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Rapid Note

Expression of soluble T-cell receptor fragments derived from a T-cell clone associated with murine collagen-induced arthritis

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The majority of T cells bear T-cell receptors (TCRs) that comprise $\alpha\beta$ heterodimers [1,2], and these TCRs are invariably membrane bound. $\alpha\beta$ TCRs recognise antigenic peptides bound to the cleft of polymorphic major histocompatibility complex (MHC) class I or class II glycoproteins and are 'MHC restricted' [3]. The TCR–peptide–MHC interaction is of central importance in the immune system, and an understanding of this interaction at the molecular level would be of value in, for example, treating autoimmune diseases in which autoreactive T cells are involved in pathogenesis. Towards this aim, a number of groups have recently described systems for the expression of soluble TCRs in mammalian [4–6] and prokaryotic systems [7–9] and these provide valuable material for use in structure–function studies.

In this study we have expanded the use of an *Escherichia coli* secretion system developed in our laboratory [7,9] to the production of the variable domains of a TCR derived from a T-cell hybridoma [10] that is associated with murine collagen-induced arthritis (CIA). CIA is an instructive model for rheumatoid arthritis in humans [reviewed in 11]. The T-cell hybridoma recognises an epitope within peptide 245–270 of type II collagen (cII) associated with the class II molecule I-A^q [10]. Residues contained within cII 260–270 have been shown to be important in both T-cell recognition and suppression of CIA [12]. Furthermore, analogues of this peptide prevent disease [10]. Thus, study of this TCR which is representative of pathogenic T cells in both recognition specificity [10] and TCR gene usage [13] could give valuable insight into the molecular mechanism by which disease-causing T cells interact with target autoantigens. In addition, the production of soluble TCRs that are associated with CIA could provide reagents for use as immunomodulating reagents.

The T-cell hybridoma qCII85.33 [10] was used as a source of Vα and Vβ domain genes for this study and was a generous gift of Dr. E. Rosloniec (University of Tennessee, Memphis). The gene families (Vβ, Dβ and Jβ and Vα, Jα) from which the Vα and Vβ domain genes were assembled were identified and provided to us by Dr. Rosloniec.

PCR primers were designed to clone the genes as *Nco*I-*Bst*EII fragments into VαpelBHis and VβpelBHis [9], using primer design analogous to that described for the in-frame cloning of the 1934.4 TCR genes into these vectors [9]. Total RNA was isolated from the hybridoma, and cDNA synthesis carried out as described previously [7]. Aliquots of the cDNA syntheses were used in the PCR with appropriate Vα and Vβ domain-specific primers. The Vα and Vβ domain PCR products were restricted with *Nco*I and *Bst*EII and ligated into VαpelBHis and VβpelBHis, respectively (Fig. 1). Resulting ligation mixes were transformed into *E. coli* BMH71-18 and the recombinant clones used as a source of DNA for sequencing. For each Vα and Vβ domain gene, several independent cDNA and PCR isolates were made to eliminate the possibility of PCR errors in the final expression constructions. To construct a plasmid for the expression of the Vα and Vβ domains as a single-chain TCR (scTCR) in which the Vα and Vβ domains are linked by a synthetic peptide linker, the 3-step procedure shown in Fig. 1 was used. Recombinant *E. coli* cells harbouring qcII85.33 VαpelBHis, qcII85.33 VβpelBHis and qcII85.33 scTCR were grown up and induced for expression as described previously

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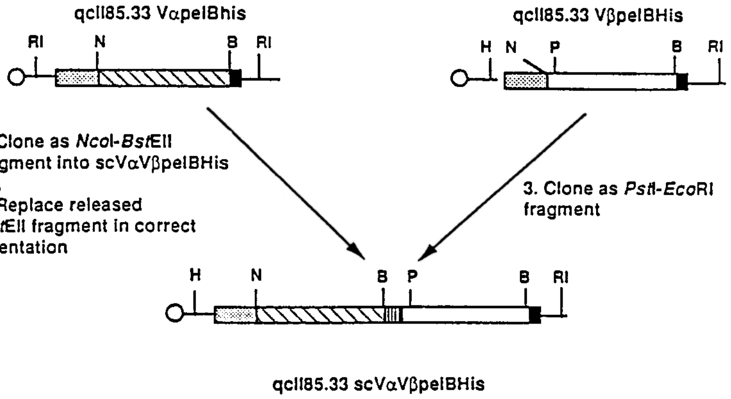

Fig. 1. Construction of the plasmids for expression of soluble TCR fragments derived from the qcII85.33 T-cell hybridoma. Open circle = lacz promoter; stippled box = pelB leader; hatched box = Vα domain gene; filled-in box = his6 peptide tag; open box = Vβ domain gene; single lines = vector backbone; vertical lines = single-chain linker peptide. B = *Bst*EII; RI = *Eco*RI; H = *Hind*III; N = *Nco*I; P = *Pst*I.

qcII85.33 Vα domain

```
                                 Vα11
          10        20        30        40        50        60
           |         |         |         |         |         |
GATCAGGTGGAGCAGAGTCCTTCAGCCCTGAGCCTCCACGAGGGAACCGATTCTGCTCTG
 D  Q  V  E  Q  S  P  S  A  L  S  L  H  E  G  T  D  S  A  L

          70        80-       90       100       110       120
           |         |         |         |         |         |
AGATGCAATTTTACGACCACCATGAGGAGTGTGCAGTGGTTCCGACAGAATTCCAGGGGC
 R  C  N  F  T  T  T  M  R  S  V  Q  W  F  R  Q  N  S  R  G

         130       140       150       160       170       180
           |         |         |         |         |         |
AGCCTCATCAGTTTGTTCTACTTGGCTTCAGGAACAAAGGAGAATGGGAGGCTAAAGTCA
 S  L  I  S  L  F  Y  L  A  S  G  T  K  E  N  G  R  L  K  S

         190       200       210       220       230       240
           |         |         |         |         |         |
GCATTTGATTCTAAGGAGCGGCGCTACAGCACCCTGCACATCAGGGATGCCCAGCTGGAG
 A  F  D  S  K  E  R  R  Y  S  T  L  H  I  R  D  A  Q  L  E

         250       260       270       280       290       300
           |         |         |         |         |         |
GACTCAGGCACTTACTTCTGTGCTGCTGAGGCCTCTTCTGGCAGCTGGCAACTCATCTTT
 D  S  G  T  Y  F  C  A  A  E  A  S  S  G  S  W  Q  L  I  F

         310       320       330              Jα17
           |         |         |
GGATCTGGAACCCAACTGACAGTTATGCCG
 G  S  G  T  Q  L  T  V  M  P
```

qcII85.33 Vβ domain

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     Vβ8.3              Dβ1                Jβ1.4
                280       290       300       310
                 |         |         |         |
       ...TGTGCCAGCAGTGCTGGGGACGAAAGATTATTTTTCGGTCATGGAACC
       ... C  A  S  S  A  G  D  E  R  L  F  F  G  H  G  T

320       330
 |         |
AAGCTGTCTGTCTTG
 K  L  S  V  L
```

Fig. 2. Nucleotide and amino acid sequences of the Vα and Vβ domain genes of the qcII85.33 T-cell hybridoma. Nucleotide numbers are shown above the sequences.

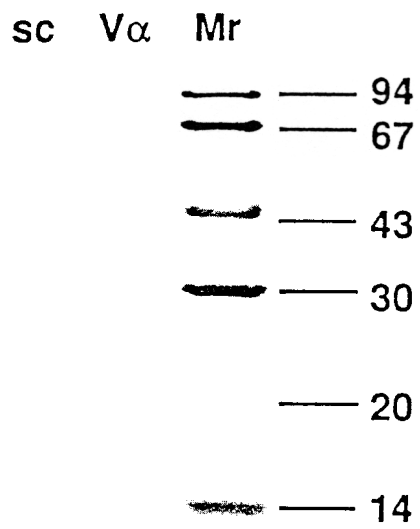

Fig. 3. 15% SDS polyacrylamide gel analysis (stained with Coomassie brilliant blue) of the purified qcII85.3 Vα and scTCR (sc). Mr = molecular weight standards with sizes shown on the right margin in kilodaltons.

[9] and soluble TCR fragments were purified from osmotic shock fractions using Ni^{2+}–NTA–agarose.

The sequences of the Vα and Vβ domain genes of the qcII85.33 hybridoma are shown in Fig. 2, together with the Vα, Jα and Vβ, Dβ, Jβ families used. The 85.33 Vα, Vβ domains and scTCR can be purified using Ni^{2+}–NTA–agarose in yields of 2–4 mg, less than 0.1 mg and 0.2–0.4 mg/l of culture, respectively. For the Vβ domain, the expression level is low and it is relatively insoluble, similar to the observations made for the 1934.4 Vβ domain [9]. Linkage of this Vβ domain to the Vα domain as a scTCR greatly improves the solubility and expression yield (Fig. 3). As a single domain, the Vα domain is expressed in high yields and is soluble at concentrations of greater than 5 mg/ml (data not shown). The reason for the significantly higher expression yield of the Vα domain relative to the Vβ domain is not clear, but earlier data [7] concerning the 1934.4 TCR indicated that the differences in yield between Vα and Vβ domains were not due to differences in translational efficiency. It therefore appears that the relative insolubility of the Vβ domain accounts for the low purification yield, due to either aggregation in the cytosol or in the periplasm following export.

In summary, the data show that a TCR associated with CIA can be secreted as soluble variable domains from recombinant *E. coli* cells. The generality of the expression system suggests that it can be used to produce V domains of any TCR of interest that may be derived from human or rodent sources. The yields of the qcII85.33 Vα domain and scTCR are high enough for use as immunomodulating reagents and in immunisations to generate anti-clonotypic antibodies. For example, the domains could be used in vaccination experiments [14,15] to treat CIA. Finally, the high solubility and expression yield of the qcII85.33 Vα domain makes it particularly attractive for use in high resolution structural studies.

Acknowledgements

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