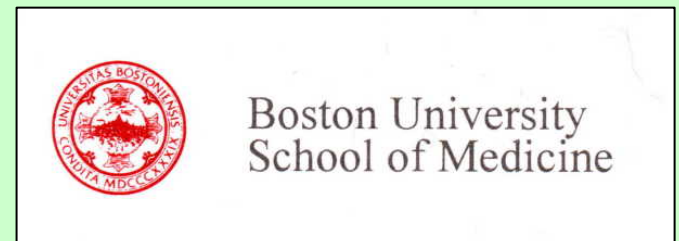


Peptides Recognized by T Cell Clones from Inflammatory Bowel Disease Patients

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Background

The etiology of inflammatory bowel disease (IBD) is unknown, and involves an interplay of the environment, genetics, and an over-reactive immunologic response. There is abundant evidence from animal models, and human IBD tissue research, for the involvement of CD4+ T cells in IBD. Due to their presence and analysis of their antigen recognition sites, the T cell receptor (TCR), it is likely that the CD4+ T cells act as promoters of the chronic intestinal inflammation. Since the majority of CD4+ T cells are stimulated by the recognition of peptide antigens, the determination of the exciting antigen(s) by these T cells could lead to the identification of either a microbial or autologous source of their stimulation.

Testing of individual CD4+ T cells for their potential antigens derived from tissues has not been feasible because their poor long term proliferative capacity. To overcome this shortfall new methodologies have been developed that allow identification of peptide antigens for TCRs. These new methods require cloning and expression of the variable regions of the TCR's alpha and beta chains in an immortal cell line, and then using a positional scanning synthetic combinatorial peptide library to identify antigenic determinants. The results of these investigations may provide not only insight into potential etiologies of cell activation, but also allow the development of new technologies designed to inhibit the cell responses.

Main Goal of this Research Project

Identify activating peptide antigens for disease relevant CD4⁺ T cells obtained from IBD intestinal tissue samples

Novel strategies and approaches used to achieve this goal:

1 – Selection of T cells that are most likely involved in the inflammatory processes in the intestinal mucosa.

For rapid identification of dominant, expanded T cell clones in diseased tissue, we use a new approach to complete TCR repertoire analysis, which combines real-time polymerase chain reaction (QPCR) with high definition agarose electrophoresis CDR3 displays. Novel sets of primers were designed to capture all known TCR α and β chain family members.

2 – Amplification and cloning of TCR α and β variable chains from molecularly phenotyped single CD4⁺ T cells.

In order to generate long-lasting TCR-expressing clones for investigations of peptide recognition, we must first identify dominant, expanded CD4⁺ single T cells and clone their TCR α and β chains. The desired cells are selected by labeling with anti-CD4-coated magnetic beads, and manually transfer single cells each into reaction well. cDNA is synthesized from each cell and amplified by SMART PCR cDNA amplification. The cDNA obtained is enough not only for cloning of the TCR α and β chains but for molecular phenotyping of the cell by QPCR, e.g. Treg, IL-2 producing, NKT cell.

Novel strategies and approaches used to achieve this goal:

3 – Construction of chimeric TCRs and their expression in a mouse hybridoma cell.

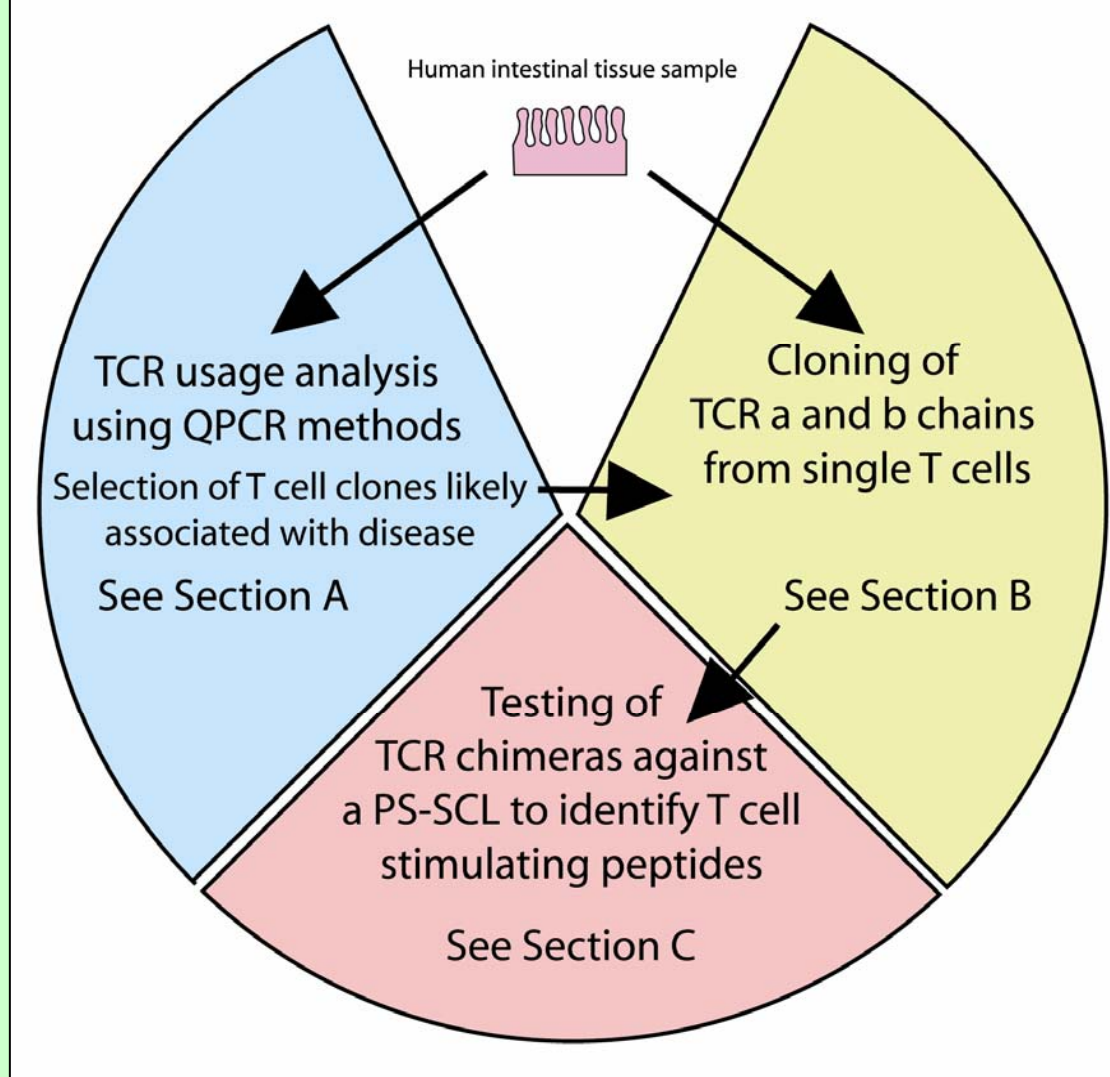
In order to obtain the large number of clonal cells necessary for TCR specificity for peptide library screening, the cloned TCR α and β chains from a single cell are fused to the mouse TCR constant region in a mammalian expression vector. These human-mouse chimeric TCRs are then expressed in a mouse hybridoma cell line which has been engineered to respond to signaling through the TCR complex with interleukin-2 production.

4 – Screening of a positional scanning synthetic combinatorial library (PS-SCL) of peptides. The IBD TCR expressing chimeric T cell hybridomas are tested against a PS-SCL of decapeptides to identify activating peptide sequences. This type of library is organized in pools and each pool consist of peptides with one specific amino acid occupying a fixed position in the sequence while all other positions are random. The results of testing of a human-mouse chimeric clone against a PS-SCL will yield the sequence for an optimal stimulating peptide.

5 – Search of protein and DNA sequence databases for peptides homologous to those capable of activating TCR-expressing hybridoma cells.

Automatic database searches of prokaryotic and eukaryotic DNA/peptide sequences using Perl scripts will be performed to identify proteins with sequence homologies to the activating peptides.

Overview of Research Project



Section A

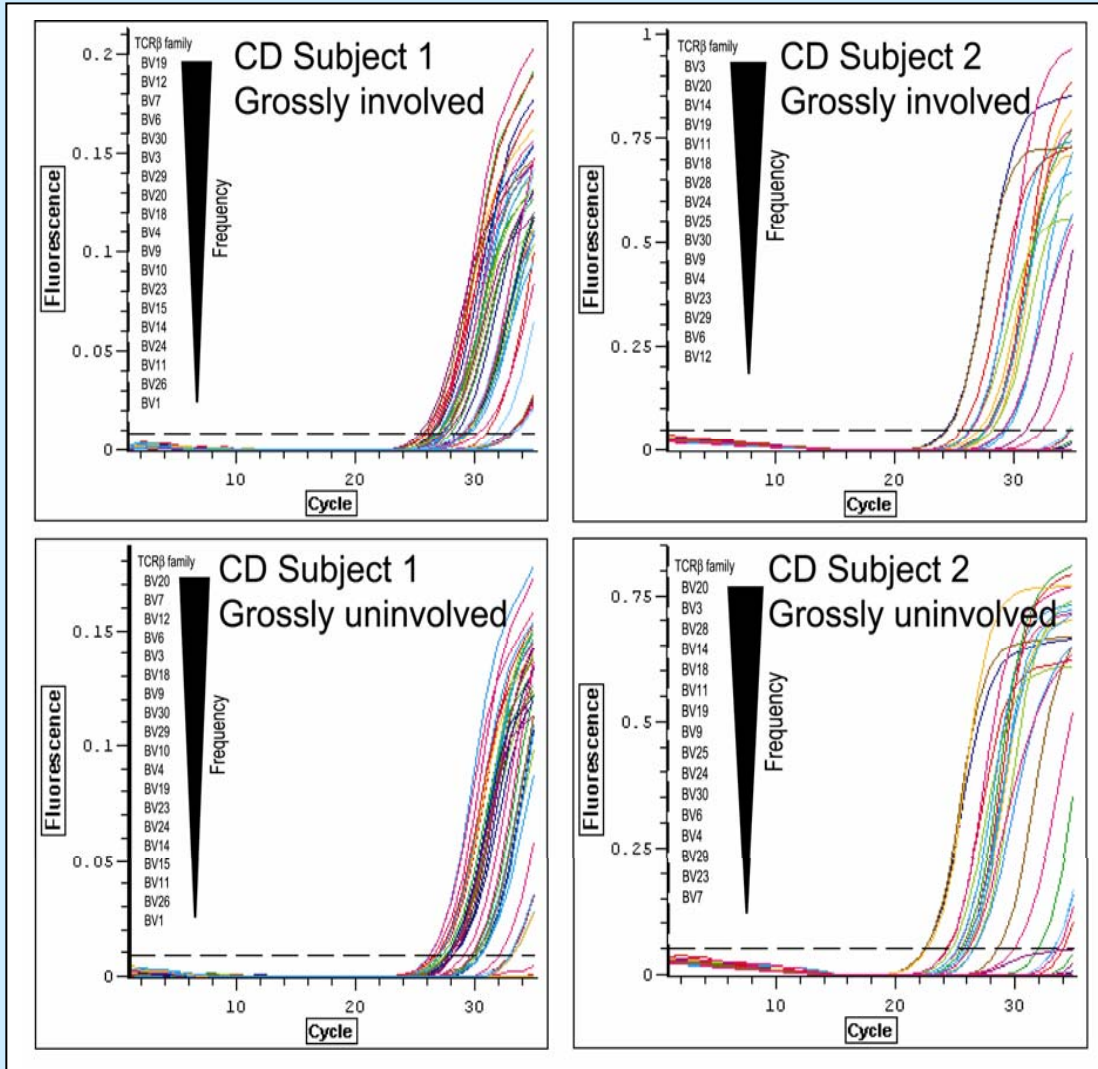


Figure 2. Example of QPCR analysis of TCRβ family usage from two CD subjects. Tissue specimens were taken from two separate sites with grossly different disease involvement and cDNA was synthesized and SMART amplified. QPCR was performed in duplicate in Qiagen Sybr Green mix using 27 individual TCRβ primers combined with a TCR constant region primer. Input cDNA amounts were normalized using β-actin QPCR estimations. The results are summarized in the list of families in decreasing order of abundance.

Section A

Figure 3. Rankings of TCR of TCR chain families in descending order of abundance. The families highlighted show more abundance in inflamed tissue. QPCR reactions were performed as described in Figure 2 with TCR α specific primers. The actual Sybr Green fluorescent curves are not shown.

CD Subject 1		CD Subject 2	
Grossly involved	Grossly uninvolved	Grossly involved	Grossly uninvolved
TRAV13	TRAV19	TRAV12	TRAV12
TRAV9	TRAV5	TRAV38	TRAV38
TRAV38	TRAV9	TRAV6	TRAV9
TRAV12	TRAV12	TRAV9	TRAV21
TRAV21	TRAV20	TRAV13	TRAV13
TRAV1	TRAV13	TRAV5	TRAV23
TRAV26	TRAV21	TRAV21	TRAV17
TRAV27	TRAV8	TRAV1	TRAV20
TRAV8	TRAV26	TRAV26	TRAV26
TRAV20	TRAV7	TRAV20	TRAV27
TRAV23	TRAV35	TRAV23	TRAV29
TRAV5	TRAV38	TRAV17	TRAV1
TRAV17	TRAV6	TRAV29	TRAV8
TRAV10	TRAV16	TRAV19	TRAV5
TRAV6	TRAV1	TRAV27	TRAV19
TRAV25	TRAV23	TRAV8	TRAV6
TRAV14	TRAV4	TRAV4	TRAV35
TRAV29	TRAV40	TRAV24	TRAV24
TRAV19	TRAV2	TRAV2	TRAV25
TRAV22	TRAV22	TRAV35	TRAV16
TRAV35	TRAV30	TRAV39	TRAV2
TRAV41	TRAV39	TRAV16	TRAV41
TRAV24	TRAV36	TRAV25	TRAV4
TRAV39	TRAV24	TRAV10	TRAV22
TRAV40	TRAV18	TRAV30	TRAV10
TRAV34	TRAV27	TRAV22	TRAV39
TRAV2	TRAV25	TRAV41	TRAV14
TRAV36	TRAV34	TRAV14	TRAV30
TRAV30	TRAV14	TRAV34	TRAV40
TRAV3	TRAV11	TRAV40	TRAV34
TRAV7	TRAV41	TRAV18	TRAV3
TRAV11	TRAV17	TRAV3	TRAV36
TRAV18	TRAV29	TRAV36	TRAV18
TRAV4	TRAV10	TRAV7	TRAV7
		TRAV11	TRAV11

Section A

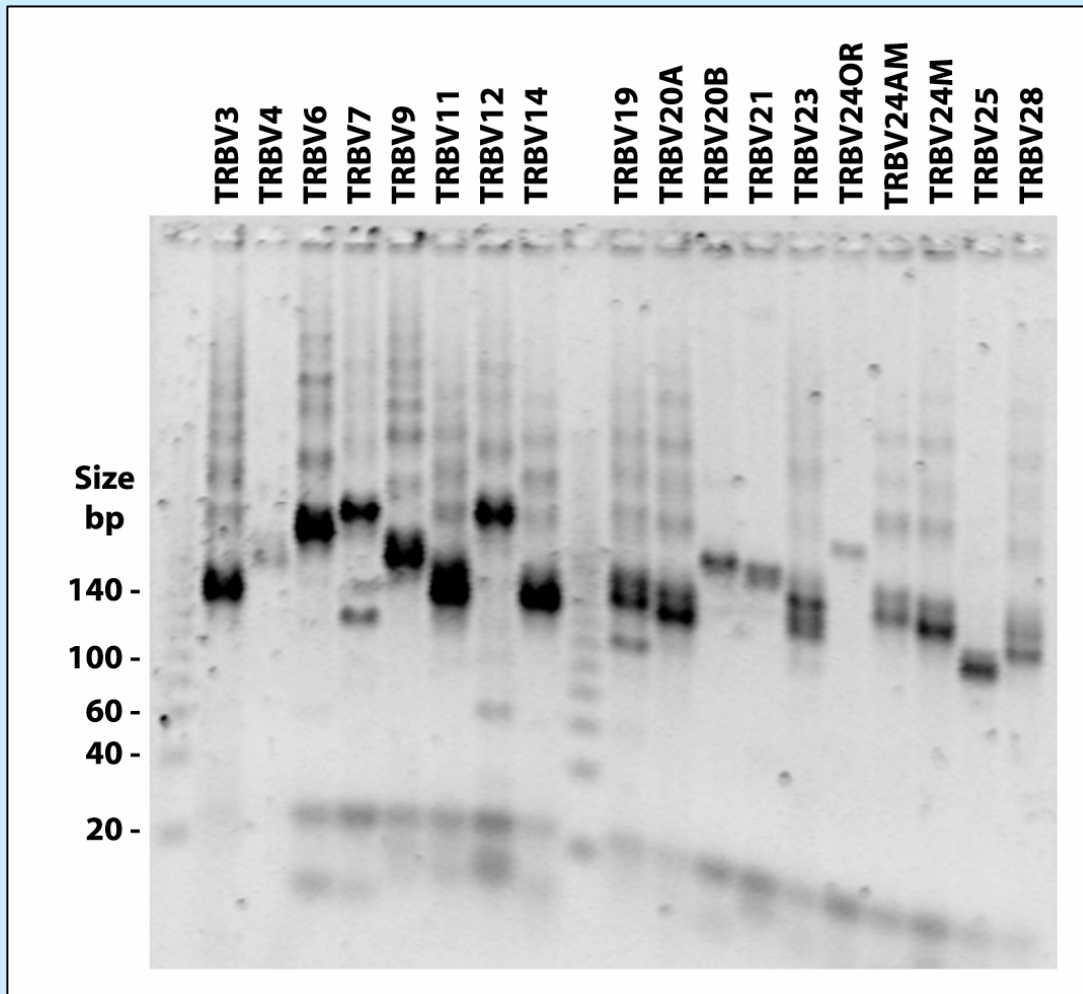
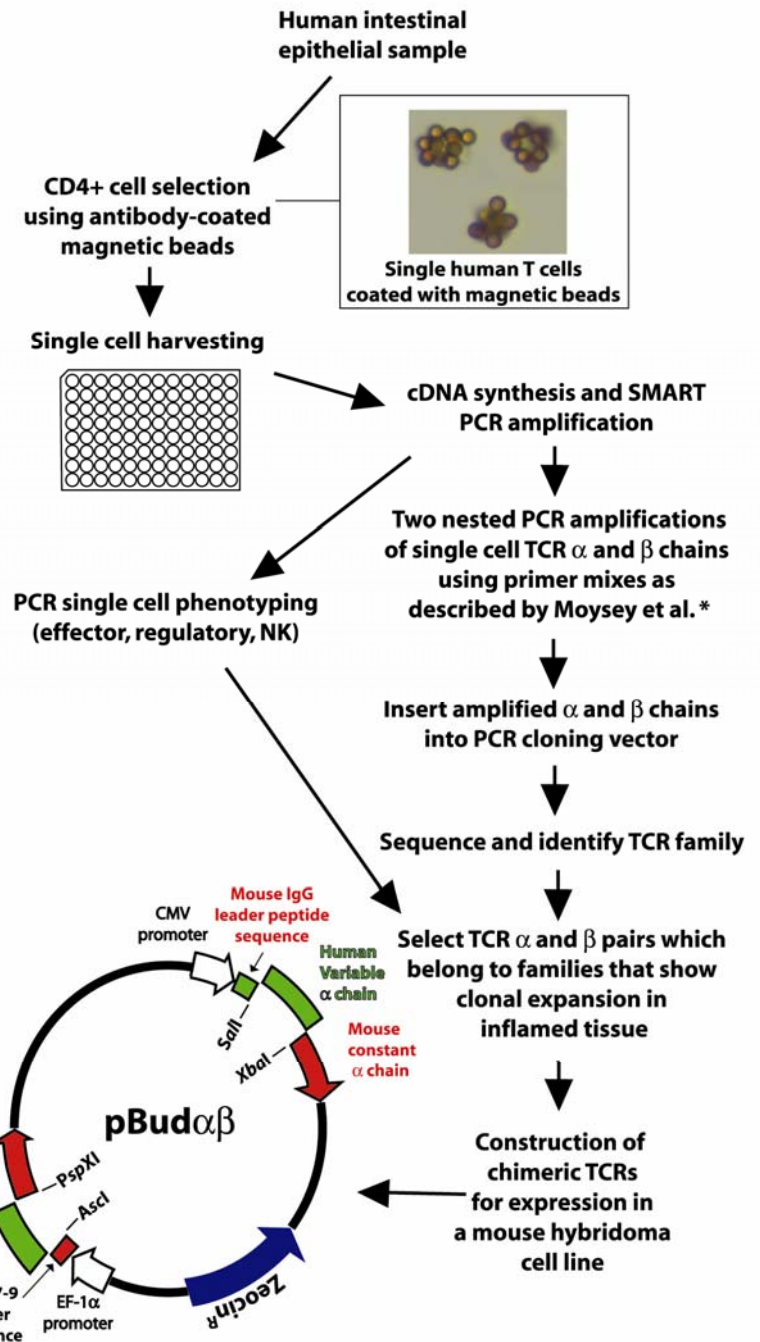


Figure 4. Third complementary determining region (CDR3) length analysis using high resolution MetaPhor agarose gel. PCR amplifications of TCR β chains were performed using cDNA obtained from an inflamed Crohn's disease tissue site. The PCR products were separated in a 2% MetaPhor agarose gel in 0.5 x TBE at 400 V for 1.5 hours. The gel was stained with Sybr Green and photographed under UV light. Notice that some TCR β families show a non-gaussian pattern of product lengths.

Experimental outline for Section B



* Moysey R, Vuidepot AL, Boulter JM. 2004. Amplification and one step expression cloning of human T cell receptors. *Anal. Biochem.* 326:284-286.

Section B

Sequence of the Chimeric TCR α chain in pBud α 1G3.

Patient 5, lane 23 in Fig5

```

sbfI
1 CCTGCAGGCAAT GTGGCCACCA TGGGATGGAG CTGTATCATC CTCTTCTTGG TAGCAACAGC TACAGGTAAG GGTTAACAG TAGCAGGCTT GAGGTCTGGA
      M G W S C I I L F L V A T A T
                                     HpaI
97 CATATATATG GGTGACAATG ACATCCACTT TGCCTTTTCTC TCCACAGCGC CGCACTCCCA GGTCCAAC TG CAGGTCGACC AGCAGCAGGT GAAACAAAGT
      G A H S Q V Q L Q V D Q Q Q V K Q S
                                     Sali
197 CCTCAATCTT TGATAGTCCA GAAAGGAGGG ATTICAATTA TAAACTGTGC TTATGAGAAC ACTGCGTTTG ACTACTTTCC ATGGTACCAA CAATCCCTG
      P Q S L I V Q K G G I S I I N C A Y E N T A F D Y F P W Y Q Q F P
297 GGAAGGCCCC TGCATTATTG ATAGCCATAC GTCCAGATGT GAGTGAAAAG AAAGAAGGAA GATTCACAAT CTCCTTCAAT AAAAGTGCCA AGCAGTTTCT
      G K G P A L L I A I R P D V S E K K E G R F T I S F N K S A K Q F
397 ATTGCATATC ATGGATTCCC AGCCTGGAGA CTCAGCCACC TACTTCTGTG CAGCAAGCAA CAGCGCTGGC CAGAAGTGC TCTTTGCAAG GGAACCATG
      S L H I M D S Q P G D S A T Y F C A A S N S A G Q K L L F A R G T M
                                     SSPI
497 TTAAAGGTGG ATCTTAATAT CCAGAACCCT GACCCGGCCG CGTACAATAT TCAGAACCCA GAACCTGCTG TGTACCAGTT AAAAGATCCT CGGTCTCAGG
      L K V D L N I Q N P D P A A Y N I Q N P E P A V Y Q L K D P R S Q
                                     ECORV
597 ACAGCACCCCT CTGCTGTTC ACCGACTTTG ACTCCCAAT CAATGTGCCG AAAACCATGG AATCTGGAAC GTTCATCACT GACAAAAC TGCTGGACAT
      D S T L C L F T D F D S Q I N V P K T M E S G T F I T D K T V L D
                                     ECORV
697 GAAAGCTATG GATTCCAAGA GCAATGGGGC CATTGCCTGG AGCAACAGA CAAGCTTAC CTGCCAAGAT ATCTTCAAAG AGACCAACGC CACCTACCCC
      M K A M D S K S N G A I A W S N Q T S F T C Q D I F K E T N A T Y P
797 AGTTCAGACG TTCCCTGTGA TGCCACGTTG ACCGAGAAAA GCTTTGAAAC AGATATGAAC CTAAACTTTC AAAACCTGTC AGTTATGGGA CTCGGAATCC
      S S D V P C D A T L T E K S F E T D M N L N F Q N L S V M G L R I
                                     XbaI
897 TCCTGTGAA AGTAGCGGGA TTTAACCTGC TCATGACGCT GAGGCTGTGG TCCAGTTGAG GTCTGCAAGA CTGACAGAGC CTCTAGA
      L L L K V A G F N L L M T L R L W S S
      Mouse IgG leader peptide Human TCR alpha Variable Mouse TCR alpha constant
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Section B

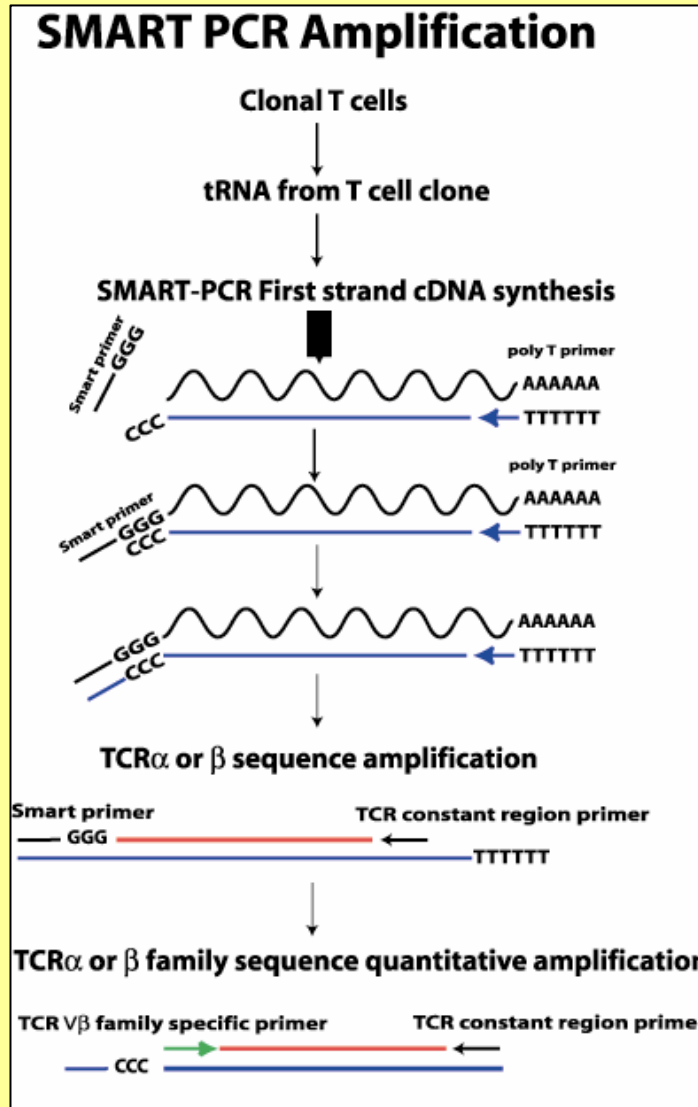
Sequence of the Chimeric TCR β chain in pBud α 1G3.

Patient 5, lane 23 in Fig5

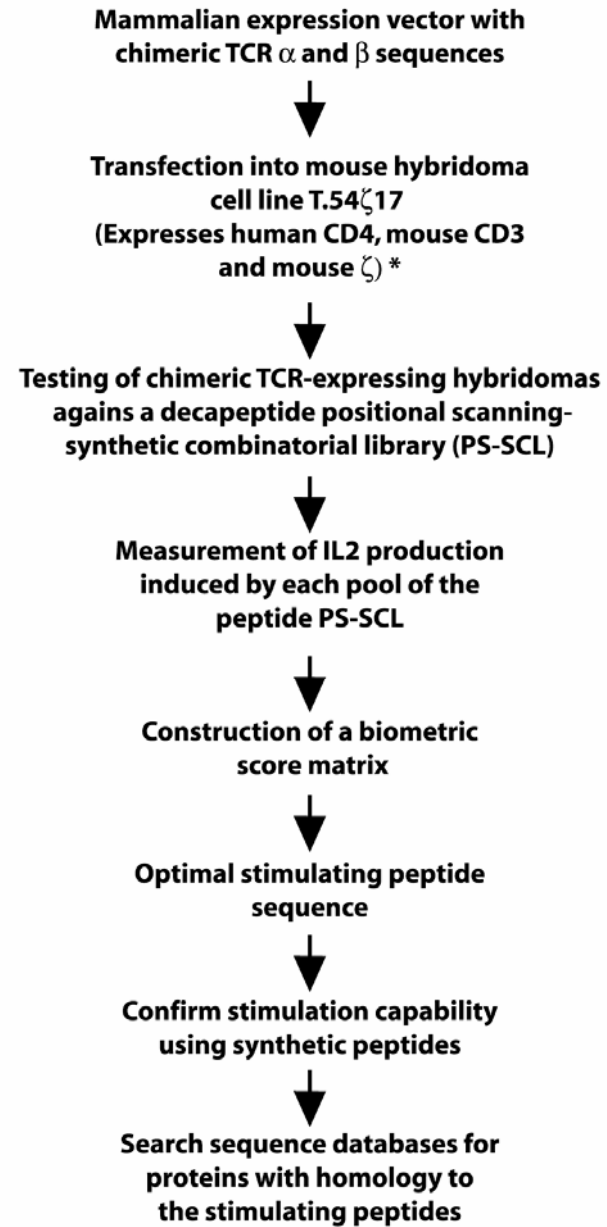
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NotI
1  GCGGCCGCTA AAGCTCCCAT CCTGCCCTGA CCCTGCCATG GGCACCAGCC TCCTCTGCTG GATGGCCCTG TGTCTCTGG GGGCAGGTGA GTCCTCAGAA
      M G T S L L C W M A L C L L G A G
101 CACCAAGCAA TCTCATGTG TCTGTGTATG TCTGTGTGTG TGTGCGTGTG TGTGTGTGTG TGTGATGACT ACAATTGTTT TCCTCCTGTT CCCAACTTGT
      AscI
201 ATCTCCACAG ATCACGCAGA TACTGGAGTC GCGGCCCCAC CCAGACACAA GATCACAAAG AGGGGACAGA ATGTAAC TTT CAGGTGTGAT CCAATTTCTG
      A D T G V G A P P R H K I T K R G Q N V T F R C D P I S
301 AACACAACCG CCTTTATTGG TACCGACAGA CCCTGGGGCA GGGCCACAG TTTCTGACTT ACTTCCAGAA TGAAGCTCAA CTAGAAAAAT CAAGGCTGCT
      E H N R L Y W Y R Q T L G Q G P E F L T Y F Q N E A Q L E K S R L
401 CAGTGATCGG TTTCTGCGAG AGAGGCCATA GGGATCTTTC TCCACCTTGG AGATCCAGCG CACAGAGCAG GGGGACTCGG CCATGTATCT CTGTGCCAGC
      L S D R F S A E R P K G S F S T L E I Q R T E Q G D S A M Y L C A S
      PspXI
501 AGCTTAGTGG CGGGGGGTAC AGATACGCAG TATTTTGGCC CAGGCACCCG GCTGACAGTG CTCGAGGACC TGAAAAACGT GTTCCCACCC GAGGTCGCTG
      S L V A G G T D T Q Y F G P G T R L T V L E D L K N V F P P E V A
601 TGTGTGAGCC ATCAGAAGCA GAGATCTCCC ACACCCAAAA GGCACACTGG GTGTGCCTGG CCACCGGTTT CGACCTGAGA AATGTGACTC CACCCAAGGT
      V F E P S E A E I S H T Q K A T L V C L A T G F D L R N V T P P K
701 CTCCTTGTTT GAGCCATCAA AAGCAGAGAT TGCAACAAA CAAAGGCTA CCCTCGTGTG CTGGCCAGG GGCTTCTTCC CTGACCAGT GGAGCTGAGC
      V S L F E P S K A E I A N K Q K A T L V C L A R G F F P D H V E L S
801 TGGTGGGTGA ATGGCAAGGA GGTCCACAGT GGGGTGAGCA CGGACCCTCA GGCCTACAAG GAGAGCAATT ATAGCTACTG CCTGAGCAGC CGCCTGAGGG
      W W V N G K E V H S G V S T D P Q A Y K E S N Y S Y C L S S R L R
901 TCTCTGCTAC CTTCTGGCAG AATCCTCGAA ACCACTTCCG CTGCCAAGTG CAGTCCATG GGCTTTCAGA GGAGGACAAG TGGCCAGAGG GCTCACCCAA
      V S A T F W H N P R N H F R C Q V Q F H G L S E E D K W P E G S P
1001 ACCTGTCACA CAGAACATCA GTGCAGAGCC CTGGGGCCGA GCAGACTGTG GAATCACTTC AGCATCCTAT CATCAGGGGG TTCTGTCTGC AACCATCCTC
      K P V T Q N I S A E A W G R A D C G I T S A S Y H Q G V L S A T I L
1101 TATGAGATCC TACTGGGGAA GGCCACCCTA TATGCTGTGC TGGTCACTGG CCTGGTCTG ATGGCCATGG TCAAGAAAAA AAATTCCTGA GACAAACTTT
      Y E I L L G K A T L Y A V L V S G L V L M A M V K K K N S -
      BSTXI
1201 TATGCATCCT GAGCCGTCTC TCACCCAGCA CAGTGG
```

Human TCR beta 7-9 leader peptide Human TCR beta Variable Mouse TCR beta constant

Section B



Experimental outline for Section C



* Caivano A *et al.* 2001. Design of cassette vectors permitting cloning of all types of human TCR variable α and β regions. *J Immunol Meth.* 255:125-134.

Section C

**Simulation of
a score
matrix
generated
from testing
a chimeric
clone**

		Amino acid position on decapeptide									
		P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Amino acid (one letter code)	A	2.17	3.54	0.34	3.47	12.39	4.20	8.83	0.08	4.75	7.48
	R	19.83	1.80	3.57	1.38	3.98	2.88	6.52	7.05	4.82	0.83
	N	4.59	1.02	4.33	0.09	3.82	4.87	0.17	2.03	5.04	7.81
	D	4.20	3.37	3.65	0.55	4.53	5.99	3.58	6.26	6.21	4.79
	C	1.59	1.26	2.90	0.26	1.83	0.69	8.57	1.61	3.90	13.60
	Q	4.14	4.20	4.56	3.28	0.01	4.96	4.73	0.89	1.29	6.72
	E	3.54	4.90	0.32	0.68	2.63	1.67	3.29	1.80	7.08	12.25
	G	3.31	3.39	19.13	3.89	3.81	0.77	12.98	5.29	4.65	2.75
	H	2.44	4.15	0.41	2.31	4.53	1.75	7.88	7.34	8.47	6.09
	I	3.92	0.43	3.63	1.49	0.78	2.69	3.57	1.63	4.59	3.04
	L	2.74	3.79	1.94	10.56	3.53	1.52	8.91	13.42	6.22	0.25
	K	2.23	6.25	1.66	4.82	1.84	2.44	7.69	5.86	10.93	7.97
	M	2.57	0.65	4.87	0.08	3.66	0.20	4.90	9.17	3.14	6.44
	F	3.30	0.30	0.20	3.07	4.20	4.01	3.61	0.09	4.10	2.63
	P	4.88	9.35	4.98	4.85	1.86	11.83	2.90	2.94	5.31	5.98
	S	3.82	18.28	3.93	0.79	1.30	0.74	0.35	0.53	1.62	3.77
	T	3.00	2.55	3.13	3.57	0.31	1.47	0.79	9.23	6.78	5.25
	W	2.13	4.43	4.53	3.58	3.73	0.04	4.96	4.67	18.42	1.45
	Y	1.90	4.53	2.47	4.78	1.46	0.51	9.82	3.01	7.16	6.61
	V	2.91	1.48	3.60	0.58	4.15	0.46	6.74	7.25	9.85	8.24

R	S	G	L	A	P	G	L	W	C
Sequence of peptide obtained from matrix									

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