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T Cell Receptor CDR3 Sequences and Recombinant T Cell Receptors

**Prerequisites for Developing Ligands to Interfere with T Cell Receptor
Binding to the MHC/Peptide Complex**

Abstract

Background: The interaction of T cell receptors (TCRs) with peptide fragments bound to major histocompatibility complex (MHC) molecules is central to the initiation and propagation of most immune responses. In order to understand and control the molecular interactions underlying T cell recognition of MHC/peptide complexes, recent efforts have focused on the production of recombinant soluble forms of the TCR heterodimer. **Methods:** TCRA variable (TCRAV) and TCRBV sequences used by human T cell clones were amplified by PCR, cloned and sequenced. The deduced amino acid sequences of the complementarity determining region (CDR) 3 loops of TCRs specific for the same allergenic epitope were compared. V region genes of a selected TCR were expressed as a single-chain (sc) molecule in the periplasm of *Escherichia coli*. **Results:** Conserved amino acid motifs specific for allergenic peptides of Bet v 1 and Phl p 1 were identified in CDR3 sequences of TCRs. A recombinant scTCR was produced. The ratio of insoluble to soluble material was 1:1. The recombinant protein was of the correct size and showed no signs of degradation. **Conclusions:** Conservation of amino acid motifs in CDR3 loops of TCRs specific for the same allergen fragments indicated that the three-dimensional structure of the CDR3 was determined by the presented peptide. The recombinant scTCR will be used to identify ligands for the CDR3s from random peptide libraries to interfere with TCR binding to the MHC/peptide complex.

Key Words

CDR3 sequences
Single-chain T cell receptors
Bet v 1
Phl p 1

Introduction

The T cell receptor (TCR)/CD3 complex is a multichain glycoprotein assembly on the surface of T cells, responsible for peptide-major histocompatibility complex (MHC) recognition and subsequent signal transduction. Recognition of MHC-presented antigen fragments by the disulfide-linked $\alpha\beta$ -heterodimer of the TCR is a critical step in the initiation of a specific immune response. The TCR α - and β -chains have variable (V) and constant domains analogous to those of antibodies. Hypervariable segments in the TCR V domains are probably functionally equivalent to the com-

plementarity-determining regions (CDRs) of antibodies. A model has been proposed in which the predicted CDR3 loops of both chains (encoded by the VDJ junctions) bind peptide, whereas the germline-encoded CDR loops contact the α -helices of the MHC molecule [1, 2].

Given the importance of understanding the process by which T cells recognize pathogens and autoantigens, recent efforts have focused on the production of recombinant soluble forms of the TCR heterodimer. As the assembly of the recombinant heterodimer is rather inefficient, single-chain (sc) TCRs have been designed in which the V regions of the heterodimer are joined by a short peptide linker.

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Bet v 1₇₇₋₉₂ TNFKYNYSVIEGGPIG

TCC	CDR3 α (8-15aa)	CDR3 β (9-10aa)
XPBP 53I	<u>MGETGGPKTI</u>	<u>DLRETNEKLF</u>
173I	<u>NTGFOKLV</u>	<u>DGPTNEKLF</u>
XPGZ 17I	<u>VRGGGADGLT</u>	<u>RLRRSTDTOY</u>
XPHC 5I	<u>ESLLIKETSGSRLT</u>	<u>MGGALEKLF</u>
8III	<u>KTGYSSASKII</u>	<u>LRANPGYEQY</u>
XPSZ 11II	<u>DEFVRNYGGATNKLI</u>	<u>LARGTLYEQY</u>
31II	<u>NNRGGVYGLT</u>	<u>AETLNEKLF</u>
31II	<u>PPRAGGTSYGKLT</u>	
MOTIF	R/K (X, X X) G G	L (X) R

Fig. 1. Deduced amino acid sequences of CDR3 loops used by TCRA and B chains specific for the Bet v 1 epitope 77–92. The underlined residues were encoded by the J segments. Restricted amino acid residues are in bold letters. TCC = T cell clones.

The production of scTCRs in bacterial expression systems and data indicating that scTCRs can in fact recognize their natural ligands have been reported [3].

Materials and Methods

CDR3 Sequences of Epitope-Mapped T Cell Clones

T cell clones were epitope mapped [4] and total RNA was isolated from 1×10^6 cells. Single-stranded cDNAs of TCRA and B sequences were directly amplified by PCR [5]. The PCR reaction products were cloned and sequenced, and the deduced amino acid sequences of the CDR3 loops of TCRs specific for the same allergenic epitope were compared.

Construction and Expression of a Single-Chain T Cell Receptor

cDNAs of TCRAV and BV chains were prepared from mRNA of a T cell clone whose TCR contained a high amount of charged amino acids. V region genes were connected by a flexible linker [(G₄S)₃ GGRASG₄S]. The construct also contained an affinity tag based on the FLAG[®] peptide at the C terminus [6]. The resulting DNA was inserted in the expression vector pSL300 which added the pelB signal sequence for secretion of the product into the *Escherichia coli* periplasm to the C terminus and a 6×His tag to the N terminus for affinity purification. The scTCR gene was expressed in *E. coli* strain SB536 (WG1 Dfhua DhhoAB). Expression of the TCR fragments in the periplasm and preparation of periplasmic fractions were carried out as described [7]. The scTCR was purified over a Ni-NTA (nickel-nitriloacetic acid) resin (Quiagen) using the 6×His affinity tag. The bound protein was eluted with increasing concentrations of imidazole. Eluted fractions were tested for the presence of the recombinant protein on immunoblots using a monoclonal anti-FLAG antibody.

Results

CDR3 Sequences of TCRs Specific for a Bet v 1 Fragment

TCRA. The motif GG was present in five of eight sequences and reduced to G in another one. A basic amino acid (R or K) preceded the G(G) motif at the C terminus of the CDR3 sequence in five of six cases where that motif was present. The gap between the basic residue and the first G was between zero and two amino acid residues wide. The T cell clone XPSZ 31II had two different mRNA species coding for functionally rearranged TCRA chains both containing the described characteristics within otherwise different CDR3 sequences (fig. 1).

TCRB. The combination of the hydrophobic residue L and the basic residue R was present at the N-terminus of the CDR3 loop in four of seven sequences. In one case, the two residues were separated by an A (fig. 1).

CDR3 Sequences of TCRs Specific for a Phl p 1 Fragment

TCRA. The motif GX(X)S could be detected in five to six sequences. In one instance, the G was separated from the S residue by two amino acid residues instead of one (XPHER 47I) (fig. 2).

TCRB. A core motif of GXG was found in four of six sequences. In three cases, this core was extended by a small amino acid residue (G or A) on one side of the motif and in two cases by the hydrophobic residue L on the other side. Remarkably, the residues of the two longest motifs (XPNO 5III and 34III) were entirely encoded by randomly added nucleotides, as was the core motif of XPNO 29III (fig. 2).

Phl p 1₁₀₃₋₁₁₄ YHFDLSGHAFGA

TCC		CDR3 α (9-11aa)	CDR3 β (8-10aa)
XPNO	5III	<u>Y</u> <u>T</u> <u>O</u> <u>G</u> <u>G</u> <u>S</u> <u>E</u> <u>K</u> <u>L</u> <u>V</u>	<u>R</u> <u>G</u> <u>G</u> <u>A</u> <u>G</u> <u>L</u> <u>N</u> <u>E</u> <u>O</u> <u>F</u>
	29III	<u>V</u> <u>I</u> <u>G</u> <u>G</u> <u>S</u> <u>G</u> <u>N</u> <u>T</u> <u>P</u> <u>L</u> <u>V</u>	<u>Y</u> <u>G</u> <u>L</u> <u>G</u> <u>Y</u> <u>G</u> <u>Y</u> <u>T</u>
	34III	<u>T</u> <u>R</u> <u>A</u> <u>A</u> <u>G</u> <u>N</u> <u>K</u> <u>L</u> <u>T</u>	<u>L</u> <u>G</u> <u>G</u> <u>G</u> <u>A</u> <u>T</u> <u>O</u> <u>Y</u>
XPEC	29I	<u>D</u> <u>A</u> <u>L</u> <u>Y</u> <u>A</u> <u>G</u> <u>K</u> <u>S</u> <u>T</u>	<u>A</u> <u>G</u> <u>T</u> <u>P</u> <u>K</u> <u>L</u> <u>E</u> <u>A</u> <u>F</u>
XPLV	24I	<u>V</u> <u>V</u> <u>G</u> <u>D</u> <u>S</u> <u>W</u> <u>G</u> <u>K</u> <u>L</u> <u>O</u>	<u>T</u> <u>S</u> <u>S</u> <u>G</u> <u>R</u> <u>D</u> <u>T</u> <u>O</u> <u>Y</u>
XPHR	47I	<u>G</u> <u>I</u> <u>I</u> <u>S</u> <u>G</u> <u>T</u> <u>Y</u> <u>K</u> <u>Y</u> <u>I</u>	<u>L</u> <u>P</u> <u>G</u> <u>G</u> <u>A</u> <u>G</u> <u>E</u> <u>L</u> <u>F</u>
MOTIF		G X (X) S	CORE G X G

Fig. 2. Deduced amino acid sequences of CDR3 loops used by TCRA and B chains specific for the Phl p 1 epitope 103–114. The underlined residues were encoded by the J segments. Restricted amino acid residues are in bold letters. TCC = T cell clones.

scTCR

After isolation of the recombinant protein, the ratio of insoluble to soluble material was 1:1. The affinity-purified scTCR detected by immunoblot had the correct molecular size and showed no signs of degradation even after prolonged storage at room temperature.

Discussion

The functionality of any protein is determined by its full three-dimensional structure. Thus, similarity of shape remains detectable even though the sequence may have changed beyond recognition [8]. Therefore, CDR3 loops may still display similar three-dimensional structures, even when the primary sequences show very little restriction of recognizable motifs. That they in fact showed some restric-

tion indicated that their three-dimensional structure was selected by the allergenic peptide. To identify ligands for CDR3 loops that will interfere with TCR binding to the presented allergen fragment, random peptide libraries displayed on the surface of filamentous bacteriophages can be used [9].

The purified rTCR will be allowed to react with a random peptides library in solution. Reactive peptides displaying phage clones will be identified and their DNA sequenced. If a limited number of related sequences arises from the screening, we will then investigate the possibility to suppress a specific T cell response with the ligands thus identified.

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