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Expression and Secretion of T-Cell Receptor Vα and Vβ Domains using *Escherichia coli* as a Host

E. S. WARD

Cancer Immunobiology Center and Department of Microbiology, The University of Texas Southwestern Medical Center at Dallas, Dallas, Texas, USA

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An expression system for the production of recombinant T-cell receptor (TCR) variable domains would, *inter alia*, allow structural studies to be carried out and provide protein for the generation of anti-clonotypic antibodies. In this report the Vα and Vβ domain genes have been isolated from a T-cell hybridoma which is associated with the pathogenesis of experimental allergic encephalomyelitis (EAE) in the H-2^u mouse. These have been expressed as secreted domains in *Escherichia coli*, using secretion vectors previously used for the production of immunoglobulin fragments. Both Vα and Vβ domains are secreted in milligram quantities into the culture supernatant, although the levels of the Vα domain are about 10–20 fold higher than those of the Vβ domain. This expression system offers a rapid route for the production of recombinant TCRs in soluble form.

E. Sally Ward, Cancer Immunobiology Center and Department of Microbiology, The University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd, Dallas, TX 75235-8576, USA

The majority of T cells recognize antigenic peptides bound to class I or II proteins of the major histocompatibility complex (MHC) and are thus 'MHC restricted' [1]. The recognition of peptide-MHC complexes is mediated by surface-bound T-cell receptors (TCRs), which comprise heterodimeric αβ glycoproteins [2–5] and constitute the vast majority of TCRs. A minority (1–10%) of T cells bear γβ [6–8] or γγ [9] polypeptides whose function is less well characterized than that of the αβ TCRs; a common feature of these three types of different TCRs is that they are associated with at least five components of the CD3 complex on the T-cell surface [10–12] to form TCR-CD3 complexes. TCR diversity is required for the recognition of an almost unlimited number of different peptide antigens associated with the highly polymorphic MHC molecules, and this diversity is achieved by somatic recombination of TCR V, (D), and J elements and extensive N-region addition (reviewed in Ref. 13). To date there is no evidence to suggest that somatic mutation contributes to TCR diversity.

A detailed understanding at the molecular level of the binding of TCRs to cognate peptide-MHC complexes would greatly expand the existing knowledge for one of the key interactions of the immune system, and would for example assist in the development of immunotherapy for T-cell mediated autoimmune diseases. To date, structural studies such as X-ray crystallography or NMR have not been reported for TCRs. This is probably due to the problems which have been encountered in the expression of TCRs in suitable form and yield to allow such analyses. Several expression systems for the production of recombinant TCRs in mammalian cell transfectomas have been documented [14–17], but as yet expression of these proteins in a prokaryotic host has not been reported. The use of *E. coli* as a host is attractive, as a bacterial expression system offers a convenient and rapid route for TCR production.

Recently antibody Fv, Fab, and single VH domains have been expressed as secreted proteins in fully functional form in *E. coli* [18–21]. Molecular modelling has indicated that there are structural similarities between the immunoglobulin Fab fragment and the extracellular domains of TCRs [22, 23]. This suggests that it might be

possible to develop an *E. coli*-based expression system for TCRs which is analogous to that used for antibody fragments. Such a system would greatly facilitate structure–function studies of TCRs, in addition to providing an extremely useful source of TCR protein for immunization to generate anti-clonotypic antibodies.

In this report, the cloning and expression of the variable domains (Vα and Vβ) of a pathogenic TCR in *E. coli* are described. The genes encoding the TCR Vα and Vβ domains of the T-cell hybridoma 1934.4 [24] have been isolated using the polymerase chain reaction (PCR, Ref. 25), and cloned into plasmid vectors designed for the expression and secretion of immunoglobulin domains [20]. These TCR genes have been selected for this study as the 1934.4 hybridoma is derived from a pathogenic CD4+ T-cell clone [26] which is associated with induction of experimental allergic (autoimmune) encephalomyelitis (EAE) in the H-2^u mouse. EAE is a prototypic model of T-cell mediated autoimmune disease, and is believed to be a valuable model for multiple sclerosis in humans [27]. Moreover, the binding specificity of the 1934.4 T-cell hybridoma has been extensively characterized [24]. Thus the expression of the 1934.4 TCR should allow studies to be carried out which may have important implications in both the mechanism and therapy of T-cell mediated autoimmune disease.

MATERIALS AND METHODS

Bacterial strains and plasmids. E. coli BMH71-18 [28] has been used as a host for the cloning and expression of TCR domains. The plasmid pSW1-VH-poly-tag1 has been previously described [20], except that pUC19 has been replaced by pUC119 [29] as the backbone vector.

Isolation of Vα and Vβ genes. The Vα and Vβ genes were isolated from 1934.4 hybridoma cells (a generous gift of Dr D. Wraith) using the PCR [25] and the following oligonucleotides (the 1934.4 TCR Vα and Vβ sequences have been described, Ref. 26):

Vα: I: 5′ ATC CTT CCA TGG CCG ACT CAG TGA CTC AGA CGG AAG GT 3′
II: 5′ AAG GAT GGT GAC CGG TTT AAT GGT GAG TTT GGT TCC 3′
Vβ: III: 5′ ATC CTT CCA TGG CCG AGG CTG CAG TCA CCC AAA GTC CA 3′
IV: 5′ AAG GAT GGT GAC CAG AAC AGT CAG TCT GGT TCC TGA 3′

Note that for each domain, the oligonucleotides encode either an *Nco*I or *Bst*EII site (indicated by underlining) to allow restriction enzyme digestion of the PCR products, followed by gel purification (using United States Biochemical Corp. 'Geneclean') and ligation as an *Nco*I-*Bst*EII fragment into VH*Nco*-poly-tag1 (see below). For the isolation of genomic DNA from 1934.4 hybridoma cells, 5×10^5 cells were pelleted, washed once with phosphate buffered saline and resuspended in 500 μl sterile distilled water. The cell suspension was then boiled for 5 min, and then centrifuged for 3 min at room temperature at 11,000 rpm [30]. Two to ten microlitres (2–10 μl) of the supernatant was used in each PCR. PCR conditions were as follows: a typical reaction comprised 3 units Promega Taq polymerase, 5 μl 10 × Promega reaction buffer, 25 pmol of each oligonucleotide primer, 0.2 mM dNTPs, 2–10 μl 1934.4 hybridoma supernatant, water to 50 μl. Cycling conditions were 94°C (0.5 min), 55°C (0.5 min), 72°C (1 min), and Taq polymerase was added at the end of the first cycle i.e. at 72°C. Thirty cycles of the PCR were performed, and an additional 3 units of Taq polymerase were added after 15 cycles to minimize the occurrence of PCR errors.

Construction of plasmids for expression of TCR Vα and Vβ domains. pSW1-VH-poly-tag1 [20] has been modified by the insertion of a unique *Nco*I site into the pelB leader sequence using site-directed mutagenesis [31] and the oligonucleotide 5′ GGC CAT GGC TGG TTG GG 3′ to generate VH*Nco*-poly-tag1. The genes, isolated and tailored by the PCR, were then cloned (in translational frame) as *Nco*I-*Bst*EII fragments into VH*Nco*-poly-tag1 to generate VαpelBtag1 and VβpelBtag1 (Fig. 1). To construct VαVβpelBtag1, VαpelBtag1 was modified by replacement of the 5′ *Hind*III site of pUC119 [29] with an *Eco*RI site (by ligation of oligonucleotide V. 5′ AGC TGA ATT C 3′ as a duplex into *Hind*III restricted VαpelBtag1; the *Eco*RI site is shown underlined and the ligation destroys the *Hind*III site) and then cloned as an *Eco*RI fragment into *Eco*RI restricted VβpelBtag1 (Fig. 1). Recombinants were then analysed for the correct orientation of the Vα gene with respect to the Vβ gene by restriction enzyme analysis. Dideoxynucleotide sequencing [32] was carried out to confirm the DNA sequences of the plasmid constructions shown in Fig. 1.

Expression analysis of Vα and Vβ domains. E. coli recombinants harbouring VαpelBtag1, VβpelBtag1, and VαVβpelBtag1 were grown up in 2 × TY plus 100 μg/ml ampicillin and 1% (wt/vol) glucose to early stationary phase, pelleted by centrifugation, washed once in either 2 × TY or 50 mM NaCl and then induced by resuspension in 2 × TY plus 100 μg/ml ampicillin and 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 14–16 h. Cultures were grown and induced at 37°C with shaking at 250 rpm. Culture supernatants were analysed by western blotting [33] as described [20], using the monoclonal antibody 9E10 [34] followed by anti-mouse Fc conjugated to horseradish peroxidase (ICN Immunobiologicals) for detection. Diaminobenzidine (Sigma, St Louis, MO, USA) was used as the horseradish peroxidase substrate.

RESULTS AND DISCUSSION

Expression of the single Vα and Vβ domains

The 1934.4 genes were cloned into VH*Nco*-poly-tag1 to generate VαpelBtag1 (Vα gene only)

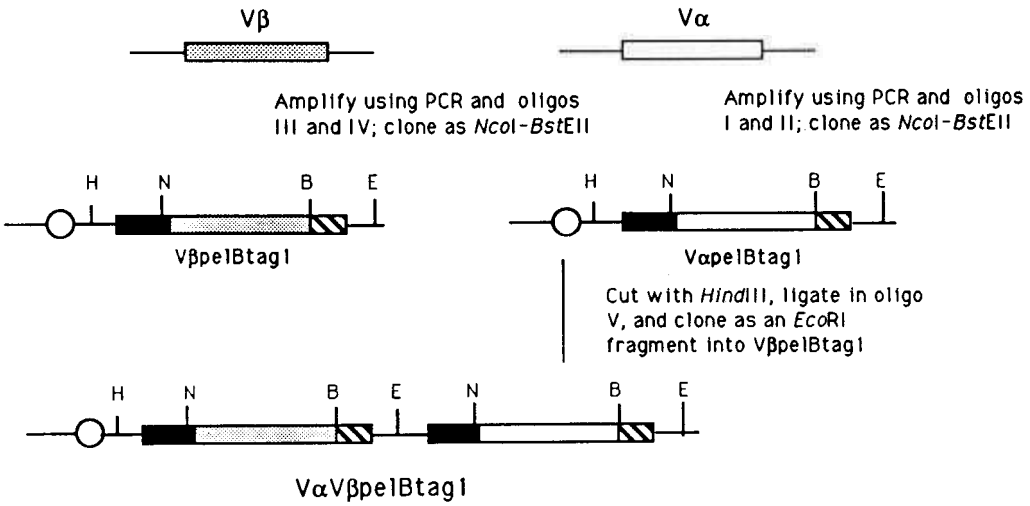

FIG. 1. Construction of plasmids for expression of the Vα domain (VαpelBtag1), Vβ domain (VβpelBtag1) and co-expression of the two domains (VαVβpelBtag1). H = *Hind*III, N = *Nco*I, B = *Bst*EII and E = *Eco*RI. Oligonucleotide numbers are as in the Materials and Methods section. Filled in box = pelB leader, stippled box = Vβ gene, striped box = five 3′ codons of VH gene in pSW1-VH-poly-tag1 [20] plus tag1 (c-myc) codons [34], open box = Vα gene and open circle = *lacz* promoter [29].

and VβpelBtag1 (Vβ gene only) and transformed into *E. coli* BMH71-18 (Fig. 1). The nucleotide sequences of the constructions were confirmed by DNA sequencing prior to growing up and inducing *E. coli* recombinants for expression, as described in the Materials and Methods section. Culture supernatants were then analysed by western blotting (Fig. 2). The Vα and Vβ domains are expressed individually, which is a similar result to that observed for the expression and secretion of single immunoglobulin VH domains [20]. The molecular weights, from SDS gel analysis, are estimated to be 17 kDa (Vα–tag1) and 14.5 kDa (Vβ–tag1). For the Vα domain this is significantly higher than that predicted by amino acid analysis, but in this respect anomalously low gel mobilities have been observed for single antibody VH domains (Ward, unpublished). Of interest is the particularly high level of secretion of the Vα domain: the expression level of this domain is similar to, if not greater than, that observed for the immunoglobulin FvD1.3 fragment expressed and secreted in *E. coli* [20]. The level is estimated to be 10 mg per litre of culture, by comparison with culture supernatants of *E. coli* recombinants harbouring pSW1-VHD1.3-VκD1.3-tag1 [20] using western blotting. The relatively high expression level of the Vα domain may reflect a propensity of this domain to form homodimers, as an earlier report describes the expression of Vα-immunoglobulin Cκ chimaeras as homodimers in mammalian cells [15]. Such homodimer formation could mask the hydrophobic residues of the Vα domain which, in a native TCR, interact with analogous Vβ residues during Vα:Vβ pairing, and this could increase the solubility (and secretion levels) of the homodimer. The oligomeric state of the secreted Vα domains is currently being assessed.

In contrast to the Vα domain, the Vβ domain is secreted into the culture supernatant at levels which are similar to those observed for single immunoglobulin VH domains (about 0.5–1 mg per litre of culture, Ref. 20), despite the observation that the intracellular/periplasmic levels of the Vβ domain are similar to those of the Vα domain (data not shown). The reasons for the lower secretion levels of the Vβ domain are currently unclear, but suggest that this protein does not fold into a soluble form as readily as the Vα domain. Thus it may be possible to increase the amount of secreted Vβ by altering the induction conditions. Alternatively, high levels of soluble Vβ domain may be obtainable by osmotically

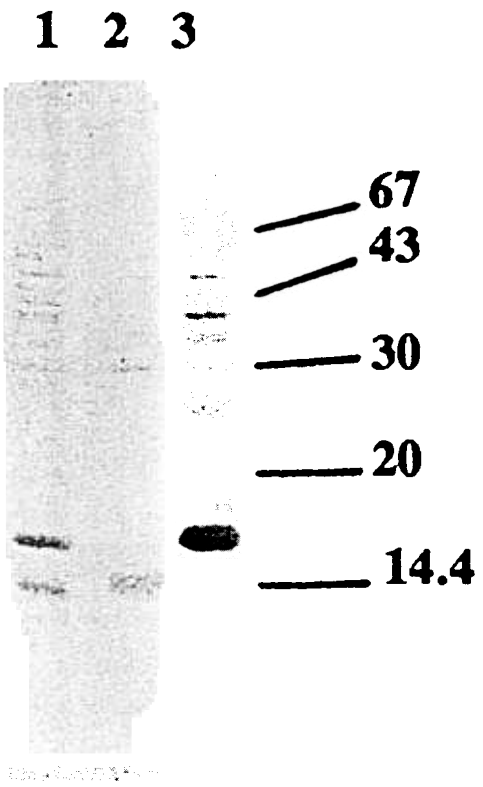

FIG. 2. Expression analysis of Vα and Vβ domains, by western blotting of culture supernatants electrophoresed on a 15% SDS polyacrylamide gel. *E. coli* recombinants harbouring the following plasmids were analysed: lane 1, VαVβpelBtag1; lane 2, VβpelBtag1, and lane 3, VαpelBtag1. The mobilities of molecular weight size standards, run on an equivalent gel which was stained with Coomassie brilliant blue rather than transferred onto nitrocellulose, are indicated (in kDa) on the right margin.

shocking the recombinant *E. coli* cells, followed by denaturation and refolding of the released Vβ protein.

Co-expression of Vα and Vβ domains

The Vα and Vβ domains can be co-expressed and secreted from *E. coli* recombinants harbouring VαVβpelBtag1 (Fig. 2). The Vα polypeptide is secreted in excess over the Vβ domain, suggesting that at least some of the recombinant TCR protein is not heterodimeric. Interestingly, however, the Vα domain secretion levels are lower when co-expressed with the Vβ polypeptide than when expressed and secreted as a single domain (compare lanes 1 and 3, Fig. 2). The possible reasons for this are currently under investigation. This may be due, for example, to limitations on the amount of protein which can be secreted into the *E. coli* periplasm i.e. Vβ secretion may compete with Vα secretion. Alternatively, there may be some polarity effects on the expression of the Vα domain, which is 3′ to the Vβ gene in VαVβpelBtag1 (Fig. 1).

In summary, the 1934.4 TCR Vα and Vβ polypeptides can be expressed and secreted from recombinant *E. coli* cells as either individual domains or co-expressed. This secretion system can be used as a rapid and economically favourable alternative to the existing methods for the production of TCRs or TCR-immunoglobulin chimaeras in mammalian cell transfectomas [14–17]. The yields of about 10 mg/l for Vα and 0.5–1 mg/l for Vβ can be readily scaled up to produce relatively large quantities of these TCR domains in a matter of days, using the peptide tag as an epitope for affinity purification with the 9E10 monoclonal antibody [34]. Thus the expression system could provide a valuable source of soluble TCR protein for use in immunizations for the generation of anti-clonotypic antibodies, which could be used for example in passive immunization for the treatment of disease. It is also conceivable that such soluble TCRs (derived from pathogenic T cells) could be used in vaccination to generate a specific anti-TCR response in vivo in a similar way to that reported using peptides derived from TCR V-regions [35–37]. Moreover, the recombinant Vα or Vβ domains, or even subfragments thereof, could be extremely useful for mapping the TCR residues which are functionally important in binding peptide-MHC complexes in murine EAE.

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