```
                10         20         30         40         50
GAATTCCAGTTTAATTTATTAAGAGCAAAATTAAATGATGTATGTGCTAATGATTAT
GluPheGlnPheAsnLeuLeuArgAlaLysLeuAsnAspValCysAlaAsnAspTyr

      60         70         80         90        100        110
TGTCAAATACCTTTCAATCTTAAAATTCGTGCAAATGAATTAGACGTACTTAAAAAACTT
CysGlnIleProPheAsnLeuLysIleArgAlaAsnGluLeuAspValLeuLysLysLeu

     120        130        140        150        160        170
GTGTTCGGATATAGAAAACCATTAGACAATATTAAAGATAATGTAGGAAAAATGGAGATT
ValPheGlyTyrArgLysProLeuAspAsnIleLysAspAsnValGlyLysMetGluIle

     180        190        200        210        220        230
ACATTAAAAAAAATAAAAAAACCCATAGAAAATATAAATTGAATTAATTTGAAGAAAGTG
ThrLeuLysLysIleLysLysProIleGluAsnIleAsn***

     240        250        260        270        280        290
TAAGAAAACAATTTGATTAAAAATTAAGAATTGCCAACTAAAAAAAAAAAAAAAAAAAAA

AAAAAGGAATTC
```

Fig. 5 Nucleotide sequence of λp190-1 cDNA. Sequence determination was carried out by the chain termination method[32] after subcloning *Eco*RI fragments of λp190-1 cDNA into M13mp9 (ref. 33) in both orientations. The putative polyadenylation signal is underlined. The reading frame is shown in phase with β-galactosidase. The two *Eco*RI linkers are indicated by a line above the sequence.

genetically pure clone Tak9/96 (4.7 kb). Kl and Tak9/96 are from Thailand[10,21], whilst MAD20 is from Papua, New Guinea[3]. This result may be particularly important as MAD20 and Tak9/96 differ from Kl with respect to the antigenic properties of p190[2,3].

We have estimated the frequency of the cloned p190 sequence in the *P. falciparum* DNA from the intensity of the 4.5 kb band in Southern blots of total genomic digests. Standard copy-number analysis[22,23] using the λp190-1 clone suggests that the number of copies of the p190 sequence per parasite genome is between one and five (data not shown). The part of the gene in p190-1 does not contain tandem repeats (see below and Fig. 5) as has recently been reported in coding sequences for *P. falciparum* S-antigens[13] and *P. knowlesi* circumsporozoite protein[12].

Sequencing studies (Fig. 5) in this laboratory on the p190-1 insert reveals a single open reading frame encoding 70 amino acids in phase with β-galactosidase, terminating with a UGA codon followed downstream by two other stop codons. The clone has a run of 26 A residues at its 3′ terminus. In addition, the sequence ATTAAA starts 24 bases upstream from the first terminal adenine, the expected position for a polyadenylation signal. This function has recently been attributed to the above sequence in several different systems[24–26]. We therefore suggest that the cloned sequence encodes the 3′-terminus of the p190 mRNA and the C-terminus of the polypeptide.

Our conclusion affects the interpretation of the mechanism by which p190 is processed from a primary product of 190,000 M_r to apparently smaller products. Examination of the parasite proteins reacting to the antibodies selected by the p190 clone reveals that in addition to p190, large product bands are also found (Fig. 3, lanes 3, 5). This strongly suggests that epitopes in the C-terminus are not removed by processing. The simplest interpretation of our result is that processing removes the N-terminus of the p190 polypeptide.

We have been unable to detect in the polypeptide encoded in p190-1 epitopes defined by monoclonal antibodies, including 2.2 and 7.3. This may simply reflect the fact that p190-1 contains less than a tenth of the total coding sequence. An alternative explanation is that the C-terminus of p190 cannot elicit an immune response because it is sequestered inside the parasite, as the monoclonal antibodies were obtained after immunization Hwith a whole parasite preparation[2].

The present report describes a cloned gene for a *P. falciparum* protein known to be both strongly immunogenic in man and a candidate for a subunit vaccine against malaria. We now have clones with larger p190 sequences which will test whether, HHlike the native protein (and its homologue in *P. yoelii*[5]), p190 synthesized in bacteria can elicit a protective response against blood stages of the parasite.

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Human antisera detect a *Plasmodium falciparum* genomic clone encoding a nonapeptide repeat

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***Plasmodium falciparum* causes malaria infections in its human host. Its wide distribution in tropical countries is a major world health problem. Before a vaccine can be produced, the identification and characterization of parasite antigens is necessary. This can be achieved by the cloning and subsequent analysis of genes coding for parasite antigens[1–4]. Recently established cDNA banks allow the expression of cDNA derived from the simian parasite *Plasmodium knowlesi*[5] and *P. falciparum*[6,7] in *Escherichia coli*. Recombinants encoding parasite antigens have been identified by immunodetection in both banks. Two of them contain repetitive units of 11 (ref. 7) or 12 (ref. 5) amino acids. We describe here the construction of an expression bank made directly from randomly generated fragments of *P. falciparum* genomic DNA. We detect several clones which react strongly with human African immune sera. One clone expresses an antigenic determinant composed of occasionally degenerated repeats of a peptide nonamer.**

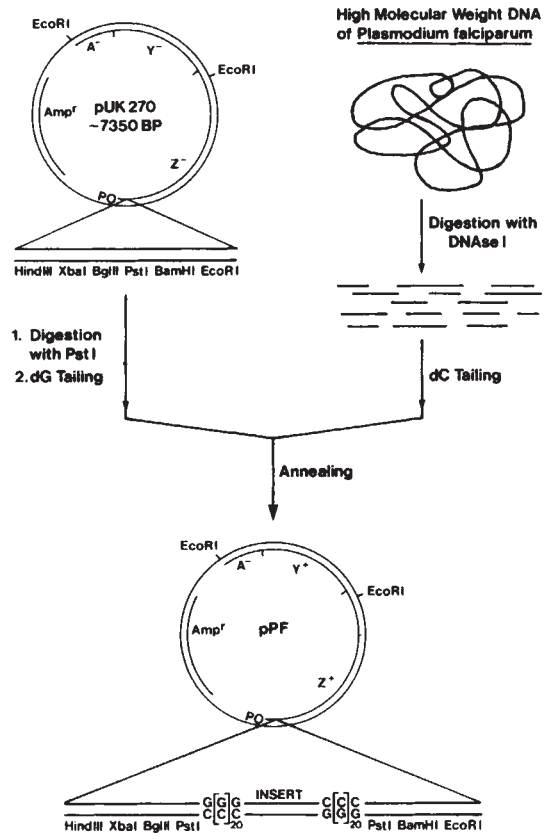

Fig. 1 Cloning of genomic DNA from *P. falciparum* in the expression vector pUK270. The vector pUK270 carries the *lacZ* and *lacY* genes with six unique restriction sites and a frameshift mutation in the promoter proximal part of the *lacZ* gene. Introduction of DNA inserts into the *Pst*I site after dG/dC tailing may overcome the frameshift mutation. Such plasmids will confer a Lac^+ phenotype on *E. coli* hosts which carry a deletion in the *lacZ* gene[8]. Such Z^+ clones can be isolated either as lactose-positive colonies on lactose minimal agar or as blue colonies on rich plates containing isopropyl-1-thio-β-D-galactoside as inducer and 5-bromo-4-chloro-3-indolyl-β-D-galactoside as indicator. The vector carrying no insert gives a pale blue colour to *E. coli* strains carrying a *lac* deletion on lactose indicator plates but does not allow growth on lactose plates. The lactose-positive recombinants express the inserted DNA as part of the N-terminus of β-galactosidase. Such chimaeric antigen-producing clones can be easily identified if antibodies directed against the expressed antigen are available[8]. Here we have modified the immunoenzymatic detection method described previously[8]. We have used nitrocellulose filters placed on lactose agar for colony replication and growth. Colonies grown on filters were lysed by dipping the filters for 1 min in chloroform at room temperature. Chloroform was removed from the filter by exposure to air for 5 min at 37 °C. Polyvinyl chloride sheets (80 mm diameter) were coated with antibodies and saturated with serum albumin[10]. We used IgG raised in rabbits immunized with Triton X-100 soluble *P. falciparum* asexual blood-stage antigens. The antibody-coated sheets were placed on the lysed colonies. After incubation for 1 h at 4 °C, the sheets were taken off and soaked in wash solution containing 0.5% Triton X-100 for 20 min. The adhering cell debris was removed by squirting with a 20-ml syringe containing wash solution over the sheets. To remove the last traces of cell debris the sheets were placed once more in fresh wash solution. The immunocomplexes were identified as described previously[8]. We used Kemp's method[6], which needs less antibody, to screen the Lac^+ colonies with a pool of four human African sera with high IFA titres directed against *P. falciparum* (gift of Professor Mannweiler). The mixture of antisera was cleared of antibodies directed against *E. coli* with sonicated extracts of *E. coli*. Immunocomplexes were detected with iodinated Protein A from *S. aureus*[6]. The lysed colonies were incubated with the treated human antisera after a ×25 dilution.

a

	5'.... G_nGGA GAC GTT GTG CCT
Repeat 1.	GAA GAA GTT GTT GAA GAA GTT GTG CCT
2.	... ... T.A ... ...A ..A
3.	.T. ... C.A ..A ... ...A ...
4.	.T. ... C.A ..A ... ...A ..A
5.	..G ... C.A ... .G ... A.A ...
6.	... ... A.. ..G ... ... A.A ...
7.	... .C ... ...A ..A ...
8.	..G ... A.A ... ...G A.A ...
9.	... ... T.A A.. ... ...A
10.	.T. ... C.A T.A ... ... A.A ..A
11.	.T. ... ..A T.A ... ..T ... A.A ...
12.	... ... A.A ..A
13.	... ... A.. A.. ... ... A.A ...
14.	... ... A.. A.. ... ..G .CG A.A ..A
15.	.T. ... A.. ... .G ... A.A ..A
16.	... ..T A.. ... ... A.A ..A
17.	... ... A.. ... .T ... A.A ..A
18.	... ... A.A ...
19.	... ...A ..A
20.	... ... T.A ..A ... ... A.A ..A
21.	... ... C.A ... .G ..G A.A ...
22.	... ... CC. ... ..G ... ..A ..A
23.	GAA GAA CTC C_n ....3'

b

	--- Glu Val Val Pro
Repeat 1.	Glu Glu Val Val Glu Glu Val Val Pro
2.	... ... Leu ... ...
3.	Val ... Leu ... ...
4.	Val ... Leu ... ...
5.	... ... Leu ... ... Ile ...
6.	... ... Ile ... ... Ile ...
7.	... ...
8.	... ... Ile ... ... Ile ...
9.	... ... Leu Ile ... ...
10.	Val ... Leu Leu ... . Ile ...
11.	Val ... ... Leu ... Asp ... Ile ...
12.	... ... Ile ...
13.	... ... Ile Ile ... ... Ile ...
14.	... ... Ile Ile ... ... Ala Ile ...
15.	Val ... Ile ... ... Ile ...
16.	... Asp Ile ... ... Ile ...
17.	... ... Ile ... ... Asp ... Ile ...
18.	... ... Ile ...
19.	... ...
20.	... ... Leu ... ... Ile ...
21.	... ... Leu ... ... Ile ...
22.	... ... Pro ... ...
23.	Glu Glu Leu ---

Fig. 2 *a*, DNA sequence of the insert of clone pPF11-1; *b*, the derived protein sequence.

We digested high-molecular weight DNA from the Palo Alto Uganda (FUP) strain[4] of *P. falciparum* with DNase I. Fragments of 50–1,000 base pairs (bp) were separated on a polyacrylamide gel and cloned into the 5' end of the *lacZ* gene by dG/dC tailing (see Fig. 1 legend). We used the vector pUK270 that carries in the non-essential 5' end of the *lacZ* gene several unique restriction sites. These sites introduce a frameshift into the *lacZ* gene which renders the *Z*, *Y* and *A* genes phenotypically Z^-, Y^- and A^-. After cutting the vector with *Pst*I, dG-tailing and annealing with genomic fragments, only recombinants allowing read-through or restart in phase are phenotypically Lac^+. We identified these positive recombinants and isolated and screened 10,000 Lac^+ clones with antisera (Fig. 1). We screened the clones with rabbit antiserum, obtained after immunization with Triton-

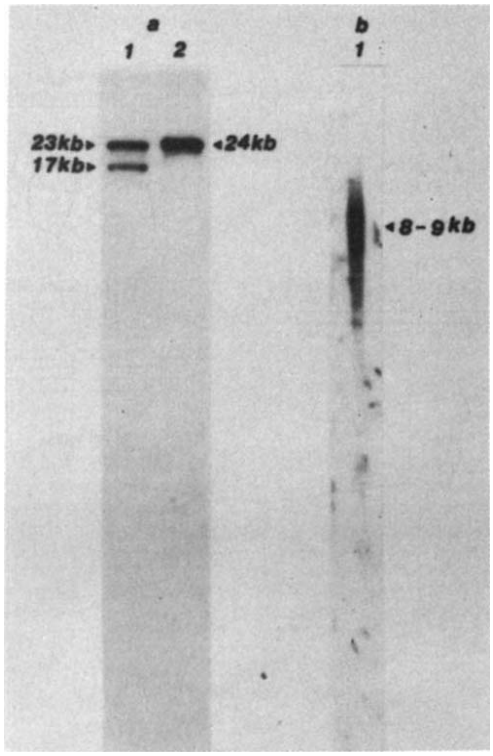

Fig. 3 *a*, Hybridization of genomic DNA of *P. falciparum* (FUP strain) with the 620-bp insert of clone pPF11-1. The genomic DNA (2 μg DNA per track) was cut with *Eco*RI (track 1) and *Hin*dIII (track 2). The DNA was fractionated on a 0.8% agarose gel, and transferred from the gel to nitrocellulose paper[13], hybridized with ^{32}P-labelled pPF11-1 probe, and autoradiographed. *b*, Hybridization of polyadenylated mRNA isolated from *P. falciparum* (blood stage) with the 620-bp insert of clone pPF11-1. 1.5 μg of polyadenylated mRNA was fractionated on a 1% agarose–formaldehyde gel, transferred to nitrocellulose paper, hybridized, washed and autoradiographed[15].

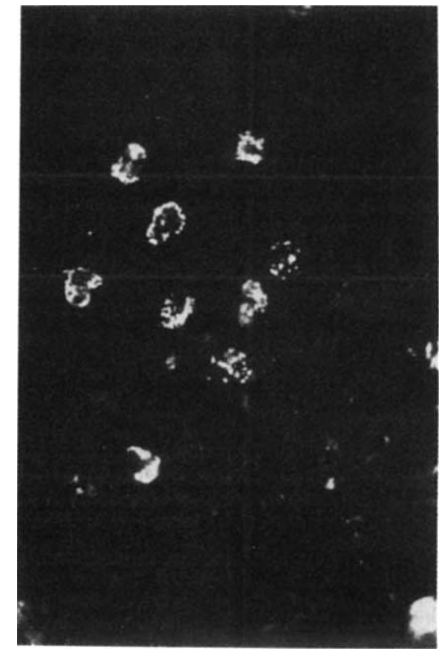

Fig. 4 Indirect immunofluorescence of *P. falciparum* antigen with anti-pPF11-1 antibodies. Thin smears of cultured parasites were fixed by acetone. The parasites were incubated with a 1/20 dilution of mouse antiserum raised against SDS-denatured 11-1 proteins and then revealed by fluorescein-conjugated anti-mouse IgG rabbit antibodies. Examination by phase-contrast microscopy (not shown) indicates that the immunofluorescence is not associated with the mature intracellular parasite but with the surrounding red blood cell membrane.

extracted proteins from *P. falciparum* asexual blood stages, using an immunoenzymatic technique[8–10]. Colonies producing antigen are visualized directly; we identified 36 positive clones with the rabbit antibodies.

We then screened 10,000 Lac$^+$ recombinants with a mixture of human sera from adult Africans living in areas endemic for malaria. Here, we visualized immunocomplexes with iodinated Protein A from *Staphylococcus aureus*[6]. Several positive clones were identified, most of them identical to those previously detected with the rabbit antisera. We present here the analysis of one antigen-producing clone, pPF11-1, which reacts strongly with both the rabbit and the human antisera in both tests. It produces a β-galactosidase fusion protein of about 140,000 molecular weight (MW) which is found in cytoplasmic extracts as a stable soluble protein and accounts for approximately 10% of the total protein. This clone contains 620 bp of *P. falciparum* genomic DNA. An insert of this length is consistent with the increase in size of the β-galactosidase fusion protein.

We next determined the complete sequence of the insert by the method of Maxam and Gilbert[11], and we found a 27-bp repeat running throughout the entire fragment. DNA sequence analysis reveals that only one reading frame contains no stop codons (Fig. 2). This frame codes for the ideal sequence Glu-Glu-Val-Val-Glu-Glu-Val-Val-Pro, but shows little similarity with the 12-amino acid repeat reported for the CS protein of *P. knowlesi*[5] or the 11-amino acid repeat found in *P. falciparum* S-antigen[7]. There is some variation in our polypeptide sequence as alanine, isoleucine or leucine can replace valine and aspartic acid or valine can replace glutamic acid. We conclude that the polypeptide probably exists as an α-helix, possibly interrupted by the prolines. The representation of this presumed α-helix according to Schiffer and Edmundson[12] reveals alternating amphipathic nonapeptides separated by proline residues, which suggests a superstructure of intertwined α-helices.

The pattern generated when pPF11-1 was hybridized[13] to FUP genomic DNA (Fig. 3*a*, track 2) demonstrates homology to a single *Hin*dIII fragment of approximately 24 kilobases (kb). We can identify two *Eco*RI fragments of 23 and 17 kbbbb (Fig. 3*a*, track 1), and single large *Bam*HI, *Aha*III, *Nde*I, *Hin*fI and *Bgl*II fragments. Sequences homologous to pPF11-1 are found in DNA extracted from a Thai isolate, K1 (ref. 14), and clone T9.96 (ref. 15)(data not shown). Our finding that the inside of pPF11-1 contains no *Eco*RI site, whereas two *Eco*RI fragments are observed in both FUP and T9.96[15] DNA, suggests that the repeated sequence spans more than 620 bp. Furthermore, a transcript of blood-stage *P. falciparum* of approximately 8–9 kb is identified[16] using pPF11-1 DNA as a probe (Fig. 3*b*). A mRNA of this apparent size could encode a protein of approximately 250,000 MW.

We raised rabbit and mice antisera against the purified fusion protein. In late trophozoites and schizont-infected red blood cells (RBC), a parasite protein can be detected by indirect immunofluorescence using these sera. The combination of phase-contrast and fluorescence microscopy indicates that the protein is located in patches associated with the membrane of the infected RBC (Fig. 4) and is not detected in ring-infected RBC or in merozoites. Using the same sera we are unable to immunoprecipitate an antigen from boiled culture supernatant in conditions where immune monkey serum immunoprecipitates an S-antigen of 200–220,000 MW. Preliminary data suggest that the 11-1 antigen has a higher molecular weight and is a minor constituent of the parasite protein. Several antigens are associated with the surface of erythrocytes infected with late stage parasites: schizont-infected cell agglutination antigen[17] which undergoes antigenic variation may be related to sequestration on endothelial cells[18]. We are currently investigating whether pPF11-1 is an antigen of this type.

Finally, concerning the difference between the commonly used cDNA expression banks (see refs 5–7) and our genomic

expression bank, in a cDNA bank the most commonly expressed gene products are overrepresented. In searching for cDNA coding for a rare protein, the overrepresented cDNAs of frequent gene products have to be accounted for. In a genomic bank all genes are equally represented, therefore only one bank is needed for one organism. In cDNA there is a bias for clones coding for the C-terminal end of the particular protein; a genomic bank shows no such bias.

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Rearrangements of T-cell receptor gene *YT35* in human DNA from thymic leukaemia T-cell lines and functional T-cell clones

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An essential property of the immune response is its ability to distinguish between self and non-self and to generate enormous diversity in antibody and T-cell immune responses. Although the genetic and molecular mechanisms responsible for antibody diversity have now largely been elucidated, the structure of the T-cell receptor and the diversification of the receptor repertoire have only recently become amenable to study. One approach has involved immunochemical studies of the protein precipitated by monoclonal antibodies which react specifically with the immunizing T-cell clones[1–3]. Another approach has been to clone and sequence a human[4] or a murine[5] T-cell specific message that may specify part of the T-cell receptor. We present here results of Southern blot analysis of non-T, immature T, and mature T-cell genomic DNA, and provide evidence that rearrangements of the *YT35* sequences do occur in the DNA of thymic leukaemia T cells. This suggests that *YT35* codes for at least part of the T-cell receptor and that rearrangements occur at this early stage of thymic ontogeny. Furthermore, DNA rearrangements are present in lymphocytes with phenotypic and functional characteristics of helper, killer, or suppressor T cells. We conclude that the three subpopulations of T cells operate via receptor molecules encoded by the same gene family.

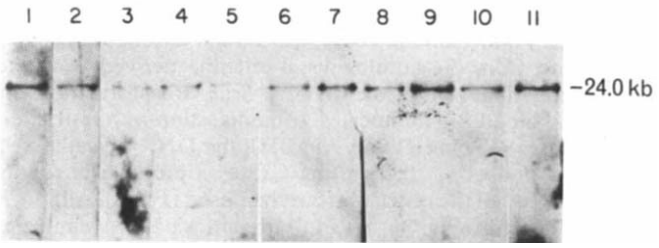

Fig. 1 Southern gel analysis of the T-cell receptor gene *YT35* in non-T-cell DNA. DNA was extracted from 11 independent samples of non-T cells and digested with 3 units of *Bam*HI per μg DNA overnight. DNA was then electrophoresed through 0.8% agarose gel as previously described[19]. DNA was transferred to nitrocellulose filters and hybridized to ^{32}P-dCMP-labelled nick-translated C35, a *Hpa*I (nucleotide 430) to *Eco*RV (nucleotide 1,087) subclone of *YT35* (ref. 4) by the method of Southern[6]. For detailed procedure, see ref. 19. Lane 1, DNA from granulocytes of a donor; lane 2, DNA from fibroblasts of the donor; lane 3, DNA from B-cell line RPMI 3638; lane 4, DNA from myeloid leukaemia cell line HL-60; lanes 5–11, DNAs from fibroblast cultures from seven different donors.

In order to determine the germ-line organization of the gene(s) corresponding to the putative T-cell receptor cDNA *YT35* and to estimate the frequency of DNA polymorphism among individuals, we have used Southern blot analysis[6] on 23 samples of genomic DNA extracted from fibroblasts (15 samples), B-cell lines (five lines), a bladder tumour cell line MGHU-1 and myeloid cell lines (two lines). The DNA samples were digested with the restriction enzyme *Bam*HI and probed with C35, a constant-region subclone of *YT35*. One 24-kilobase (kb) restriction fragment band was observed in each of the non-T cells examined (see Fig. 1). We also obtained similar results showing unchanging patterns and intensities of restriction fragment bands in non-T-cell DNA using four other restriction enzymes, *Bst*EII, *Hind*III, *Eco*RI and *Sph*I. These data establish that the non-T-cell DNAs from a variety of sources show no rearrangement of sequences corresponding to the putative T-cell receptor cDNA clone *YT35*. It also appears that DNA polymorphism between individuals is rare for sequences homologous to the *YT35* constant region.

To determine whether DNA rearrangements occur during or after intrathymic differentiation, we next investigated the rearrangement patterns of several thymic leukaemia lines. DNA was extracted from leukaemic T-cell lines and treated as described above. Our results show that DNA of non-T cells (Fig. 2*a*, lanes 4 and 5) contain the same restriction fragment band typical of germ-line DNA as summarized in Fig. 1. DNA from the thymic leukaemia cell lines (Fig. 2*a*, lanes 1, 2 and 3; Fig. 2*b*, lanes 2, 3 and 4) contains bands not found in the germ-line DNA lanes. When hybridization was carried out using the entire *YT35* cDNA, data consistent with DNA rearrangement in T-cell DNA were also observed, but the number of bands increased to 6 or 7 (Fig. 2*c*, lanes 2–4). In addition to the germ-line bands presented by the C35 probe, hybridization with the entire cDNA *YT35* probe reveals four new bands corresponding to the homologous variable gene segments.

Regulatory (helper and suppressor) and effector (killer) T-lymphocytes probably cooperate via complex interactions to establish a successful immune response, but the existence and relative role of the T-cell receptor in each of these subpopulations is unknown. In order to ascertain the presence of the T-cell receptor in functionally distinct T-cell subpopulations, we analysed four mature T-cell clones with helper, killer or suppressor function for possible gene rearrangements of sequences corresponding to the putative T-cell receptor clone *YT35*. All these T-cell clones were derived from the same individual and were generated by *in vitro* alloactivation against stimulating cells from a single donor[7,8].