cell Receptor Fragments From Recombinant *Escherichia coli* Cells

E. Sally Ward

Cancer Immunobiology Center and Department of Microbiology
University of Texas Southwestern Medical Center
5323 Harry Hines Blvd, Dallas, TX 75235-8576, U.S.A.

(Received 18 October 1991; accepted 20 December 1991)

This study describes the secretion and purification of T cell receptor (TCR) Vα, Vβ domains and single chain Vα-Vβ fragments (scTCRs) from recombinant *Escherichia coli* cells. The TCR Vα and Vβ genes are derived from a T cell hybridoma that is associated with disease pathogenesis in murine experimental allergic encephalomyelitis (EAE). Circular dichroism (c.d.) analyses of the single domains and the scTCR indicate that they are folded into β-pleated sheet structures similar to those of immunoglobulin variable domains. The secreted TCR fragments can be purified in milligram quantities, and could therefore be used in high-resolution structural studies, in immunization to generate anti-clonotypic antibodies or in vaccination.

Keywords: T cell receptor; *E. coli* secretion; murine EAE; circular dichroism

T cell recognition is mediated by surface bound T cell receptors (TCRs†), and the majority (90 to 95%) of T cells bear TCRs that comprize αβ heterodimers (Meuer *et al.*, 1983; Allison *et al.*, 1982). In contrast, a much smaller proportion (5 to 10%) bear γδ heterodimers (Brenner *et al.*, 1986; Borst *et al.*, 1987). The αβ TCRs recognise antigenic peptides bound to the cleft of the highly polymorphic MHC class I or class II glycoproteins, and are therefore "MHC restricted" (Zinkernagel & Doherty, 1979). An understanding at the molecular level of the interaction of TCRs with cognate peptide–MHC complexes would expand the available data concerning this key interaction of the immune system. Towards this aim, TCR αβ heterodimers have recently been expressed as phosphatidylinositol linked polypeptides (Lin *et al.*, 1990) or TCR-immunoglobulin chimeras (Gregoire *et al.*, 1991) in mammalian transfectomas, and the production of Vα-Cκ homodimers (Mariuzza & Winter, 1989) and Vβ-Cβ monomers (Gascoigne, 1990) in mammalian cells has also been described. The use of *Escherichia coli* as an expression host offers a convenient and rapid route for the production of recombinant TCRs, and this is the aim of this study. The expression and secretion of immunoglobulin VH domains (Ward *et al.*, 1989), Fv fragments (Skerra & Plückthun, 1988) and Fab fragments (Better *et al.*, 1988) has been reported. Analysis by molecular modeling indicates that there are structural similarities between immunoglobulin Fab fragments and the extracellular domains of TCRs (Novotny *et al.*, 1986; Chothia *et al.*, 1988). These similarities led to the use of an *E. coli* secretion system for the expression of TCR Vα and Vβ domains (Ward, 1991) derived from an encephalitogenic (1934.4) T cell hybridoma (Wraith *et al.*, 1989). In this study, the purification and characterization, using c.d., of secreted Vα, Vβ domains and single chain TCR (scTCR) fragments derived from this hybridoma are described.

The plasmids VαpelBHis and VβpelBHis for the secretion and purification of the TCR fragments are shown in Figure 1, and are derived from expression plasmids described previously (Ward *et al.*, 1989; Ward, 1991). When compared with the myc tag, the His_6 tag was found to be a superior ligand for affinity purification using Ni^{2+}-NTA agarose columns (Hochuli *et al.*, 1988) (data not shown). The Vα and Vβ domains with the carboxy-terminal His_6 tags can be purified in yields of approximately 1 to 2 mg/litre of culture and 0·1 to 0·2 mg/litre of culture, respectively. Shorter induction times were used in preference to those described previously (Ward *et al.*, 1989; Ward, 1991), since for longer induction periods the purification yields were lower, possibly due to proteolytic loss of the His_6 tag.

Figure 2 shows SDS/polyacrylamide gel analyses

† Abbreviations used: TCRs, T cell receptors; MHC, major histocompatibility complex; c.d., circular dichroism; sc, single chain

0022-2836/92/080885-06 $03.00/0

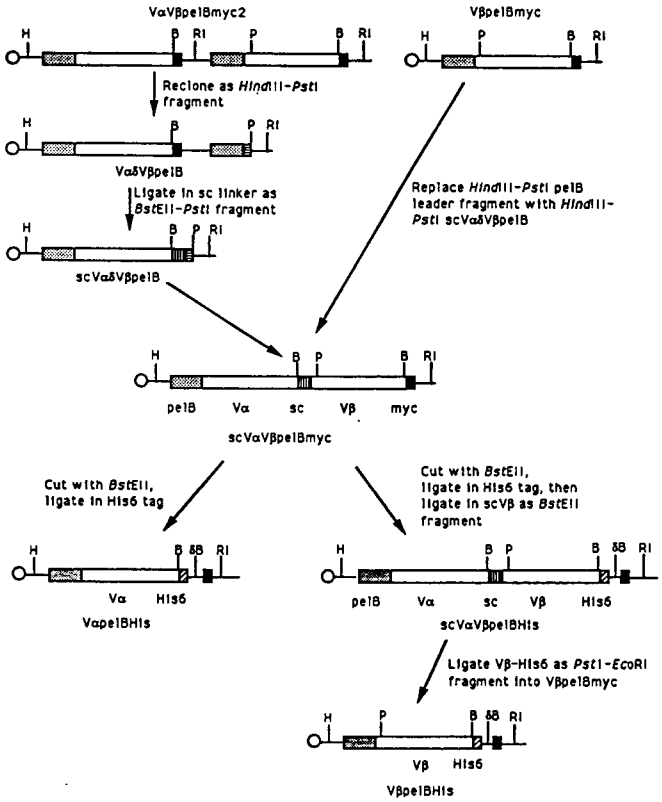

Figure 1. Strategy used for the construction of plasmids for expression and purification of single Vα, Vβ domains and scTCR fragments. H, *Hin*dIII; B, *Bst*EII; P, *Pst*I and RI, *Eco*RI restriction sites. δB = *Bst*EII site removed by insertion of the DNA duplex encoding the His_6 tag. Open circles represent the *lacz* promoter of pUC119 (Viera *et al.*, 1987), open boxes represent the Vα and Vβ genes. The pelB leader is indicated by stippled boxes, the single chain linker (sc) sequence by vertical hatching, the 5′ end of the Vβ gene in VαδVβpelB and scVαδVβpelB by horizontal hatching, the His_6 tag by diagonal hatching and the myc tag (which is the same as tag1 in Ward, 1991) by a filled-in box. Single lines represent pUC119 sequences. The isolation of the Vα and Vβ genes from the 1934.4 T cell hybridoma, and the cloning of these genes to generate the expression plasmids VαpelBmyc and VβpelBmyc have been described (Ward, 1991). The plasmid VαVβpelBmyc2 was constructed in a similar way to VαpelBmyc (Ward, 1991), except that the VβpelBmyc gene has been cloned 3′ to the VαpelBmyc gene. The plasmid scVαVβpelBmyc was constructed as indicated. The single chain linker, $(Gly_4Ser)_3$, is the same as that used previously for immunoglobulin scFvs (Huston *et al.*, 1988), and was ligated into *Bst*EII–*Pst*I restricted VαδVβpelB as the following DNA duplex:

5′ GTC ACC GGT GGA GGC GGT TCA GGC GGA GGT GGC TCT GGC GGT GGC GGA TCG GAG GCT GCA 3′
3′ G CCA CCT CCG CCA AGT CCG CCT CCA CCG AGA CCG CCA CCG CCT AGC CTC CG-5′

(coding strand indicated by underlining).

To construct scVαVβpelBmyc, the resulting *Hin*dIII–*Pst*I fragment (scVαδVβpelB) encoding the pelB leader, the Vα gene, the single chain linker and the 5′ end of the Vβ gene was ligated into *Hin*dIII–*Pst*I restricted VβpelBmyc (to replace the pelB leader). To insert the His_6 peptide tag into scVαVβpelBmyc, this plasmid was restricted with *Bst*EII and the following DNA duplex ligated into the construct;

5′ GTC ACC CAT CAC CAT CAC CAT CAC TAA TAA 3′
3′ G GTA GTG GTA GTG GTA GTG ATT ATT CAG TG 5′

(coding strand indicated by underlining).

Recombinant clones with the correct orientation of the His_6 tag were identified by PCR screening (Güssow & Clackson, 1989). Ligation of the duplex in the correct orientation into *Bst*EII cut scVαVβpelBmyc will remove the 3′ *Bst*EII site. In addition, the presence of 2 stop codons at the 3′ end of the histidine codons prevents readthrough into the downstream myc tag sequences. VαpelBHis and VβpelBHis were constructed using the strategy shown. Prior to expression analyses, all DNA constructs were sequenced using the dideoxynucleotide method (Sanger *et al.*, 1977).

of the purified proteins. In addition to the much lower purification yields, the Vβ domain has a tendency to precipitate out of solution at a concentration of approximately 0·2 milligrams per ml. In contrast, the Vα domain appears to be soluble at concentrations of several milligrams per ml. Chemical crosslinking experiments using dithiobis-(succimidylpropionate) indicate that the Vα domain has a propensity to form homodimers (data not shown). This observation is not unexpected since

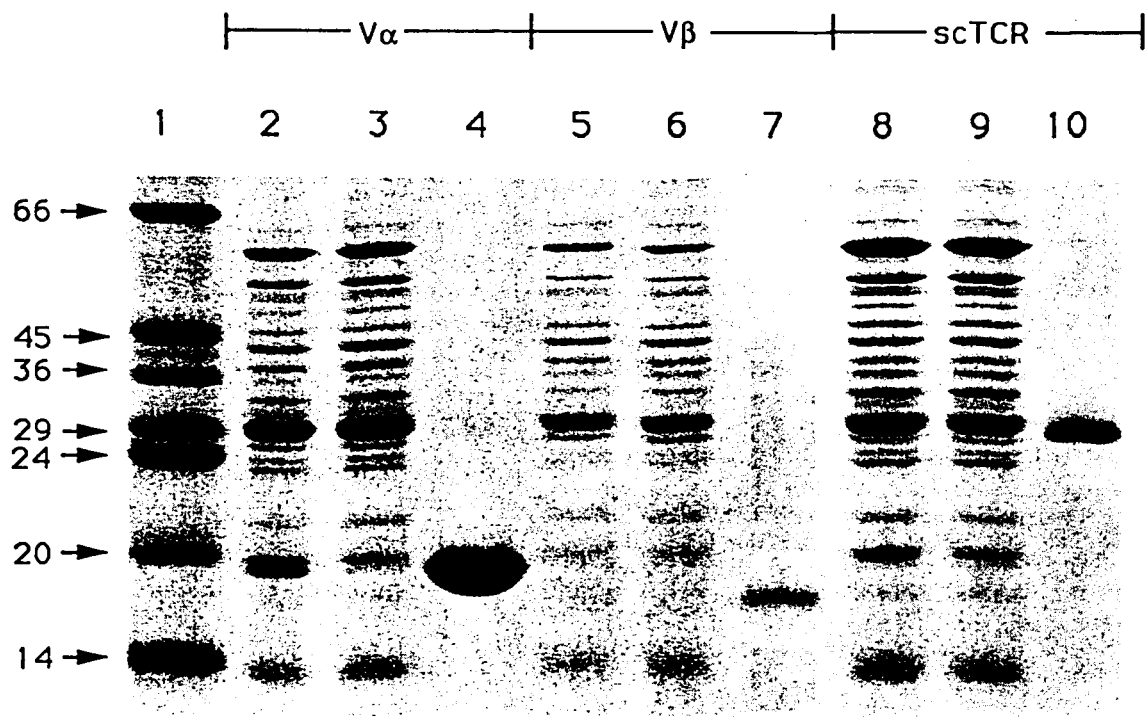

Figure 2. Purification of the recombinant proteins. 15% (w/v) SDS/polyacrylamide gel analysis of the purified single lomains (lanes 2 to 7) and scTCR (lanes 8 to 10). Lane 1, molecular weight markers (with sizes shown on the left margin n kDa); lane 2, osmotic stock fraction of *E. coli* harboring VαpelBHis; lane 3, flowthrough from VαpelBHis osmotic hock fraction after passage through Ni^{2+}-NTA agarose column; lane 4, purified Vα domain; lanes 5 to 7, same as lanes 2 o 4, respectively, but for *E. coli* harboring VβpelBHis; lanes 8 to 10, same as lanes 2 to 4, respectively, but for *E. coli* ıarboring scVαVβpelBHis. Recombinant plasmids were transformed into *E. coli* BMH71-18 (Rüther *et al.*, 1981) and the esulting transformants grown and induced under conditions that were optimized to maximize the expression levels. For his optimization, the plasmid scVαVβpelBmyc was used, since the myc tag allows detection of unpurified recombinant protein in culture supernatants or osmotic shock fractions, using Western blotting and the 9E10 monoclonal antibody (Towbin *et al.*, 1979; Evan *et al.*, 1985) as described (Ward *et al.*, 1989). Following optimization, recombinants harboring VαpelBHis, VβpelBHis and scVαVβpelBHis were grown up and induced, using the following procedure: 1 to 2 litre cultures of transformants were grown up in 4 × TY media (double strength 2 × TY; Miller, 1972) plus 100 μg ampicillin/ml plus 1% (w/v) glucose for 15 h at 30°C. Cells were pelleted by centrifugation, washed once in 4 × TY and resuspended in 1 litre of 4 × TY plus 100 μg ampicillin/ml, 0·1 mM-IPTG, 10 μg leupeptin/ml and 100 μg PMSF/ml, and grown at 25°C for 5 to 5·5 h. At this stage the majority of the recombinant protein is located in the periplasm, and can be isolated by osmotically shocking the cells. The osmotic shock fraction (40 ml/litre of culture) was dialyzed against 3 changes of phosphate buffered saline, and then passed through an Ni^{2+}-NTA agarose column. Bound protein was batch eluted in 1 to 2 ml fractions with 250 mM-imidazole (pH 9·2). To reduce non-specific binding of additional proteins, the column was washed with 500 mM-NaCl/100 mM-Tris·HCl (pH 8) prior to elution. The purified protein was dialyzed extensively against 10 mM-NaH_2PO_4 (pH 7·0) prior to c.d. analysis. Purity of TCR fragments was assessed by 15% SDS/polyacrylamide gel electrophoresis (Laemmli, 1970) followed by staining with Coomassie brilliant blue.

Mariuzza & Winter (1989) have reported the expression of Vα-Cκ homodimers in mammalian cell transfectomas. Such dimerization may contribute to the increased solubility and expression yields of the Vα domain, since the hydrophobic residues (Chothia *et al.*, 1988), which in a native TCR interact with corresponding Vβ residues, may no longer be exposed in the homodimer.

These observations prompted the construction of the plasmid scVαVβpelBHis (Fig. 1) in which the Vα domain is covalently linked to the Vβ domain as a scTCR fragment, using the same linker peptide that has been used to link immunoglobulin heavy and light chain variable domains as single chain Fv fragments (Huston *et al.*, 1988). In this construct, the aims were: (1) to stabilize the Vα-Vβ association, since recent work suggests that co-expressed Vα and Vβ domains derived from the 1934.4 hybridoma (Ward, 1991) do not associate (unpublished results). This lack of heterodimer formation may be in part, at least, due to the dimerization of the Vα domain. It may also be that for this particular Vα-Vβ pair the association of the Vα and Vβ domains is not stable. In this respect, variations in the stability of VH-VL association for different immunoglobulin fragments have been observed (Skerra & Plückthun, 1988; Ward *et al.*, 1989). (2) To improve the solubility properties of the Vβ domain since association with the Vα domain, driven and stabilized by the single chain linker, may "cap off" the exposed hydrophobic residues (Chothia *et al.*, 1988).

As a single chain polypeptide, the 1934.4 derived scTCR could be secreted into the periplasm and

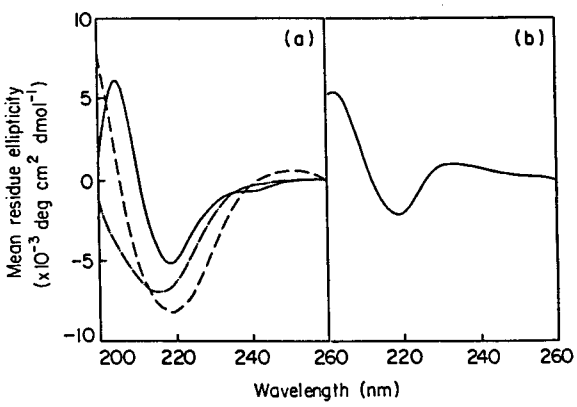

Figure 3. Analysis using circular dichroism of the recombinant TCR proteins. (a) The spectrum for the Vα domain is represented by a continuous line, for the Vβ domain by a broken line and for the scTCR by a dashed and dotted line. (b) The spectrum for the D1.3 scFv fragment. All spectra were smoothed and baseline corrected. The construction of a plasmid derivative of pSW2 (Ward *et al.*, 1989) for the secretion of the D1.3 scFv has been described (McCafferty *et al.*, 1990). Transformants harboring this plasmid were grown up, induced for expression and protein purified from the culture supernatant using lysozyme-Sepharose (Ward *et al.*, 1989). c.d. analyses were carried out using an AVIV model 60DS circular dichroism spectrophotometer at 25°C and a cell of 0·2 cm path length. Concentrations of proteins (in 10 mM-NaH_2PO_4 (pH 7·0)) used varied from 1 μM to 7·8 μM. For the determination of concentration, purified proteins (Vα, Vβ, scTCR and D1.3 scFv) were subjected to quantitative amino acid hydrolysis.

purified in yields of approximately 0·5 to 1 mg/litre of culture using Ni^{2+}-NTA agarose. Figure 2 shows an SDS/polyacrylamide gel analysis of the purification of this protein. For the scTCR in particular, lower growth and induction temperatures of 25 to 30°C resulted in higher expression yields. In this respect, similar observations have been made for the expression of other recombinant proteins in *E. coli* (Takagi *et al.*, 1988). The scTCR could also be purified using an affinity column, made by linking the monoclonal antibody KJ16 (specific for murine Vβ8: Kappler *et al.*, 1988) to Sepharose, which indicates that the epitope recognized by this monoclonal antibody is in the correct conformational state in the recombinant scTCR.

To assess the folded state of the recombinant TCR fragments, c.d. spectral analyses were carried out (Fig. 3(a)). The D1.3 single chain Fv fragment (D1.3 scFv; McCafferty *et al.*, 1990) has also been purified and characterized using c.d. (Fig. 3(b)). c.d. spectra were obtained for each of the recombinant proteins at several different concentrations, and Figure 3 shows representative spectra. The minima in the curves at 218 nm for all of these proteins indicates the presence of a high proportion of β-pleated sheet structure (Brahms & Brahms, 1980; Johnson, 1990). The spectrum for the Vα domain also has a peak at 205 nm, and this may indicate the presence of β-turns (Brahms & Brahms, 1980). In addition, from these spectra it can be concluded that there are no α-helical regions in the recombinant TCR fragments, nor the D1.3 scFv. This latter observation is consistent with the proposed structural models for TCR extracellular domains (Novotny *et al.*, 1986; Chothia *et al.*, 1988), and the structure of the crystallographically solved D1.3 Fv fragment (Bhat *et al.*, 1990).

Thus, the data indicate that single Vα, Vβ domains and single chain heterodimeric TCRs derived from an encephalitogenic T cell hybridoma can be expressed and purified in yields ranging from 0·1 to 2 mg/litre of bacterial culture. Novotny *et al.* (1991) have recently reported the expression of a single chain anti-fluorescein TCR in *E. coli*. In contrast to the findings with the 1934.4 TCR, however, the anti-fluorescein scTCR could not be isolated from the periplasm, although the leader sequence had been cleaved from the N terminus of the recombinant protein. This scTCR was found to be relatively insoluble, and therefore genetic manipulation was used to replace five of the "exposed" hydrophobic residues with hydrophilic ones (Novotny *et al.*, 1991). The observed solubility differences between the anti-fluorescein scTCR and the 1934.4 derived scTCR could be due to: (1) sequence differences between the two scTCRs. Sequence comparisons indicate that there are relatively hydrophilic residues in the 1934.4 TCR at the positions corresponding to three out of five of the hydrophobic residues that were replaced by more hydrophilic residues in the anti-fluorescein TCR. (2) The different orientation of the Vα domain with respect to the Vβ domain in the two constructions used to express the scTCRs. (3) The different conditions (particularly growth/induction temperature) used for expression of the recombinant proteins in the two studies.

Structural analysis using c.d. indicates that the recombinant 1934.4 derived TCR fragments contain a high proportion of β-pleated sheet structures. Although molecular modeling has indicated that the extracellular domains of TCRs may resemble immunoglobulin Fv and Fab fragments in structure (Novotny *et al.*, 1986; Chothia *et al.*, 1988), to date this has not been demonstrated experimentally. The ability of the Vα, Vβ domains and heterodimeric scTCR to inhibit the binding of the 1934.4 T cell hybridoma to cognate peptide–MHC complexes (N terminus residues 1 to 11 of myelin basic protein associated with the MHC class II protein $I\text{-}A^u$; Wraith *et al.*, 1989) is of particular interest, and is currently being assessed. It is conceivable, however, that soluble TCR fragments are ineffective inhibitors of the multivalent, high avidity, interaction (Harding & Unanue, 1990) of T cell borne antigen receptors with peptide–MHC complexes. Data also suggest that the tripartite interaction of "native" TCR on $CD4^+$ T cells with peptide–MHC complexes is stabilized by contacts between CD4 residues and the MHC class II molecule (Sleckman *et al.*, 1987; Fleury *et al.*, 1991). The absence of this "co-receptor" in the recombinant TCRs will therefore decrease the avidity of the interaction further.

This rapid method for the production of soluble, heterodimeric TCRs can be readily extended to the production of soluble TCRs of different specificities, derived from other species such as man. This opens up new avenues for immunotherapy and diagnosis, particularly in relation to T cell-mediated autoimmune diseases. For example, recombinant TCR fragments (single domains or heterodimers) could be used in vaccination to protect and reverse disease induction. A similar approach, in which synthetic TCR-derived peptides were used for the treatment of experimental allergic encephalomyelitis (EAE) in Lewis rats has been reported (Vandenbark *et al.*, 1989; Howell *et al.*, 1989). The soluble TCRs could also be used in immunization to generate anti-clonotypic antibodies (Devaux *et al.*, 1991), which could have uses in both passive immunization and diagnosis. Finally, in addition to the use of single chain, heterodimeric TCRs for structure–function studies directed towards the mapping of the residues that are involved in binding to cognate peptide–MHC complexes, the TCR fragments are produced in sufficient quantities to allow high resolution structural analyses.

I am grateful to Dr David Wraith for his collaboration, to Dr Kirsten Fischer-Lindahl for suggesting the use of circular dichroism, and to Dr Sam Landry, Dr Lila Gierasch and Zhi-Ping Liu for providing invaluable assistance with these analyses. The technical assistance of Greg Hubbard is gratefully acknowledged. Dr Andrew Griffiths was involved in the construction of the D1.3 scFv expression plasmid in the MRC Laboratory of Molecular Biology, Cambridge, UK. I thank Dr Richard Baer for critically reading the manuscript. The work was supported by the Department of Microbiology, an NIH Biomedical Research Support grant and an NIH 'FIRST' Award.

References

Allison, J. P., MacIntyre, B. W. & Block, D. (1982). Tumour specific antigen of murine T-lymphoma defined with monoclonal antibody. *J. Immunol.* **129**, 2293–2300.

Better, M., Chang, C. P., Robinson, R. R. & Horwitz, A. H. (1988). *Escherichia coli* secretion of an active chimeric antibody fragment. *Science*, **240**, 1041–1043.

Bhat, T. N., Bentley, G. A., Fischmann, T. O., Boulot, G. & Poljak, R. J. (1990). Small rearrangements in structures of Fv and Fab fragments of antibody D1.3 on antigen binding. *Nature (London)*, 483–485.

Borst, J., van de Griend, R. J., van Oostveen, J. W., Ang, S. L., Melief, C. J., Seidman, J. G. & Bolhuis, R. L. H. (1987). A T cell receptor γ/CD3 complex found on cloned functional lymphocytes. *Nature (London)*, **325**, 683–688.

Brahms, S. & Brahms, J. (1980). Determination of protein secondary structure in solution by vacuum ultraviolet circular dichroism. *J. Mol. Biol.* **138**, 149–178

Brenner, M. B., McLean, J., Dialynas, D. P., Strominger, J. L., Owen, F. L., Seidman, J. G., Ip, S., Rosen, F. & Krangel, M. S. (1986). Identification of a putative second T cell receptor. *Nature (London)*, **322**, 145–149.

Chothia, C., Boswell, D. R. & Lesk, A. M. (1988). The outline structure of the T-cell αβ receptor. *EMBO J.* **7**, 3745–3755.

Devaux, B., Bjorkman, P. J., Stevenson, C., Grief, W., Elliott, J. F., Sagerström, C., Clayberger, C., Krensky, A. M. & Davis, M. M. (1991). Generation of monoclonal antibodies against soluble human T cell receptor polypeptides. *Eur. J. Immunol.* **21**, 2111–2119.

Evan, G. I., Lewis, G. K., Ramsay, G. & Bishop, J. M. (1985). Isolation of monoclonal antibodies specific for human c-myc proto-oncogene product. *Mol. Cell. Biol.* **5**, 3610–3616.

Fleury, S., Lamarre, D., Meloche, S., Ryu, S.-E., Cantin, C., Hendrickson, W. A. & Sekaly, R.-P. (1991). Mutational analysis of the interaction between CD4 and Class II MHC: Class II antigens contact CD4 on a surface opposite the gp120-binding site. *Cell*, **66**, 1037–1049.

Gascoigne, N. R. J. (1990). Transport and secretion of truncated T cell receptor β-chain occurs in the absence of association with CD3. *J. Biol. Chem.* **265**, 9296–9301.

Gregoire, C., Rebai, N., Schweisguth, F., Necker, A., Mazza, G., Auphan, N., Millward, A., Schmitt-Verhulst, A.-M. & Malissen, B. (1991). Engineered secreted T-cell receptor αβ heterodimers. *Proc. Nat. Acad. Sci., U.S.A.* **88**, 8077–8081.

Güssow, D. & Clackson, T. P. (1989). Direct clone characterisation from plaques and colonies by the polymerase chain reaction. *Nucl. Acids Res.* **17**, 4000.

Harding, C. V. & Unanue, E. R. (1990). Quantitation of antigen-presenting cell MHC class II/peptide complexes necessary for T-cell stimulation. *Nature (London)*, **346**, 574–576.

Hochuli, E., Bannwarth, W., Döbeli, H., Gentz, R. & Stüber, D. (1988). Genetic approach to facilitate purification of recombinant proteins with a novel metal chelate adsorbent. *Bio/technol.* **6**, 1321–1325.

Howell, M. D., Winters, S. T., Olee, T., Powell, H. C., Carlo, D. J. & Brostoff, S. W. (1989). Vaccination against experimental allergic encephalomyelitis with T-cell receptor peptides. *Science*, **246**, 668–670.

Huston, J. S., Levinson, D., Mudgett-Hunter, M., Tai, M.-S., Novotny, J., Margolies, M. N., Ridge, R. J., Bruccoleri, R. E., Haber, E., Crea, R. & Oppermann, H. (1988). Protein engineering of antibody binding sites: recovery of specific activity in an anti-digoxin single-chain Fv analogue produced in *Escherichia coli*. *Proc. Nat. Acad. Sci., U.S.A.* **85**, 5879–5883.

Johnson, W. C. (1990). Protein secondary structure and circular dichroism: a practical guide. *Proteins*, **7**, 205–214.

Kappler, J. W., Staerz, U., White, J. & Marrack, P. C. (1988). Self-tolerance eliminates T cells specific for Mls-modified products of the major histocompatibility complex. *Nature (London)*, **332**, 35–40.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)*, **227**, 680–685.

Lin, A. Y., Devaux, B., Green, A., Sagerström, C., Elliott, J. F. & Davis, M. M. (1990). Expression of T-cell antigen receptors in a lipid-linked form. *Science*, **249**, 677–679.

Mariuzza, R. A. & Winter, G. P. (1989). Secretion of a homodimeric VαCκ T-cell receptor–immunoglobulin chimeric protein. *J. Biol. Chem.* **264**, 7310–7316.

McCafferty, J., Griffiths, A. D., Winter, G & Chiswell, D. J. (1990). Phage antibodies: filamentous phage

displaying antibody variable domains. *Nature (London)*, **348**, 552–554.

Meuer, S. C., Fitzgerald, K. A., Hussey, R. E., Hodgdon, J. C., Schlossman, S. F. & Reinherz, E. L. (1983). Clonotypic structures involved in antigen-specific human T cell function. Relationship to the T3 molecular complex. *J. Expt. Med.* **157**, 705–719.

Miller, J. H. (1972). *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Novotny, J., Tonegawa, S., Saito, H., Kranz, D. M. & Eisen, H. N. (1986). Secondary, tertiary and quaternary structure of T-cell specific immunoglobulin-like polypeptide chains. *Proc. Nat. Acad. Sci., U.S.A.* **83**, 742–746.

Novotny, J., Ganju, R. K., Smiley, S. T., Hussey, R. E., Luther, M. A., Recny, M. A., Siciliano, R. F. & Reinherz, E. L. (1991). A soluble, single-chain T-cell receptor fragment endowed with antigen-combining properties. *Proc. Nat. Acad. Sci., U.S.A.* **88**, 8646–8650.

Rüther, U., Koenen, M., Otto, K. & Müller-Hill, B. (1981). pUR322, a vector for cloning and rapid chemical sequencing of DNA. *Nucl. Acids Res.* **9**, 4087–4098.

Sanger, F., Nicklen, S. & Coulson, A. R. (1977). DNA sequencing with chain terminating inhibitors. *Proc. Nat. Acad. Sci., U.S.A.* **74**, 5643–5647.

Skerra, A. & Plückthun, A. (1988). Assembly of a functional immunoglobulin Fv fragment in *Escherichia coli*. *Science*, **240**, 1038–1041.

Sleckman, B. P., Peterson, A., Jones, W. K., Foran, J. A., Greenstein, J. L., Seed, B. & Burakoff, S. J. (1987). Expression and function of CD4 in a murine T-cell hybridoma. *Nature (London)*, **328**, 351–353.

Takagi, H., Morinaga, Y., Tsuchiya, M., Ikemura, H. & Inouye, M. (1988). Control of folding of proteins secreted by a high expression secretion vector, pIN-III-*ompA*: 16-fold increase in production of active subtilisin E in *Escherichia coli*. *Bio/technol.* **6**, 948–950.

Towbin, H., Staehelin, T. & Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Nat. Acad. Sci., U.S.A.* **76**, 4350–4354.

Vandenbark, A. A., Hashim, G. & Offner, H. (1989). Immunization with a synthetic T-cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis. *Nature (London)*, **331**, 541–544.

Viera, J. & Messing, J. (1987). In *Methods in Enzymology* (Wu, R. & Grossman, L., eds), vol. 153, pp. 3–11, Academic Press, New York.

Ward, E. S., Güssow, D., Griffiths, A. D., Jones, P. T. & Winter, G. (1989). Binding activities of a repertoire of single immunoglobulin variable domains secreted from *Escherichia coli*. *Nature (London)*, **341**, 544–546.

Ward, E. S. (1991). Expression and secretion of T-cell receptor Vα and Vβ domains using *Escherichia coli* as a host. *Scand. J. Immunol.* **34**, 215–220.

Wraith, D. C., Smilek, D. E., Mitchell, D. J., Steinman, L. & McDevitt, H. O. (1989). Antigen recognition in autoimmune encephalomyelitis and the potential for peptide-mediated immunotherapy. *Cell*, **59**, 247–255.

Zinkernagel, R. M. & Doherty, P. C. (1979). MHC-restricted cytotoxic T cells: studies on the biological role of polymorphic major transplantation antigens determining T cell restriction specificity, function and responsiveness, *Advan. Immunol.* **27**, 52–77.

Edited by A. R. Fersht