

**Proposal No.** IBD-0094

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**Project Title:** Peptides recognized by T cell clones from inflammatory bowel disease patients

**Award Period:** February 1, 2004 – September 30, 2006

## I- Summary of project aims.

*Aim 1 – Determination of dominant expanded Crohn's disease (CD) tissue T cell receptors (TCR).* To identify TCRs from T cells that may play a role in the initiation or maintenance of the disease, the TCR usages in involved and uninvolved tissues are compared. Single T cells with TCR sequences corresponding to TCR families that are more common in inflamed tissue than in non-involved portions and that show signs of increased clonality will be selected for further study.

*Aim 2 – Cloning and expression of relevant human T cell variable regions in a murine hybridoma cell line.* In order to circumvent the difficulty of obtaining human intestinal T cell clones, chimeric TCRs, consisting of the human variable region ( $\alpha$  or  $\beta$ ) fused to the corresponding murine constant region ( $\alpha$  or  $\beta$ ), are constructed. The TCR $\alpha$  and  $\beta$  chains that originated from the same human cell will be co-expressed in a murine T cell hybridoma cell line. This murine T cell hybridoma has been engineered to express the human CD4 and the murine CD3 $\zeta$  chain and it responds to TCR stimulation with production of murine IL2.

*Aim 3 – Testing for specific peptides that activate T cells from CD patients.* Each T cell hybridoma transfectant expressing a chimeric TCR will be used to scan a positional scanning synthetic combinatorial peptide library (PS-SCL). Antigen presenting cells (APCs) that express the HLA ClassII alleles from the tissue donor will be constructed. The APCs will be exposed to pools of the PS-SCL for 18 to 24 hours before mixing them with chimeric TCR-expressing murine T cell hybridomas. The IL2 production induced by each pool of the PS-SCL will be recorded, arranged into a matrix and used to deduct the optimal stimulatory peptide sequence. Optimal stimulatory peptide sequences will be synthesized and tested to confirm the library scanning results.

*Aim 4 – Search of protein and DNA data libraries for proteins containing peptides homologous to those capable of activating TCR expressing hybridoma cells.* A Perl script will be used to search for proteins containing homologous peptides in databases such as GenBank, PIR, microbial genomes (TIGR) and others.

## II- Accomplishments towards meeting aims.

**Aim 1 – Determination of dominant expanded Crohn's disease (CD) tissue T cell receptors (TCR).**

**a- Repertoire analysis by real-time polymerase chain reaction (RT-PCR).** The primers described in the International ImMunoGeneTics primer database were tested for suitability for RT-PCR; however, most of them either produced too large products or amplified additional unrelated sequences. Only 4 of the ImMunoGeneTics TCR $\beta$  primers (4 families) were found suitable for RT-PCR. In response, we manually designed a RT-PCR primer set for each of the 34 TCR $\alpha$  families and for each of 21 TCR $\beta$  families. Each primer set specifically amplifies a sequence encompassing the CDR3 hypervariable region of all the subfamilies of a given family of TCRs. We used degenerate sequences in the 5' half of the primer, in order to reduce the number of unique primers per TCR chain family set. The new set of primers gave consistent results and produced a set of amplicons closely similar in size with a minimum of background

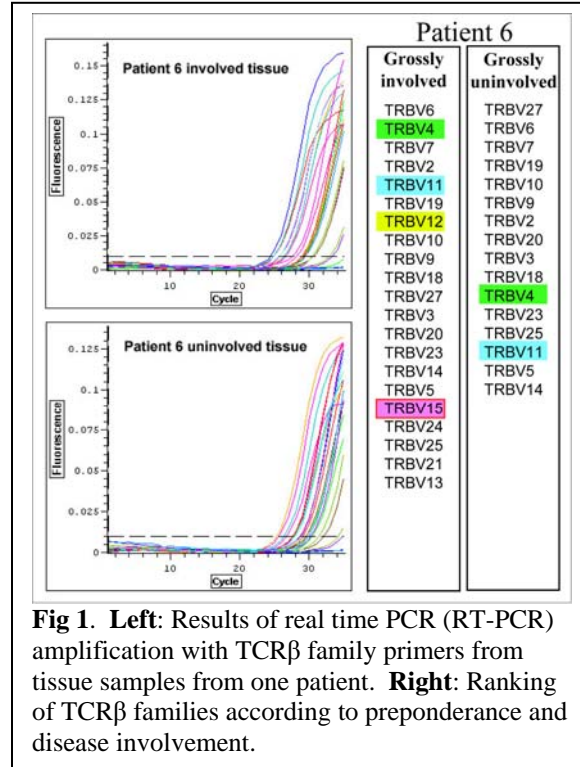
amplification. This technique allowed us to rank the usage of each TCR family in tissues and detect shifts in preponderance between disease involved and uninvolved sites (see Fig. 1).

The clonality of the T cell population was estimated by separation of the amplification products in a high definition MetaPhor agarose gel (Cambrex, Rockland, ME) (Fig. 2). This method provides enough information on the amplicon size distribution more quickly and inexpensively than spectratyping.

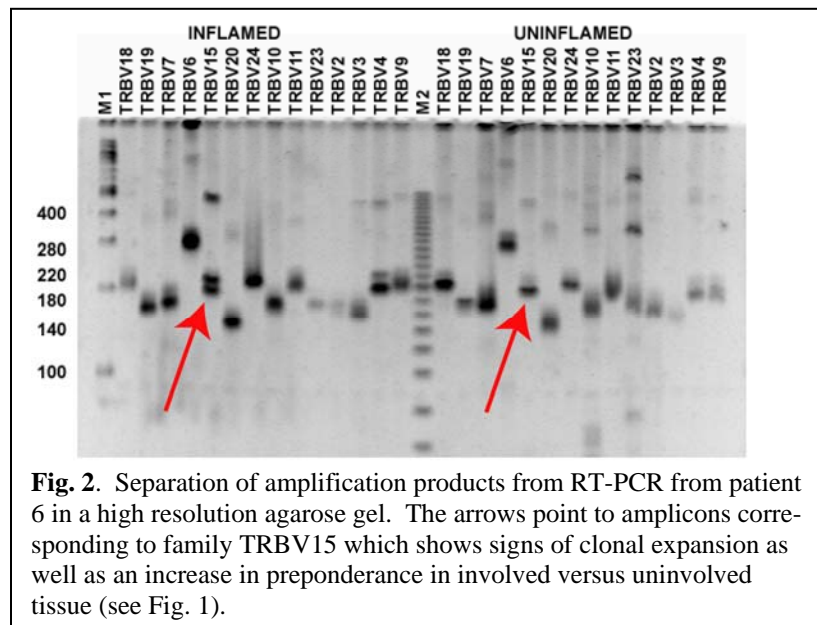
We received surgical tissue samples from 6 and biopsies from 2 patients. Some of these samples were used for development of methodology. We studied the TCR usage extensively from 3 patients.

**b- Amplification and cloning of TCR $\alpha$  and  $\beta$  chains from single CD4<sup>+</sup> cells.** We used single cell PCR because of the inherent low yield of T cell clones that can be obtained from human intestinal tissue, the time necessary to obtain such clones and the uncertainty about their single cell clonality. One disadvantage of this method is the short window of opportunity for isolation of live cells after the surgical removal of the tissue; although this can be circumvented by the use of amplification from single cells dissected from preserved tissue samples[1].

Small pieces of tissue from each of these 3 patients were incubated for 6 to 8 hours in R10 (RPMI with 10% fetal calf serum) at 37°C and 5% CO<sub>2</sub> to allow cells to crawl out. After washing the cells with MACS buffer (phosphate buffered saline with 0.5% fetal calf serum), anti-CD4 coated magnetic beads were added and cells that bound magnetic beads were manually selected using a micro-manipulator. These CD4<sup>+</sup> cells were transferred into 96 well reaction plates, one cell per well. For each cell, cDNA was synthesized in a 10  $\mu$ L reaction volume, followed by SMART-cDNA amplification [2] (and Douek, DC personal communication) for not more than 8 cycles. The single cell cDNA synthesis and amplification yields enough for 8 to 10 PCR reactions which allows not only the amplification of the sequences for the TCR $\alpha$  and  $\beta$  chains but of other genes. It is possible;

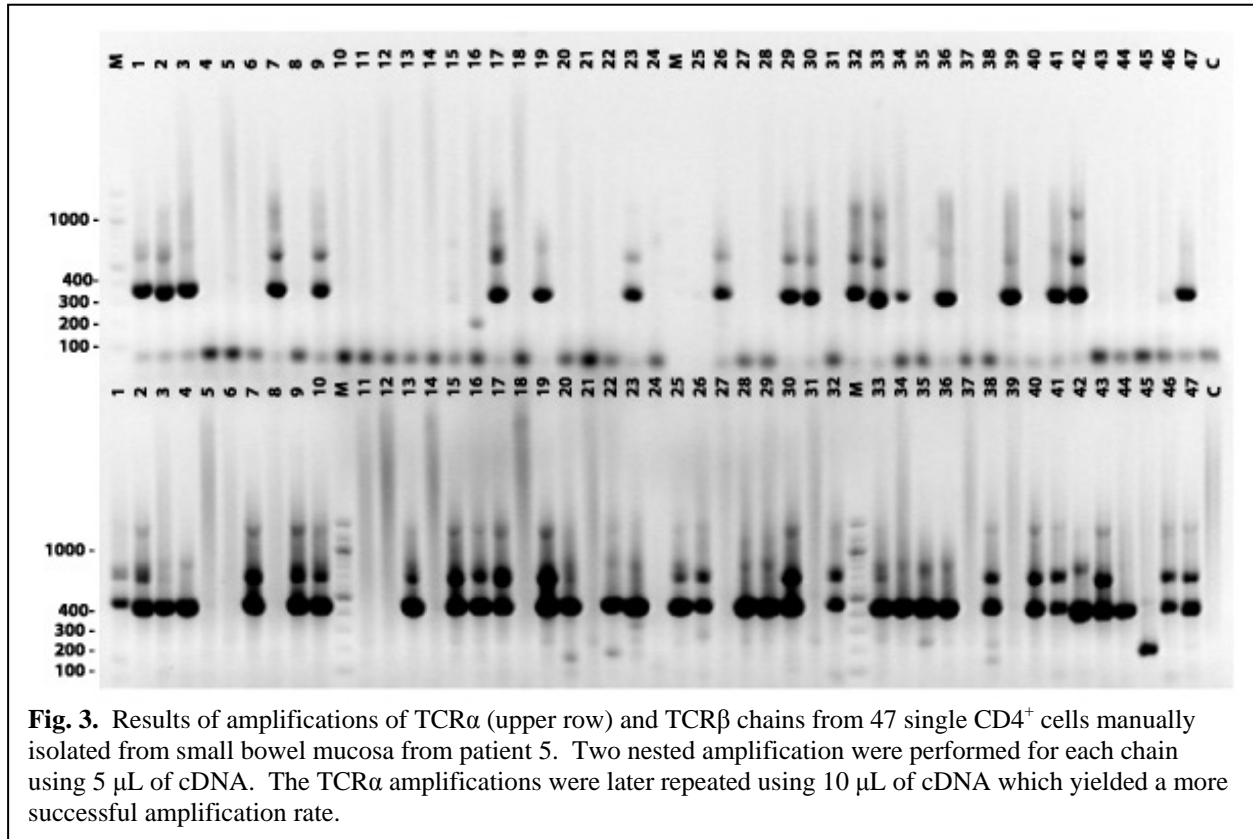


**Fig 1. Left:** Results of real time PCR (RT-PCR) amplification with TCR $\beta$  family primers from tissue samples from one patient. **Right:** Ranking of TCR $\beta$  families according to preponderance and disease involvement.



**Fig. 2.** Separation of amplification products from RT-PCR from patient 6 in a high resolution agarose gel. The arrows point to amplicons corresponding to family TRBV15 which shows signs of clonal expansion as well as an increase in preponderance in involved versus uninvolved tissue (see Fig. 1).

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**Fig. 3.** Results of amplifications of TCR $\alpha$  (upper row) and TCR $\beta$  chains from 47 single CD4<sup>+</sup> cells manually isolated from small bowel mucosa from patient 5. Two nested amplification were performed for each chain using 5  $\mu$ L of cDNA. The TCR $\alpha$  amplifications were later repeated using 10  $\mu$ L of cDNA which yielded a more successful amplification rate.

herefore, to characterize the cell whose cDNA has been amplified to a certain extent, to distinguish the immunophenotype of the cell, e.g. CD4<sup>+</sup> NK T cells from CD4<sup>+</sup> Tr1 cells.

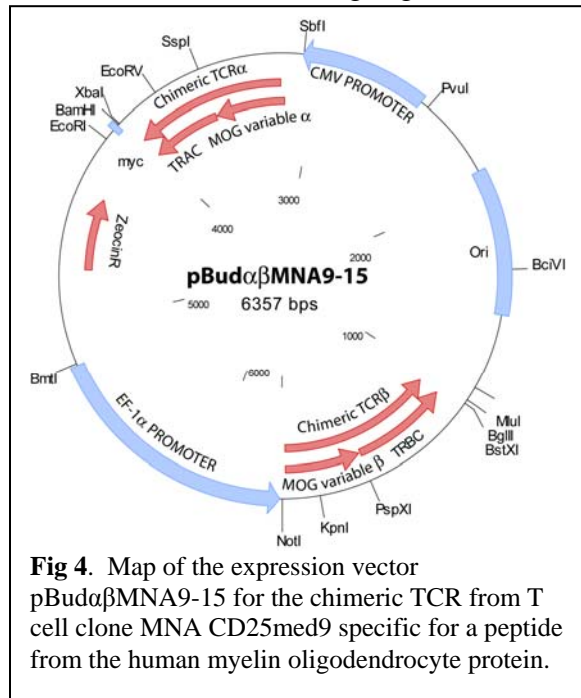
The TCR $\alpha$  and  $\beta$  chains can be amplified from the single cell cDNA by two nested PCR amplifications as described by Moysey et al [3]. After optimization of the amplification reactions, successful amplification of the TCR $\beta$  was obtained for 50 to 60% of the cells and 70% of these TCR $\beta$ <sup>+</sup> were successful at amplifying the TCR $\alpha$  chain. It is noteworthy to indicate that not all CD4<sup>+</sup> cells are TCR $\alpha\beta$  T cells, which is consistent with the literature noting CD4 expression on non-T cells [4]. The TCR $\alpha$  is more difficult to amplify for some unknown reason, perhaps lower mRNA levels or lower primer efficiency than for the TCR $\beta$ . An example of results for TCR $\alpha$  and  $\beta$  chains amplifications are shown in Fig 3. Primer sets were also designed for amplification of cDNAs coding for FoxP3, CD25 [same as IL2 $\alpha$ ] and GITR (three genes expressed in regulatory T cells), IL2R $\gamma$  and CD161 which is expressed in NK cells. The results with these primer sets, however, were unreliable with too many false negatives for some and too many false positives for others. Perhaps these genes have a much lower level of transcription compared to the TCR chains, indicating that more stringent PCR approaches are necessary such as nested PCR or TaqMan probe techniques.

#### **Aim 2 - Cloning and expression of relevant human T cell variable regions in a murine hybridoma cell line.**

**a- Single cell PCR for TCR $\alpha$  and  $\beta$  chains.** After developing the single cell PCR technique we had suitable samples from 3 patients and from these samples we proceeded to isolate cells, amplify TCR chains, clone and sequence on a larger scale than previous. For these 3 patients we isolated single CD4<sup>+</sup> cells and amplified their TCR sequences for an average of 150 cells (100 to 300 range) The amplified TCR $\alpha$  and  $\beta$  chains were cloned into the vector pSTBlue-1 (Novagen, San Diego, CA) and sequenced. The TCR sequences were analyzed using the

alignment software QUEST available in the ImMunoGeneTics web site. T cells that used a TCR family with indications of clonal expansion in involved as compared to uninvolved tissues, such as cell 1B3 from patient 6 (Figs 1 and 2) were selected for further study. One cell from each of these 3 patients was selected for construction of chimeric TCRs. Additionally, as a positive control, we obtained, from Dr. David Hafler (Harvard Medical School, Boston, MA), RNA from a T cell clone (MNA CD25<sup>med9</sup>) isolated from a multiple sclerosis patient. This T cell clone responds to a peptide derived from human myelin oligodendrocyte glycoprotein (MOG). The TCR chains from this MOG T cell clone were also amplified, cloned and sequenced. The sequences of the amplified chains appear in supplementary Fig. 8.

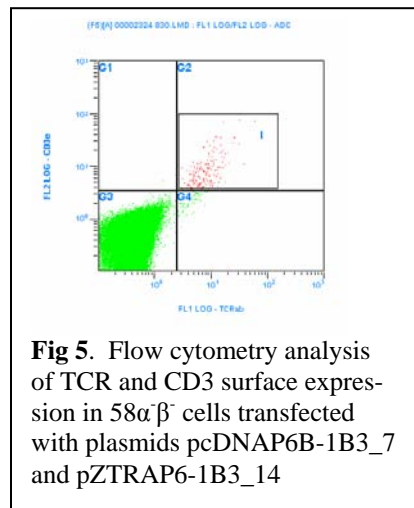
**b- Construction of chimeric TCR expression vectors.** In the initial construction the cloned TCR $\alpha$  and  $\beta$  chains from cell 1G3 from patient 5 (referred from here on as P5-1G3) were fused to the mouse constant TCR region by ligation into vectors pG $\alpha$  and pG $\beta$  [5]. The fused TCR $\alpha$  and  $\beta$  chains were then moved into the bicistronic expression vector pBudCE4.1 (Invitrogen, Carlsbad, CA)(Details of the construction appears in supplementary Fig. 9). Since the products of the TCR amplification reactions do not include the leader peptide, which is necessary for export of the protein to the cell surface, the leader peptide for a mouse IgG was used for the  $\alpha$  chain. This leader peptide sequence includes the first intron of the IgG gene and it has been successfully used in our lab to express and secrete protein-Fc fusions. The leader peptide that corresponds to the TCR $\beta$  family TRBV7-9, which is used by P5-1G3, was amplified using specific primers from total cDNA from the same patient, cloned and inserted in front of the sequence of the  $\beta$  chain amplified from the single cell P5-1G3. The plasmid produced was named pBud $\alpha\beta$ P5-1G3A. This plasmid was then used to construct pBud $\alpha\beta$ P6-1B3K2, the TCR expression vector for cell 1B3 from patient 6 (referred from here on as P6-1B3) by exchanging the variable regions using the NotI and PspXI sites for the variable TCR  $\beta$  and the SbfI and SspI sites for the variable TCR $\alpha$  (see supplementary Fig. 10). The plasmid pBud $\alpha\beta$ P6-1B3 was in turn used to construct the pBud $\alpha\beta$ MNA9\_15 which expresses the TCR from the MOG T cell clone MNA CD25<sup>med9</sup> as shown in Fig. 4.



**c- Transfection of chimeric TCR expression vectors into mouse T cell hybridoma cells.** The plasmids pBud $\alpha\beta$ P5-1G3A and pBud $\alpha\beta$ P6-1B3K2 were electroporated into the mouse hybridoma cell line T54 $\zeta$ 17 (kindly provided by Dr. P. De Berardinis, ). There was, however, no surface expression of the TCR or the mouse CD3 complex as shown by flow cytometry analysis. Since we suspected that the problem was transport and assembly of the TCR complex to the surface of the cell, we changed the following possible problems in these plasmids: 1- An intron present in the mouse IgG leader peptide used for the TCR $\alpha$  chain in pBud $\alpha\beta$ P5-1G3A was removed. 2- The junction between the  $\alpha$  variable and constant regions in pBud $\alpha\beta$ P5-1G3A was reconstructed to remove a 10 amino acid repeat in the protein. 3- The untranslated 5' region of the TCR $\beta$  chain in pBud $\alpha\beta$ P6-1B3K2 was also modified to delete an ATG in front of the ORF that we suspected was acting as a false translational start. The modified plasmids were named pBud $\alpha\beta$ P5-1G3C3 and pBud $\alpha\beta$ P6-1B3L. These plasmids were introduced into T54 $\zeta$ 17 cells by nu-

cleoporation using the Amaxa nucleoporation apparatus (Amaxa, Gaithersburg, MD) which yields better rates of transfection than regular electroporation. However, again there was no surface expression of the TCR with either of the plasmids.

We suspected now the EF-1 $\alpha$  promoter that drives the TCR $\beta$  chain expression (we found no previous record of usage of this promoter in T cells) or just the fact of trying to express both chains from the same plasmid for the failure in TCR expression. Therefore, we separated the two TCR chains using the expression vectors pcDNA3.1Hygro+ and pcDNA3.1Zeo+ which uses the CMV promoter for expression of a gene. The whole chimeric TCR $\alpha$  and  $\beta$  chains from pBud $\alpha\beta$ P6-1B3L were amplified and ligated to these vectors as described in supplementary Fig. 11 to yield plasmids pcDNAP6B-1B3\_7 and pZTRAP6-1B3\_14. These plasmids again failed to induce surface expression of the TCR in T54 $\zeta$ 17 cells causing us to suspect the T cell hybridoma. Dr. Klaus Dornmair agreed with us that the reason for failure was most likely due to the T cell hybridoma, which is very unstable. He also kindly sent us a derivative of this T cell hybridoma named 58 $\alpha\beta^-$  to test this hypothesis. We tested this cell line with both bicistronic and the monocistronic plasmids pcDNAP6B-1B3\_7 and pZTRAP6-1B3\_14. We detected a small but definite population with surface TCR and CD3 expression after nucleoporation with the monocistronic set of plasmids but none or very few with the bicistronic plasmids (Fig. 5). We used two techniques for isolation or enrichment of these TCR $\alpha\beta^+$  CD3 $^+$  transfectants: Selection by anti-TCR or anti-CD3-antibody covered magnetic beads or limited dilution. Neither of the techniques was successful. Antibody binding of the



**Fig 5.** Flow cytometry analysis of TCR and CD3 surface expression in 58 $\alpha\beta^-$  cells transfected with plasmids pcDNAP6B-1B3\_7 and pZTRAP6-1B3\_14

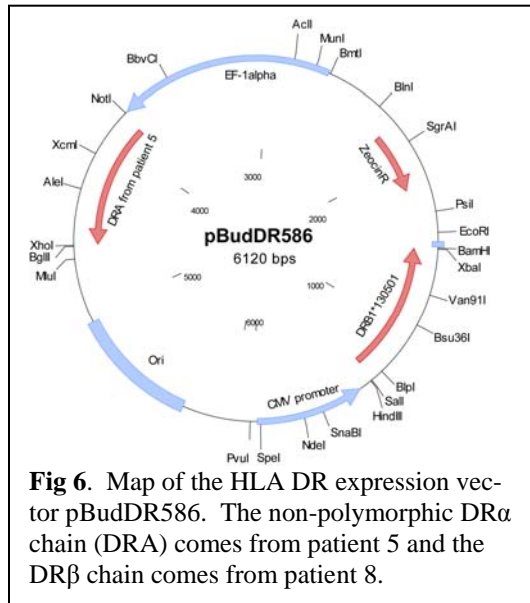
TCR or the CD3 complex may trigger apoptosis, after transient stimulation, like they do in normal T cells. We checked more than 200 single cell clones from this transfection for surface expression of TCR or CD3 complex without success. Further research is necessary in order to develop reliable methods for transfection and selection of high expressers of TCRs in T cell hybridomas.

**Aim 3. – Testing for specific peptides that activate T cells from CD patients.**

**a- Construction of HLA DR expression vectors.** We decided to concentrate on the DR alleles of the HLA since they are the most polymorphic and more often reported in association with CD. We designed primers to amplify the entire coding regions for the DRA ( $\alpha$  chain) and DRB ( $\beta$  chain) alleles. These alleles were amplified using a high fidelity polymerase from cDNA obtained from total RNA from

TABLE 1 - Alleles present in Patients		
Patient 5	Patient 6	Patient 8
DRB3*0201	DRB1*070101	DRB1*010201
DRB3*020201	DRB1*130201	DRB1*130501
DRB1*030101	DRB1*130501	DRB1*030201
DRB1*030102		

the 3 patients for whom chimeric TCR were being constructed. The DRA alleles are not polymorphic; therefore, we cloned the two known alleles from patients 5 and 8. Table 1 shows the results of the genotyping for the DRB. Each of the complete coding sequences for DRA were inserted into vector pBudCE4.1 under the EF-1 $\alpha$  promoter creating pBud57 and pBud818 (which contain DRA allele from patient 5 and patient 8 respectively). The DRB chains were then inserted into each of these two vectors creating expression plasmids such as pBudDR586 (see Fig. 6) which expresses a HLA Class II using the  $\alpha$  chain from patient 5 and the  $\beta$  chain

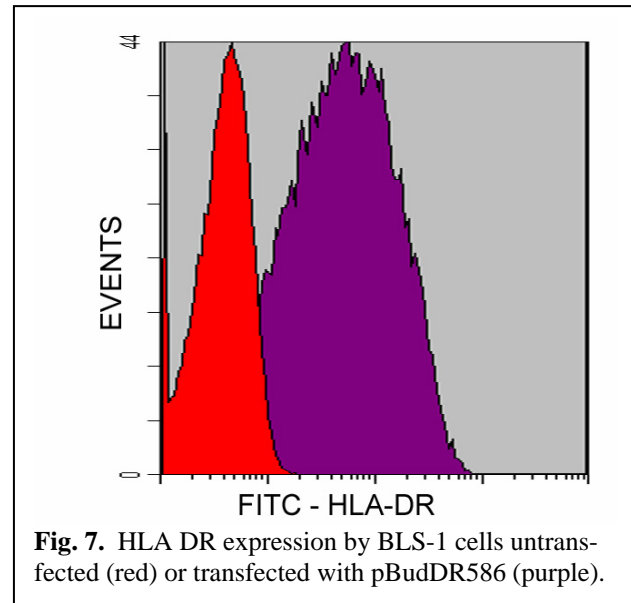


by Dr. Gerald Nepom (Benaroya Research Institute at Virginia Mason, Seattle, WA). After selection with Zeocin, single cell clones were selected by limiting dilution. The pattern of HLA Class II expression of BSL-1 cells transfected with plasmid pBudDR586 is shown in Fig. 7.

**c- Scanning of a PS-SCL for stimulation of chimeric TCRs.** The scanning of the peptide library could not be accomplished because of the failure of stable expression of the chimeric TCRs in T54 $\zeta$ 17 or 58 $\alpha$  $\beta$ <sup>-</sup> cells.

(allele 8W41 which corresponds to DRB1\*130501) from patient 8. We did not construct all the possible combinations but concentrated on those alleles common in the North American population. In total we constructed 14 different DR allele expression plasmids.

**b- Transfection and expression of patients HLA DR alleles.** The HLA DR-expression plasmids were electroporated into BSL-1 cells. BSL-1 is a human B cell line originated from a patient with Bare Lymphocyte syndrome and they do not express HLA Class II antigens. This cell line was kindly provided



*Aim 4 – Search of protein and DNA data libraries for proteins containing peptides homologous to those capable of activating TCR expressing hybridoma cells.*  
This aim could not be accomplished without transfection results from aim 3.

#### Bibliography and References cited

1. Seitz, S., et al., *Reconstitution of paired T cell receptor alpha- and beta-chains from microdissected single cells of human inflammatory tissues.* Proc Natl Acad Sci U S A, 2006. **103**(32): p. 12057-62.
2. Douek, D.C., et al., *A novel approach to the analysis of specificity, clonality, and frequency of HIV-specific T cell responses reveals a potential mechanism for control of viral escape.* J Immunol, 2002. **168**(6): p. 3099-104.
3. Moysey, R., A.L. Vuidepot, and J.M. Boulter, *Amplification and one-step expression cloning of human T cell receptor genes.* Anal Biochem, 2004. **326**(2): p. 284-6.

4. Lynch, G.W., et al., *Marked differences in the structures and protein associations of lymphocyte and monocyte CD4: Resolution of a novel CD4 isoform*. Immunology and Cell Biology, 2006. **84**(2): p. 154-165.
5. Caivano, A., et al., *Design of cassette vectors permitting cloning of all types of human TCR variable alpha and beta regions*. J Immunol Methods, 2001. **255**(1-2): p. 125-34.

### **III- List of significant results:**

- a- Development of techniques for single cell amplification of TCR $\alpha$  and  $\beta$  chains.
- b- Construction of chimeric TCRs in mammalian expression vectors.
- c- Lack of surface expression of TCR from bicistronic expression vectors and difficulty obtaining stable surface expression of TCRs from 2 monocistronic expression vectors.
- d- Development of techniques for amplification and cloning of HLA DR alleles from cDNA.
- e- Surface expression of recombinant HLA DR molecules in Bare Lymphocyte syndrome human B cells using bicistronic expression vectors.

### **IV- List of publications resulting from the grant.**

There are no publications yet resulting from the grant.

### **V- List of applications submitting to other funding agencies to continue work on the project.**

NIH

Status: Not funded

Type: Research grant - R01

Title of grant proposal: "Molecular approach to antigen recognition of regulatory T cells in IBD".

Total amount requested: \$1,000,000.00

### **VI- Lay summary.**

When T cells recognize protein segments (peptides) that are given away by microbes (bacteria, fungi or virus) they promote inflammation which is the first step in mounting an immune defense. However, if they mistakenly recognize peptides from the normal gut microbes as if they were pathogens, they may trigger a chronic inflammation with damage to the tissues. The purpose of the research project was the identification of peptides that stimulate certain immune cells, the T cells, which are involved in the chronic inflammation that characterize Crohn's disease. If these inflammatory peptides were known it would be possible then to develop therapies to block or ameliorate their impact. We developed techniques that allowed us to amplify, clone and sequence the genes coding for the T cell receptor (TCR) from single isolated T cells. The TCR is the surface receptor by which the cells recognize the protein segments that are given away by microbes. We also developed techniques to study the population dynamics of these T cells in order to estimate which T cells were more likely involved in the maintenance of chronic inflammation. In order to be able to find the peptides that stimulate the particular TCRs that we isolated from the patients, we had to express these TCRs in mouse cells which reproduce very rapidly. However, we could not obtain mouse cells that produced the constructed TCRs on their surface. The technical problems are not insurmountable and we will solve them and continue with the research.

## Supplemental Figures

### **Supplementary figure 8. Sequences of the chimeric TCRs.**

- A1- Sequence of the chimeric TCR $\beta$  in expression vector pBud $\alpha\beta$ P5-1G3C3
- A2- Sequence of the chimeric TCR $\alpha$  in expression vector pBud $\alpha\beta$ P5-1G3C3
- B1- Sequence of the chimeric TCR $\beta$  in expression vector pBud $\alpha\beta$ P6-1B3L
- B2- Sequence of the chimeric TCR $\alpha$  in expression vector pBud $\alpha\beta$ P6-1B3L
- C1- Sequence of the chimeric TCR $\beta$  in expression vector pBud $\alpha\beta$ MNA9-15
- B1- Sequence of the chimeric TCR $\alpha$  in expression vector pBud $\alpha\beta$ MNA9-15



B1

### Sequence of the chimeric TCR $\beta$ chain in pBud $\alpha$ P6-1B3L

TRBV15\*02 J2-2\*01

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1      NotI                               AscI
   cgcggccgcg ccaatgggtc ctgggcttct ccaactggatg gccctttgtc tcttggaac aggtcatggc gcgcccatgg tcattccagaa cccaagatac
      |----- TCR $\beta$ V15*02 leader peptide -----|----- TRBP6-1B3 -----|
101  caggttacc agtttggaaa gccactgacc ctgagttgtt ctcagacttt gaaccataac gtcattgact ggtaccagca gaagtcaagt caggccccaa
      q v t q f g k p v t l s c s q t l n h n v m y w y q q k s s q a p
      ----- human TCR $\beta$  chain from P6-1B3 -----
201  agctgtgtt ccaactactat gacaagatt ttaacaatga agcagacacc cctgataact tccaatccag gaggccgaac acttctttct gctttcttga
      k l l f h y y d k d f n n e a d t p d n f q s r r p n t s f c f l
      ----- human TCR $\beta$  chain from P6-1B3 -----
301  catccgctca ccaggcctgg ggaagcagc catgtacctg tgtgccacca gcagagatcg taaggcctcg aacaccgggg agctgttttt tggagaaggc
      d i r s p g l g d a a m y l c a t s r d r k a s n t g e l f f g e g
      ----- human TCR $\beta$  chain from P6-1B3 -----
401  tctaggtgta cgtcctcga ggaactgaga aatgtgactc caccgaaggt ctcctgtttt gagccatcaa aagcagagat tgcaaacaaa caaaaggcta
      PspXI
      s r l t v l e d l r n v t p p k v s l f e p s k a e i a n k q k a
      -- human TCR $\beta$  chain from P6-1B3 -|----- mouse TRAC -----|
501  cctctgtgtg cttggccagg gctcttctc ctgaccactg ggagctgagc tgggtgggtga atggcaagga ggtccacagt ggggtcagca cggaccctca
      t l v c l a r y g f f p d h v e l s w w v n g k e v h s g v s t d p
      ----- mouse TRAC -----
601  ggcctacaag gagagcaatt atagctactg cctgagcagc cgcctgaggg tctctgtctac cttctggcac aatcctcgaa accacttccg ctgccaagtg
      q a y k e s n y s y c l s s r l r v s a t f w h n p r n h f r c q v
      ----- mouse TRAC -----
701  cagttccatg gcttttccga ggagacaag tggccagagg gctcaccaca acctgtcaca cagaacatca gtgcagagcg ctggggccga gcagactgtg
      q f h g l s e e d k w p e g t s p k p v t q n i s a e a w g r a d c
      ----- mouse TRAC -----
801  gaatcactc agcactctat catcaggggg tctgtctgc aaccatctc tatgatctc tactgggaa ggcacccta tatgtctgc tggctcagtg
      g i t s a s y h q g v l s a t i l y e i l l g k a t l y a v l v s
      ----- mouse TRAC -----
901  cctgtgtctg atggccatgg tcaagaaaaa aaattctcga gacaaactt tatgcatctt gagccgttct tcaccagca cagtggactc gagagatctg
      BstXI                               BglII
      g l v l m a m v k k k n s -
      ----- mouse TRAC -----|

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B2

### Sequence of the chimeric TCR $\alpha$ chain in pBud $\alpha$ P6-1B3L

TRAV2\*01 J28\*01

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3101  acccaactgct tactggctta tcgaaattaa taagactcac tatagggaga cccaagcttg cattcctgca ggcaatgtgg ccaccatggc tttgcagagc
      |----- m a l q s -----|
3201  actctggggg cgggtgtggct agggcttctc ctcaactctc tctggaaggt tcagaaaagc gtcgaccgag tgtttcagcc ttccacagtg gcactctcag
      Sali
      t l g a v w l g l l l n s l w k v a e s v d q v f q p s t v a s s
      ----- Human TCR $\alpha$ V2*01 leader peptide -----|----- human TCR $\alpha$  chain in P6-1B3 -----
3301  agggagctgt ggtggaactc tctgtaatc actctgtctc caatgcttc aacttcttct ggtaccctca cttcccggga tgtgcaccaa gactcctgtt
      e g a v e i f c n h s v s n a y n f f w y l h f p g c a p r l l
      ----- human TCR $\alpha$  chain in P6-1B3 -----
3401  taaaggctca aagccttctc agcagggagc atacaactag acctatgaac ggttctctc atcgtctctc atctccagg tgcgggagcg agatgtctgt
      v k g s k p s q q g r y n m t y e r f s s l l i l q v r e a d a a
      ----- human TCR $\alpha$  chain in P6-1B3 -----
3501  gtttactact gtctgtgga ggcaccactc ggggctgga gttaccaact cactttcggg aaggggacct cgctcactaa tattcagaac ccagaactcg
      XcmI                               SspI
      v y y c a v e a h s g a g s y q l t f g k g t s l t n i q n p e p
      ----- human TCR $\alpha$  chain in P6-1B3 -----|-----
3601  ctgtgtacca gttaaaagat cctcgtctc aggcagcac cctctgctg ttcaccgact ttgactccca aatcaatgtg ccgaaaacca tggaactctgg
      a v y q l k d p r s q d s t l c l f t d f d s q i n v p k t m e s
      ----- mouse TRAC -----
3701  aacgttcatc actgacaaaa ctgtgctgga catgaaagct atgatttcca agagcaatgg ggccattgac tggagcaacc agacaagctt cacttgccaa
      g t f i t d k t v l d m k a m d s k s n g a i a w s n q t s f t c q
      ----- mouse TRAC -----
3801  gatatcttca aagagaccaa cgccactac ccagttcag acgttccctg tgatgccagc ttgaccgaga aaagcttga aacagatag aacctaaact
      d i f k e t n a t y p s s d v p c d a t l t e k s f e t d m n l n
      ----- mouse TRAC -----
3901  ttcaaaactc gtcagttatg ggaactcgaa tctcctgct gaaagttagc ggatttaacc tgctcatgac gctgaggctg tggctcagtt gaggtctgca
      f q n l s v m g l r i l l l k v a g f n l l m t l r l w s s
      ----- mouse TRAC -----|
4001  agactgacag agcctctaga ggatccgaac
      BamHI
      XbaI

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C1

### Sequence of the chimeric TCR $\beta$ chain in pBud $\alpha$ BMNA9-15

TRBV15\*01 J1-2\*01

```

NotI
1  cgcgggccga tctaccatg ggtcctgggc tctccaactg gatggccctt tgtctccttg gaacaggtea tggggatgcc atggtcatcc agaaccacaag
   m g p g l l h w m a l c l l g t g h g d a m v i q n p
   |----- human TCR $\beta$ V15*01 leader peptide -----|

101 ataccagggtt acccagtttg gaaagccagt gacctgagt tgtttcaga ctttgaacca taacgtcatg tactggtacc agcagaagtc aagtcaggcc
   r y q v t q f g k p v t l s c s q t l n h n v m y w y q q k s s q a
   |----- human TCR $\beta$  from MOG T cell clone -----|
KpnI

201 ccaaagctgc tgttccaacta ctatgacaaa gattttaaca atgaagcaga caccctgat aacttcaat ccaggaggcc gaacaattct tctgccttc
   p k l l f h y y d k d f n n e a d t p d n f q s r r p n t s f c f
   |----- human TCR $\beta$  from MOG T cell clone -----|

301 ttgacatccg ctcaccaggc ctgggggacg cagccatgta cctgtgtgcc accagcccgg acggggtaac taactatggc tacaccttcg gttcggggac
   l d i r s p g l g d a a m y l c a t s p d g v t n y g y t f g s g
   |----- human TCR $\beta$  from MOG T cell clone -----|

401 caggttaacc gtgctcgagg acctgagaaa tgtgactcca cccaaggtct ccttgtttga gccatcaaaa gcagagattg caaacaacaa aaaggctacc
   t r l t v l e d l r n v t p p k v s l f e p s k a e i a n k q k a t
   |----- mouse TRBC -----|
PspXI

501 ctogtgtgct tggccagggg cttcttccct gaccacgtgg agctgagctg gtgggtgaat ggcaaggagg tccacagtgg ggtcagcaga gacctcagg
   l v c l a r g f f p d h v e l s w v n g k e v h s g v s r d p q
   |----- mouse TRBC -----|

601 cctacaagga gagcaattat agctactgcc tgagcagccg cctgagggtc cgtgtacctt tctggcaaaa tctcgaaac cacttccgct gccaaagtca
   g gatgttctt ctggttaata tcgatgacgg actcgtcggc ggactcccag gcacgatgga agaccgtgtt aggactttg gtgaaggcga cggttccagt
   a y k e s n y s y c l s s r l r v r a t f w h n p r n h f r c q v
   |----- mouse TRBC -----|

701 gttccatggg ctttcagagg aggacaatg gccagaggcc tcaccaaac ctgtcacaca gaacatcagt gcagaggcct ggggcccagc agactgtgga
   q f h g l s e e d k w p e g s p k p v t q n i s a e a w g r a d c g
   |----- mouse TRBC -----|

801 atcaactcag catcctatca tcagggggtt ctgtctgcaa ccactcctta tgagatccta ctggggaagg ccaacctata tgctgtgctg gtcagtggcc
   i t s a s y h q g v l s a t i l y e i l l g k a t l y a v l v s g
   |----- mouse TRBC -----|

901 tgggtgctgat ggccatggtc aagaaaaaaa attcctgaga caaactttta tgcactcctg gccgttcttc acccagcaca gtggactcga gagactctggc
   l v l m a m v k k k n s -
   |----- mouse TRBC -----|
BstXI BglII

```

C2

### Sequence of the chimeric TCR $\alpha$ chain in pBud $\alpha$ BMNA9-15

TRAV13-1\*01 J13\*01

```

SbfI
3161 ttctctgcagg caatgtggcc accatgacat ccattcgagc
   m t s i r
   |-----

3201 tgtatttata ttctgtggc tgcagctgga cttgggtgaat ggagagaatg tggagcagca tccttcaacc ctgagtgtcc aggaggggaga cagcgtgtt
   a v f i f l w l q l d l v n g e n v e q h p s t l s v q e g d s a v
   |----- TCR $\alpha$ V13-1 leader peptide -----|----- human TCR $\alpha$  chain from MOG T cell clone -----|

3301 atcaagtga cttatccaga cagtgccctca aactacttcc cttggtataa gcaagaactt ggaaaaagac ctcagcttat tatagacatt cgttcaaatg
   i k c t y s d s a s n y f p w y k q e l g k r p q l i i d i r s n
   |----- human TCR $\alpha$  chain from MOG T cell clone -----|

3401 tgggogaaaa gaaagaccaa cgaattgctg ttacattgaa caagacagcc aaacatttct cctgacat cacaagagacc caacctgaag actcggctgt
   v g e k k d q r i a v t l n k t a k h f s l h i t e t q p e d s a
   |----- human TCR $\alpha$  chain from MOG T cell clone -----|

3501 ctacttctgt ggcgctggtt accagaaagt tacctttgga actggaacaa agtccaagt catcccaaat attcagaacc cagaacctgc tgtgtaccag
   v y f c g a r y q k v t f g t g t k l q v i p n i q n p e p a v y q
   |----- human TCR $\alpha$  chain from MOG T cell clone -----|-----|
SspI

3601 ttaaaagatc ctggtctca ggacagcacc ctctgctctg tcaccgactt tgactcccaa atcaatgtgc cgaaaacctt ggaatctgga acgttcatca
   l k d p r s q 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
   |----- mouse TRAC -----|

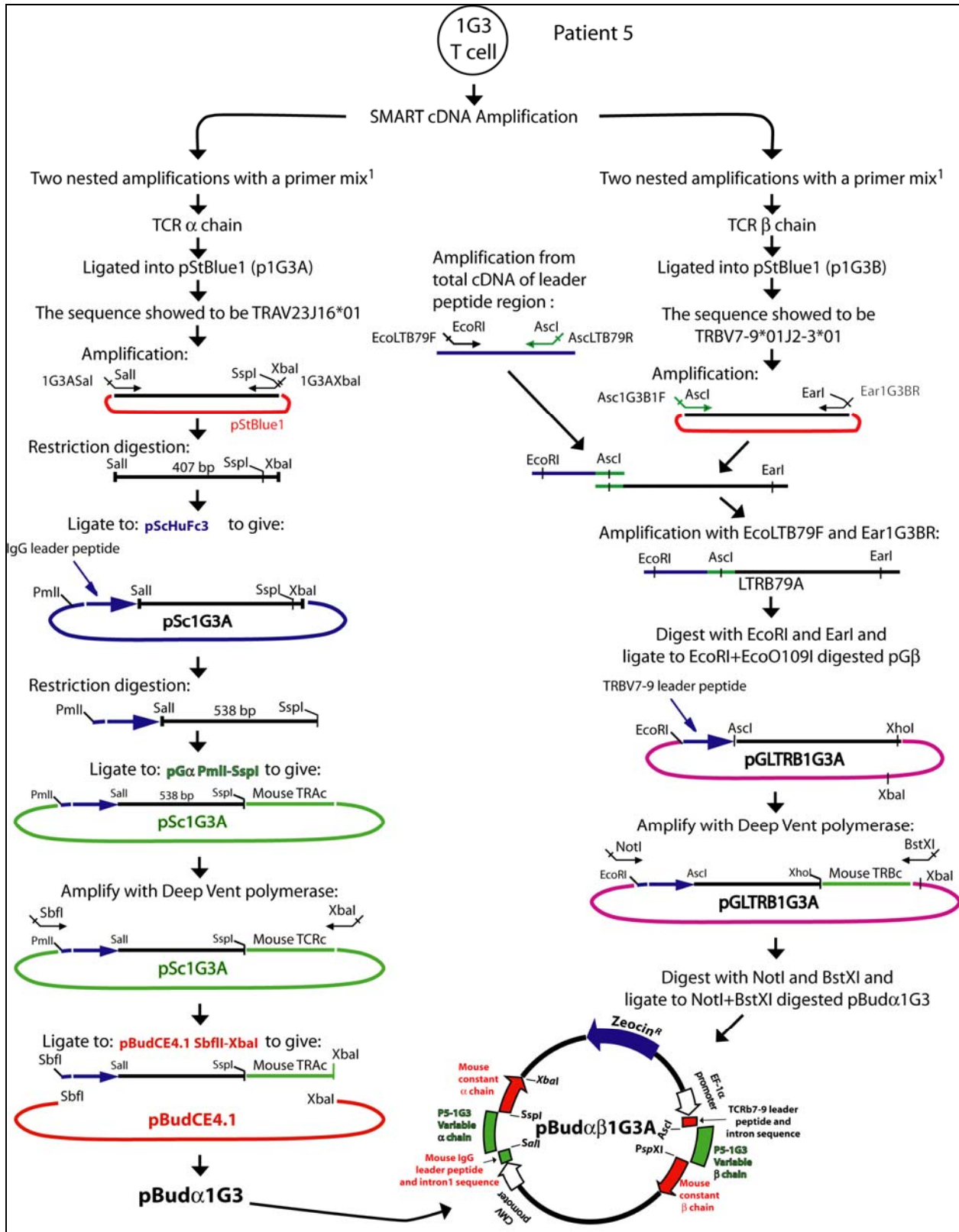
3701 ctgacaaaaa tgtgctggac atgaaagcta tggattccaa gagcaatggg gccattgctt ggagcaacca gacaagcttc acctgccaag atatctcaa
   t d k t v l d m k a m d s k s n g a i a w s n q t s f t c q d i f
   |----- mouse TRAC -----|

3801 agagaccaac gccacctacc ccagttcaga cgttccctgt gatgccactg tgaccgagaa aagctttgaa acagatatga acctaaactt tcaaaactcg
   k e t n a t y p 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
   |----- mouse TRAC -----|

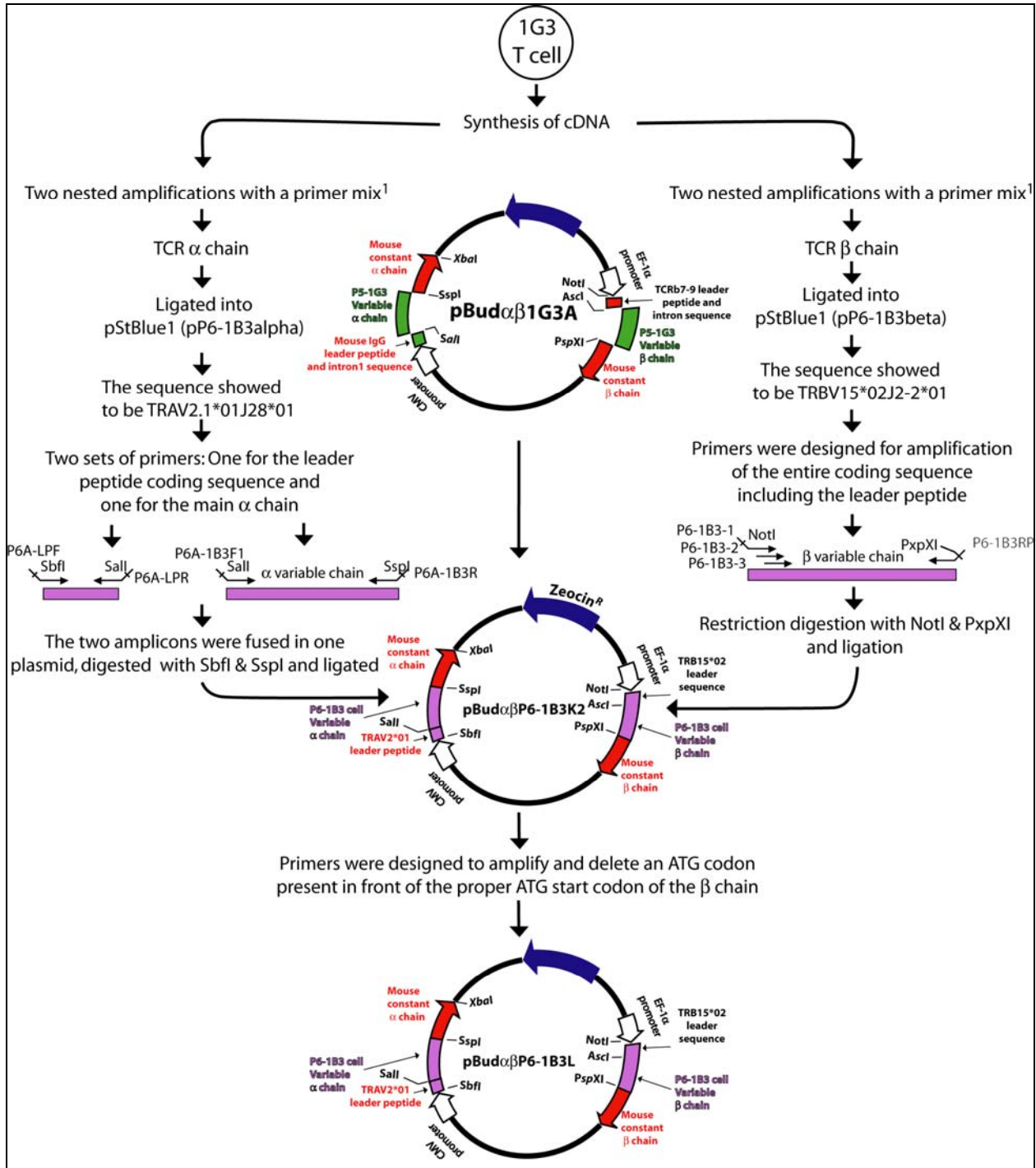
3901 tcagttatgg gactcgaat cctcctgctg aaagtagcgg gatttaacct gctcatgacg ctgaggctgt ggtccagtgt aggtctgcaa gactgacaga
   s v m g l r i l l l k v a g f n l l m t l r l w s s
   |----- mouse TRAC -----|

4001 gcctctagag gatccgaaca
BamHI XbaI

```



**Supplemental figure 9.** Scheme depicting the construction of the chimeric TCR expression vector pBudaβP6-1G3A. This vector has the TCRα and β variable regions from cell 1G3 from patient 5 fused to the mouse TCR constant region.



**Supplemental figure 10.** Scheme describing the construction of plasmid pBudαβP6-1B3L which contains the TCRα and β variable regions from the cell 1B3 of patient 6 fused to the mouse constant α and β regions.

