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Section 29

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PATENT REQUEST: CONVENTION PATENT

We, being the person identified below as the Applicant, request the grant of a patent to the person identified below as the Nominated Person, for an invention described in the accompanying standard complete specification

Full application details follow:-

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Nominated Person:	ROUSSEL-UCLAF
Address:	35 Boulevard des Invalides, 75007, Paris, France
Invention Title:	NUCLEOTIDE SEQUENCES CODING FOR ALPHA CHAIN VARIABLE REGIONS IN HUMAN LYMPHOCYTE RECEPTORS AND APPLICATIONS THEREOF
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Convention Details

Application Number	Country	Country Code	Date of Application
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rawing number recommended to accompany the abstract FIG. 1

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PATENT

NOTICE OF ENTITLEMENT

We, ROUSSEL-UCLAF, of 35 Boulevard des Invalides, 75007, Paris, France, being the applicant and the person nominated for grant of patent in respect of the Application for an invention entitled NUCLEOTIDE SEQUENCES CODING FOR ALPHA CHAIN VARIABLE REGIONS IN HUMAN LYMPHOCYTE RECEPTORS AND APPLICATIONS THEREOF
state the following:-

PCT-CONVENTION NATIONAL PHASE FILING

(a) The person nominated for the grant of the patent:

(i) has entitlement from the actual inventor(s) by virtue of being a person who would, if a patent were to be granted upon an application made by the said inventor(s), be entitled to have the patent assigned to it; and

(ii) is the applicant of the basic application(s).

(b) The basic application(s) listed on the request form and in the declaration made under Article 8 of the PCT are the first application(s) made in a Convention country in respect of the invention.

....
Michael J. Houlihan Date
Registered Patent Attorney

1 October 1992

To: The Commissioner of Patents

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(57) Claim

1. Nucleotide sequences coding for the variable regions of ∝ chains of human T lymphocyte receptors, corresponding to cDNAs containing nucleotide sequences chosen from any one of:

a - V∝ segments corresponding to one of the sequences SEQ ID No. 1 to 11, and

b - J∝ segments corresponding to one of the sequences SEQ ID No. 12, 13 ~~and~~ and 15 to 20
and the sequences which differ from them by one or more nucleotides.

8. Peptides coded by any one of the nucleotide sequences as defined in any one of claims 1 to 7, as well as the alleles and the derivatives of the latter which possess the same function.

12. Antibodies directed against an antigenic determinant of one of the peptides defined in claim 8 or claim 9.

21. Therapeutic composition containing anti sense oligonucleotides corresponding to any one of the sequences of

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a Vα segment or ~~an~~ any one of the sequences of a Jα segment as defined in any one of claims 4 to 7.

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(54) Title: NUCLEOTIDE SEQUENCES CODING FOR ALPHA CHAIN VARIABLE REGIONS IN HUMAN LYMPHOCYTE RECEPTORS AND APPLICATIONS THEREOF

(54) Titre: SEQUENCES NUCLEOTIDIQUES CODANT POUR DES REGIONS VARIABLES DE CHAINES ALPHA DES RECEPTEURS DES LYMPHOCYTES HUMAINS AINSI QUE LEURS APPLICATIONS

(57) Abstract

Novel nucleotide sequences coding for alpha chain variable regions in human T lymphocyte receptors, corresponding peptide segments and diagnostic and therapeutic applications thereof.

(57) Abrégé

La présente invention concerne de nouvelles séquences nucléotidiques codant pour des régions variables de chaines α des récepteurs des lymphocytes T humains, les segments peptidiques correspondants et les applications diagnostiques et thérapeutiques.

Nucleotide sequences coding for variable regions of the α chains of human T lymphocyte receptors, corresponding peptide segments and the diagnostic and therapeutic uses.

The present invention relates to new nucleotide sequences coding for variable regions of α chain T-cell receptors, corresponding peptide segments and the diagnostic and therapeutic uses.

It is known that the receptors recognizing antigens at the surface of mature T lymphocytes (hereafter designated T-cell receptors) possess a structure having a certain similarity with those of immunoglobulins. Therefore, they contain heterodimeric structures containing α and β glycoprotein chains or γ and δ glycoprotein chains (see Meuer et al. (1), Moingeon et al. (2), Brenner et al. (3), Bank et al. (4)).

The directory of T-cell receptors must be able to address the immense diversity of antigenic determinants. This is obtained by genetic recombination of different discontinuous segments of genes which code for the different structural regions of T-cell receptors. Thus, the genes contain V segments (variable segments), optionally D segments (diversity segments), J segments (junction segments) and C segments (constant segments). During the differentiation of T-cells, specific genes are created by recombination of V, D and J segments for the β and δ loci and V and J segments for the α and γ loci. These specific combinations as well as the pairing of two chains create the combinational diversity. This diversity is highly amplified by two supplementary mechanisms, namely the imprecise recombination of V-D-J or V-J segments and the addition of nucleotides corresponding to the N region (Davis et al. (5).

A certain number of genetic V segments are already known. These segments have been grouped into subfamilies as a function of the similarity of sequences. By definition, the segments which have more than 75% similarity in the nucleotide sequence have been considered as members of the same subfamily (Crews et al. (6)). The known Vα genetic

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segments have also been classified into 22 subfamilies, 14 of which have only one member (see Concannon et al. (7), Kimura et al. (8), Wilson et al. (9)).

Moreover, about 60 J genetic segments have been described (9).

Furthermore, monoclonal antibodies directed against specific segments of the variable parts of T-cell receptors, in particular the β or δ chains, were recently described in WO 90/06758. These monoclonal antibodies are useful not only as diagnostic tools but also as therapeutic tools, for example, vis-à-vis rheumatoid athritis.

The use of synthetic peptides corresponding to the variable regions of the α or β chains in the treatment of auto-immune diseases is also described (23 and 24).

It is also known that variations exist from one individual to another in the expression of different variable segments of the T-cell receptor in man (27 and 28).

The present inventions aims to enrich the directory of genetic segments coding for the variable regions of the chains of T-cell receptors by providing on the one hand new Vα genetic segments belonging to new subfamilies or belonging to subfamilies of which at least one member is already known, and on the other hand, new Jα genetic segments.

Therefore a subject of the present invention is nucleotide sequences coding for the variable regions of α chains of human T lymphocyte receptors, corresponding to cDNA's containing nucleotide sequences chosen from any one of the following:

a - Vα segments corresponding to one of the sequences SEQ ID No. 1 to 11, and

b - Jα segments corresponding to one of the sequences SEQ ID No. 12, 13 and 15 to 20,

and the sequences which differ from them by one or more nucleotides.

More particularly a subject of the present invention is:

- sequences coding for the variable regions of chains of human T lymphocyte receptors, corresponding to cDNAs

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containing nucleotide sequences chosen from any one of the V α segments corresponding to one of the sequences SEQ ID No. 1 to 10 and the sequences which differ from them by one or more nucleotides,

- sequences coding for the variable regions of α chains of human T lymphocyte receptors, corresponding to cDNAs containing nucleotide sequences chosen from any one of the J α segments corresponding to one of the sequences SEQ ID No. 12, 13 and 15 to 20 and the sequences which differ from them by one or more nucleotides.

The expression "and sequences which differ from them by one or more nucleotides", encompasses alleles which differ by up to 8 nucleotides, but more often differ by 1 or 2 nucleotides or which can differ by the deletion or addition of one or two codons.

Also a more particular subject of the invention is:

- nucleotide sequences coding for the variable regions of α chains of human T lymphocyte receptors, corresponding to cDNAs corresponding to all or part of the nucleotide sequences chosen from any one of the V α segments corresponding to one of the sequences SEQ ID No. 2 to 5, and the sequences which differ from them by one or two nucleotides,

- nucleotide sequences coding for the variable regions of the α chains of human T lymphocyte receptors, corresponding to cDNAs corresponding to all or part of the nucleotide sequences chosen from any one of the Vα segments corresponding to one of the sequences

1 to 200 of SEQ ID No. 1
1 to 467 of SEQ ID No. 6
1 to 77 of SEQ ID No. 7
1 to 151 of SEQ ID No. 8
291 to 386 of SEQ ID No. 9
1 to 260 of SEQ ID No. 10

and the sequences which differ from them by one or two nucleotides,

- nucleotide sequences coding for the variable regions of the α chains of human T lymphocyte receptors,

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corresponding to cDNAs corresponding to all or part of the nucleotide sequence corresponding to SEQ ID No. 11 and which contain the 108 nucleotide,

- nucleotide sequences coding for the variable regions of the ∝ chains of human T lymphocyte receptors, corresponding to cDNAs corresponding to all or part of the nucleotide sequences chosen from any one of the J∝ segments corresponding to one of the sequences SEQ ID No. 12, 13 and 15 to 20 and the sequences which differ from them by one or two nucleotides.

By the expression "nucleotide sequences corresponding to cDNAs corresponding to all or part of the nucleotide sequences" is also designated the complete sequences as well as fragments of these sequences including short fragments (oligonucleotides) which can be used as probes (generally containing at least 10 nucleotides) or as primers (generally containing at least 15 nucleotides). In a general fashion, the invention encompasses the group of new oligonucleotides which are fragments of V∝ and J∝ sequences according to the invention.

As to the sequences which differ by one or two nucleotides, they correspond to variations which are observed experimentally at the time of determination of the nucleotide sequence of several cDNAs.

Also a subject of the present invention is the peptides coded by the nucleotide sequences according to the invention as well as the alleles and the derivatives of the latter which have the same function.

Also a subject of the present invention is the peptides constituted by or composed of a peptide sequence coded by all or part of the sequence 108 to 364 of SEQ ID No. 11.

In a general fashion, the present invention encompasses the peptides constituted by or composed of a peptide sequence coded by the nucleotide sequences according to the invention as well as fragments of these peptides. It also encompasses the peptides which differ from the latter by one or more amino acids and which have the same function. These peptides can correspond to modifications such as those known with

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muteins or to allelic variations. In fact it has been shown in particular that certain genetic segments coding for the variable regions of chains of T receptors in man were subjected to a phenomenon of genetic polymorphism called allelic variation (25). The present invention encompasses the peptides resulting from this phenomenon.

The nucleotide sequences according to the invention have been obtained according to the following stages:

- isolation of the RNA's of peripheral lymphocytes of an individual;
- obtaining the complementary DNA using reverse transcriptase and a primer A which is specific to the Cα region (SEQ ID No. 21);
- genetic amplification (by Anchored Polymerase Chain Reaction or A-PCR) using a DNA polymerase, a poly C primer (SEQ ID No. 22) and a primer B which is specific to the Cα region (SEQ ID No. 23);
- a new amplification by A-PCR using DNA polymerase and a primer C which is specific to the Cα region (SEQ ID No. 24);
- insertion in a plasmid vector;
- transformation of a bacterial host with the recombinant vector;
- screening of recombinant bacterial colonies with a labelled oligonucleotide D which is specific to Cα (SEQ ID No. 25);
- extraction of plasmids from positive colonies;
- and sequencing of DNA fragments containing the C region.

The present invention can be reproduced, in particular, by bispecific genetic amplification (polymerase chain reaction or PCR) by starting with the peripheral lymphocytes which express the mRNA including the variable or junctional segments corresponding to sequences ID No. 1 to 13 and 15 to 20 of the invention or alternatively by applying this PCR technique to genomic DNA of any somatic cell of an individual taken at random. The invention can also be reproduced by preparing the above genetic sequences by the chemical

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synthesis of oligonucleotides.

The peptides according to the invention can be obtained by standard peptide synthesis. They can also be obtained by the application of known genetic engineering techniques including the insertion of a DNA sequence coding for a peptide according to the invention into an expression vector such as a plasmid and the transformation of cells with this expression vector.

Therefore a subject of the present invention is also plasmids and expression vectors containing a DNA sequence coding for a peptide according to the invention as well as the hosts transformed with this vector.

Also a subject of the present invention is antibodies, and, in particular, monoclonal antibodies directed, against an antigenic determinant belonging to or composed of a peptide according to the invention.

The monoclonal antibodies may be obtained by any of the techniques which allow the production of antibody molecules from cell line culture. These techniques include different techniques using hybridomas.

The antibody production may be obtained in animals by the immunization of the animals by injection with the peptides or fragments according to the invention, whether they be natural, recombinant or synthetic, optionally after coupling to an immunogen such as tetanic anatoxin, or also by injection of human T lymphocytes expressing the corresponding sequences at their surface, including recombinant cells transfected with the corresponding coding sequences.

Also a subject of the present invention is hybridomas producing monoclonal antibodies directed against the polypeptides according to the invention.

The present invention also encompasses the fragments and the derivatives of monoclonal antibodies according to the invention which are reactive with defined variable regions of T-cell receptors. These fragments are, in particular, the $F(ab')_2$ fragments which can be obtained by the enzymatic cleavage of antibody molecules with pepsin, the Fab'

fragments which can be obtained by reduction of the disulphide bridges of $F(ab')_2$ fragments and the Fab fragments which can be obtained by the enzymatic cleavage of antibody molecules with papain in the presence of a reducing agent. These fragments can also be obtained by genetic engineering.

The monoclonal antibody derivatives are for example antibodies or fragments of these antibodies to which labellers such as a radio-isotope are attached. The monoclonal antibody derivatives are also antibodies or fragments of these antibodies to which therapeutically active molecules are attached, in particular, cytotoxic compounds.

The products of the invention have several uses in the field of diagnostics and in the field of therapeutics.

1 - Uses in the field of diagnostics

The oligonucleotides contained in the nucleotide sequences according to the invention can be used to constitute detection probes (generally at least 10 nucleotides) which are capable of hybridizing with a variable region of the chain or primers for the amplification of DNA (generally containing at least 15 nucleotides and preferably at least 17 nucleotides) which are capable of being linked to a sequence to be amplified.

Thus the oligonucleotides are used in the diagnosis of immune disorders by detecting the presence of nucleic acid sequences which are homologues of a gene coding for the variable regions of chains of T-cell receptors in the mRNA of a sample from a patient. Different methods can be used to establish a connection between the expression of T-cell genes and an illness. These methods include:

a - the production and analysis of cDNA expression libraries obtained from T-cells connected with the illness to determine the frequency of dominant genes;

b - Southern blot analysis of samples of genomic DNA to determine whether genetic polymorphisms or rearrangements of the genes coding for the T-cell receptors exist;

c - the analysis of samples by obtaining cDNA, amplification by PCR and hybridization with labelled probes;

d - the hybridization in situ of T-cells without

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culture of T-cells beforehand.

The primers are used in PCR reactions in a method such as that defined in c above.

The monoclonal antibodies, the fragments or the derivatives of these antibodies according to the invention, in particular the anti Vα antibodies, can be used to study T-type immune responses, for example in the field of the auto-immune diseases of cancerology, of allergies, of transplants and of infectious diseases. In particular, the directory of different variable α segments of the T receptor can be studied, whether it be blood or tissue T-cells. In a general fashion the techniques used can be in vitro or in vivo methods.

With in vitro methods, the samples used can be samples of body fluids or tissue samples. The techniques used can include in particular flow cytofluorimetry to analyse blood T lymphocytes or labelling with immunoperoxidase on an anatomopathological section to study the lymphocytes infiltrating the tissues.

With in vivo methods, the antibodies, their fragments or their derivatives are administered by the usual routes, for example by intravenous route, and the immunospecific linkages are detected. This can be obtained for example in the case where an antibody is used which is labelled with a radio-isotope.

2 - Uses in the therapeutic field

The oligonucleotides contained in the nucleotide sequences according to the invention can be used in therapeutics as anti sense oligonucleotides. In fact it is known that it is possible in vitro to inhibit the expression of a transcript gene in human lymphocytes by incubating these lymphocytes with an anti sense oligonucleotide specific to the gene in question (26). These anti sense oligonucleotides generally contain at least 10 and, preferably, at least 16 nucleotides. These anti sense oligonucleotides can be in particular the inverted and complemented sequences corresponding to 20 nucleotides upstream from the initiation site of the translation (ATG). The significance of the use in

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vitro of anti sense oligonucleotides specific to a Vα or Jα genetic segment is to abolish (or strongly diminish) the expression of a T receptor containing this Vα or Jα segment and thus to obtain a phenomenon of clonal deletion at the level of the specific reactivity of T lymphocytes. The anti sense oligonucleotides can not only be used in vitro on human T lymphocytes which are then reinjected, but also in vivo by local or systemic injection preferably after modification to increase the stability in vivo and the penetration into the T lymphocytes of these oligonucleotides.

The monoclonal antibodies according to the invention, in particular the anti Vα antibodies can be used to modulate the immune system. It is in this way that the antibodies can be administered to block the interaction of the effector T-cells with their specific antigen. Anti T receptor antibodies linked for example to a cytotoxic molecule or a radio-isotope can also be administered in a way so as to obtain a clonal deletion, thanks to the specific fixation on an α chain of a T-cell receptor. The monoclonal antibodies according to the invention can be used in therapeutics at low mitogenic concentrations so as to activate, in a specific fashion, certain sub-assemblies of T-cells or can be used at much higher concentrations to fix them to the receptors concerned and thus label these sub-assemblies with a view to their elimination by the reticulo-endothelial system. An important criterion in the treatment of an illness is the ability to modulate the sub-assemblies of T-cells linked with an illness. The exact nature of this therapeutic modulation, namely blocking or suppressing a particular sub-assembly of T-cells or on the contrary stimulating and activating a particular sub-assembly, will depend on the illness in question and the specific sub-assembly of T-cells concerned.

This type of treatment has an advantage over current treatments using antibodies such as the treatment with anti CD3 antibodies in patients having had a kidney transplant and having a rejection problem, given that thanks to the invention there will be no modulation of the totality of the

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T-cell population but only of the sub-assembly of T-cells expressing the α sub-family specific to the T-cell receptors.

Moreover, as the response of T-cells is often oligoclonal, it is generally convenient to use "cocktails" of several antibodies in therapeutics.

In addition anti Vα antibodies can be used to select T lyphocytes in vitro, for example by passing through a column containing spheres carrying the antibody. This separation of certain T lymphocytes can be used with a view to culturing these lymphocytes before reinjection into the patient.

Moreover, all or part of the peptide sequences according to the invention can be used in therapeutics, that is to say the peptide sequences coded by the nucleotide sequences according to the invention or fragments of these sequences (generally containing at least 8 to 10 amino acids). These sequences or these fragments, administered to humans or animals, can act as a decoy, that is to say they fix themselves on the epitope carried by the harmful antigen and stop the reaction of normal T-cells with the antigen, preventing in this way the development of an illness which is aggressive towards the self determinants. They can also be used as immunogens in the manufacture of vaccines (optionally after conjugation with protein carriers).

The present invention will be described in greater detail hereafter by referring to the annexed figures in which:

- Figures 1 A to E show in a line both a known V sequence and a partial sequence of an extension according to the invention for the respective sequences SEQ ID No. 6 to 10, marked IGRa 08 to IGRa 12. In these figures, the numbering of nucleotides starts at the ATG initiation codon (which is underlined). The dots indicate identical nucleotides. The sequences which are assumed to be the leader sequences have a line over them.

- Figure 2 shows in a line the new J sequences (SEQ ID No. 12, 13 and 15 to 20) marked IGRJa 01, 02 and 04 to 09. In these sequences the recombination signals of the germinal

line are underlined. The amino acids corresponding to highly preserved codons are marked above the sequences. The codons corresponding to a substitution in one position of a preserved amino acid are underlined twice.

- Figure 3 shows the Southern blot analyses of the genomic DNA treated with a restriction enzyme using probes specific to sequences SEQ ID No. 1 to 5. The restriction enzymes used are EcoRI (column R), Hind III (column H) and Bam III (column B). On this figure the triangles mark the position of DNA fragments hybridizing in a specific fashion with C α .

- Figure 4 represents the detection by autoradiography of amplified transcripts of TCR α chains expressed by the peripheral lymphocytes of a healthy individual and of a co-amplified β-actin control.

I - Obtaining the cDNA and amplification by PCR

The peripheral lymphocytes of an individual are used as the DNA source. The total RNA was prepared according to the method using guanidinium isothiocyanate and caesium chloride (Chirgwin (10)) or according to a one-stage method by extraction with guanidinium isothiocyanate, phenol and chloroform (Chomcyznski (11)).

The first cDNA strand was synthesized in a final volume of 50 microlitres at a temperature of 42°C for 1 hour using 5 micrograms of total RNA, reverse transcriptase and a primer A which is specific to the C α region constituted by the sequence 5'-GTTGCTCCAGGCCACAGCACTG (SEQ ID No. 21). This material was then purified by extraction with phenol/chloroform and precipitation with ammonium acetate. After selecting a 0.45/1 kb fraction on agarose gel, the addition of a dG end is carried out on the RNA/cDNA hetero complex in a $CoCl_2$ addition buffer with 14 units of terminal deoxynucleotidyl transferase (TdT) for 30 minutes at 37°C. The reaction was stopped by maintenance at 70°C for 10 minutes. 1N NaOH (1/3 volume) was added and the sample was incubated at 50°C for 1 hour to hydrolyze the RNA, then

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neutralized with Tris HCl 2M pH 8 and 1N HCl. After extraction with a phenol/chloroform mixture the first cDNA strand at end G was precipitated with ethanol and subjected to an amplification using the PCR technique (Polymerase Chain Reaction described by Saiki et al. (12)) in a final volume of 100 microlitres containing 50 mM of KCl, 10 mM of Tris-Cl pH 8.3, 1.5 mM of $MgCl_2$, 0.1% (weight/volume) of gelatine, 200 micromoles of dNTP, 2.5 units of Taq polymerase and 100 picomoles of two primers. The two primers used are, on the one hand a poly-C primer (5'-GCATGCGCGCGGCCGCGGAGG-14C) (SEQ ID No.22) described by Loh et al. (13) as well as a primer B specific to the Cα region (5'-GTCCATAGACCTCATGTCCAGCACAG) (SEQ ID No. 23).

25 amplification cycles are carried out followed by a final 15 minute elongation period at 72°C. Each cycle includes a denaturation stage at 92°C for 1 minute, a hybridization stage at 55°C for 2 minutes and an elongation period at 72°C for 4 minutes. The amplified products are then precipitated with ethanol, resuspended in 30 mM of sodium acetate pH 5, 50 mM NaCl, 1 mM $ZnCl_2$, glycerol 5% by volume and 1/10 of this material is purified as a function of size on a 1% low melting point agarose gel.

A second amplification phase is then carried out directly on approximately 10% of the band containing the agarose following the same conditions as previously, except that the primer 5'-ATACACATCAGAATTCTTACTTTG (SEQ ID No. 24) is used as primer C which is specific to the Cα region. The reaction mixture is then precipitated with ethanol and resuspended in 60 µl of H_2O.

II - <u>Cloning and sequencing of cDNA's</u>

1/3 of the product of the second amplification is digested with Sac II, separated on 1% agarose gel and purified by absorption on glass beads. The material is inserted in the Bluescript SK^+ vector (Stratagene, La Jolla, U.S.A.) and the recombinants obtained are used to transform the XL1-blue strains of E. Coli (Stratagene). After

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sedimentation in the presence of X-gal and IPTG, a test is carried out on the white colonies using a "dot blot" technique and a third oligonucleotide specific to the Cα region (5'-GTCACTGGATTTAGAGTCT) (SEQ ID No. 25) labelled with ^{32}P is used as a probe. The plasmid DNA of positive colonies is extracted and sequencing takes place under the two strands by the process of termination of the dideoxy chain (Sanger et al. (14)) with Sequenase 2.0 (United States Biochemicals, Cleveland, U.S.A.) following the supplier's recommendations. With the exception of the Sequence SEQ ID No. 5, all the nucleotide sequences were determined on the two strands using at least two distinct clones of cDNA.

The sequences obtained were compared with published Vα and Jα sequences using the method developed by Lipman and Pearson (15). The presumed start codons were identified by searching for the presence of the Kozak consensus sequence for the initiation sites of translations in the eukaryotic cells (Kozak (16)). The presence of hydrophobic leader sequences of the N-terminal side was detected by analysis of the hydrophobicity according to the method described by Kyte (17).

III - Southern blot analysis

The DNA was extracted from the human erythroleucemic cell line K562 and digested with one of the following restriction enzymes: EcoR I, BamH I or Hind III. The DNA (15 micrograms) was subjected to electrophoresis on 0.7% agarose and transferred onto Nylon membranes as described by Triebel et al. (18). The hybridizations were carried out at 65°C with 6 X SSC, 0.5% of SDS, 5 X Denhardt's and 100 micrograms of denatured salmon sperm DNA for 16 hours. The membranes were washed at 65°C with 2 X SSC, 0.2% of SDS.

As V specific probes, are used the probes obtained by amplification of V-J-C cDNA (> 500 bp) containing Vα fragments corresponding to sequences SEQ ID No. 1 to 5 using as a primer the poly-C primer and the C primer. The probes were purified on 1% agarose gel. DNA probes labelled

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with ^{32}P were prepared from fragments purified on agarose by the Feinberg method (19).

IV - Results

By using the A-PCR method, 308 cDNA which hybridize with the Cα clone were cloned, then sequenced. Among these, 172 cDNA correspond to the V-J-Cα variable regions only.

The Vα and Jα sequences of the invention are shown in the list of sequences under SEQ ID No. 1 to 11 and SEQ ID No. 12, 13 and 15 to 20 respectively. The sequences SEQ ID No. 2 to 5 correspond to the new sub-families (designated Vα 25, Vα 26, Vα 27 and Vα 29 respectively) while the sequences SEQ ID No. 1 and 6 to 11 correspond to extensions of known V segments.

1. Vα sequences corresponding to new sub-families

The Southern blot analyses of germinal line DNA subjected to digestion by endonucleases, using V-J-Cα probes containing Vα fragments corresponding to sequences SEQ ID No. 2 to 5 were carried out in "low stringency" hybridization conditions to identify the number of Vα genetic segments belonging to each family and to characterize the DNA restriction fragments carrying these Vα genetic segments. The representative results are shown in Figure 3.

These analyses show that the sub-family corresponding to the sequence SEQ ID No. 3 includes at least two genetic segments while the other sequences (SEQ ID No. 2, No. 4 and No. 5) probably correspond to unique members.

The sizes of the germinal DNA restriction fragments are as follows:

SEQ ID No. 2 : EcoR I 2.2 kb, Hind III 4.8 and 5.7 kb, BamH I 25 kb

SEQ ID No. 3 : EcoR I 4.6 and 7.5 kb, Hind III 4.2 and 6.4 kb, BamH I 23 and 4.5 kb

SEQ ID No. 4 : EcoR I 7.6 kb, Hind III 18 kb, BamH I 9 and 0.9 kb

SEQ ID No. 5 : EcoR I 5.9 and 4.8 kb, Hind III 6.6 kb,

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BamH I 6.5 kb.

2. Sequences corresponding to extensions of known V sequences

SEQ ID No. 1 (IGR a 02) corresponds to an extension of the 5' side of the LINV sequence (171 bp) (mengle-Gaw (20)): This sequence defines the sub-family provisionaly designated Vα w24.

SEQ ID No. 6 (IGR a 08) : this sequence corresponds to an extension of the 5' side of the Vα 1 AE11 clone sequence (Klein et al. (21)). The two straight line sequences are represented in Figure 1A.

SEQ ID No. 7 (IGR a 09): This sequence corresponds to an extension coding for the NH2 terminal end of the Vα 2 AF110 sequence (Klein already quoted). The two straight line sequences are represented in Figure 1B. The sequence ID No. 7 corresponds to a consensus sequence. The existence of a T instead of a C is observed in position 206.

SEQ ID No. 8 (IGR a 010): This sequence corresponds to an extension of the 5' region of the Vα HAP35 clone (Yoshikai (22)). The two straight line sequences are represented in Figure 1C. The sequence ID No. 8 corresponds to a consensus sequence. The existence of a G instead of an A in position 307 and the existence of a T instead of a C in position 360 have been observed.

SEQ ID No. 9 (IGR a 11): This sequence corresponds to an extension of the 3' side of the Vα 7 HAP12 sequence (Yoshikai already quoted). The straight line of the sequences is represented in Figure 1D. The sequence ID No. 9 corresponds to a consensus sequence. The existence of a C instead of a T in position 86 has been observed.

SEQ ID No. 10 (IGR a 12): This sequence includes the complete coding region of a gene of the Vα 22 sub-family which had been previously identified by the partial sequence (113 bp) AC9 (Klein already quoted). The two straight line sequences are represented in Figure 1E.

SEQ ID No. 11 (IGR a 13): This sequence corresponds in part to the HAVT 32 and HAVT 35 clones (belonging to the Vα 16

(8) sub-family and which have been described as pseudogenes. In fact, following the addition of a nucleotide in position 108, the SEQ ID No. 11 codes for an original variable region of a T lymphocyte receptor. Moreover, this sequence is equivalent to a sequence HSTCAYM (Klein et al. (21)) for the coding part. However, the sequence SEQ No. 11 is the only one which is complete and coding.

3. Jα sequences

The set of new Jα sequences are represented in Figure 2. Among the 8 Jα segments, the majority of them have a highly preserved amino acid sequence FGXGT of Jα segments as described by Yoshikai already quoted. However, for the IGRJa 07 segment the threonine residue is replaced by an isoleucine residue.

In addition, instead of a phenylalanine residue a cysteine residue is found in IGRJa 02G.

The present invention also aims at providing specific oligonucleotides of different Vα sub-families, which can be used as primers for the amplification of DNA corresponding to these different Vα sub-families, with a view, for example, of a study of the expression of certain Vα sub-families in a patient and finally of a diagnosis of immune disorders, as indicated above.

The predominant expression of certain Vα sub-families has already been studied using an incomplete range of oligonucleotides. In this way Nitta et al. (29) have described the predominant expression of Vα 7 genes in the lymphocytes infiltrating the tumours. Moreover, Sottini et al. (30) have described the study of the directory of Vα 's, in patients suffering from rheumatoid arthritis.

The present invention aims to provide a complete range of oligonucleotides allowing the study, of both known Vα sub-families and new Vα sub-families of the invention and which are completely specific to each sub-family. Thus the oligonucleotides have been chosen and synthesized to this end

and to the requirements of modifications of one or two nucleotides which have been introduced relative to the natural sequences to reduce the cross-reactions between sub-families.

Thus a subject of the present invention is also oligonucleotides which can be used as primers for the amplification of DNA corresponding to the variable regions of α chains of T-cell receptors, chosen from the sequences SEQ ID No. 26 to 54.

Also a subject of the present invention is the use, as primers for the amplification of DNA corresponding to the variable regions of α chains of T-cell receptors, of oligonucleotides chosen from the sequences SEQ ID No. 26 to 54.

Also a subject of the present invention is a detection process of nucleotide sequences coding for the Vα segments of T receptors or of cDNA corresponding to transcription products of the latter, in a biological sample, characterized in that it includes:

a) the amplification of DNA with at least one pair of primers formed by one of the oligonucleotides chosen from the sequences SEQ ID No. 26 to 54 and one oligonucleotide belonging to segment Cα , and

b) the detection of amplified sequences with a Cα probe.

The oligonucleotide belonging to a Cα segment used for the amplification can be, in particular, chosen from the sequences SEQ ID No. 55 and 56.

To check the efficiency of the amplification, the operation is preferably carried out in the presence of a pair of control primers and the corresponding control sequence amplified using a corresponding control probe is detected.

This pair of control primers can correspond to two Cβ segments, for example the Cβ F and Cβ K primers corresponding to sequences SEQ ID No. 61 and 62. Then a Cβ detection probe is used (corresponding for example to the sequence SEQ ID No. 63). But this pair of primers can also be constituted by two primers belonging to β-actin, notably those corresponding to sequences SEQ ID No. 58 and 59. Then a detection probe

corresponding to a sequence of β-actin, such as the sequence SEQ ID No. 60, is used.

Also a subject of the present invention is a diagnostic kit for the implementation of the process defined previously, which includes:

a) at least one oligonucleotide chosen from the sequences SEQ ID No. 26 to 54,

b) a Cα primer,

c) a Cα probe.

In addition such a kit advantageously contains:

d) a pair of control primers,

e) a control probe.

This kit can contain in particular:

a) the group of 29 oligonucleotides corresponding to sequences SEQ ID No. 26 to 54,

b) a Cα primer chosen from the sequences corresponding to sequences SEQ ID No. 55 and 56,

c) a pair of control primers for β-actin having a sequence corresponding to sequences SEQ ID NO. 58 and 59 respectively,

d) a Cα probe corresponding to the sequence SEQ ID No. 57,

e) a control probe for β-actin corresponding to the sequence SEQ ID No. 60.

In the information given in the list of sequences for the sequences 26 to 60, the sequences SEQ ID No. 26 to 47 correspond to sequences belonging to clones of known Vα1 to Vα22 sub-families (available from the EMBL database) or to sequences which differ from them by one or two nucleotides.

The sequences SEQ ID No. 49, 50, 51, 52 and 54 correspond to sequences belonging to clones of new sub-families of the invention, corresponding to sub-families provisionally designated Vαw24, Vαw25, Vαw26, Vαw27 and Vαw29 (w indicating that the designation is pending definitive designation).

The sequences SEQ ID No. 48 and 53 correspond to sequences belonging to clones IGRa01 and IGRa06 respectively of known sub-families but having not yet received definitive designation (Vαw23 and Vαw28 respectively) one member element of which has already been described (Hinkkanen A. et

al. (31) and Bernard O. et al. (32) respectively). The complete sequence of IGRa06 has not yet been published.

The sequences SEQ ID No. 55 and 56 are two examples of oligonucleotides which can be used as Cα primers for amplification.

The sequence SEQ ID No. 57 is the sequence of a C probe which can be used for the detection of amplified DNAs.

The sequences SEQ ID No. 58, 59 and 60 are respectively the sequences of a pair of oligonucleotides belonging to the sequence of β-actin which can be used to check the amplification and the sequence of a probe for detecting the corresponding amplified DNAs.

In the list of sequences the position indicated is the position of the 5' end counting from the predicted initiation site of the ATG translation. In the case where the sequences are incomplete (unknown 5' region), the position (marked with an asterisk) is given relative to the first nucleotide of the sequence. The underlined nucleotides correspond to mismatches introduced relative to the natural sequence.

The oligonucleotides were sythesized with an Applied Biosystems 381 A automated DNA synthesizer using the β-cyano-ethylphosphoramidite method (Sinha N. et al. (33)) and following the protocol recommended by the manufacturer. The oligonucleotides were detritylated in the apparatus, cleaved from the support and deprotected with ammonia (at 60°C for 5 hours). The crude products were purified by reverse phase high pressure chromatography on a μ-bondapak C18 column using an acetonitrile gradient (9 to 15%) in a 0.01M triethy' nium acetate buffer at pH 5.5.

The amplification carried out using the primers according to the invention can be, in particular, the technique of amplification by PCR (Polymerase Chain Reaction) as described by Saiki et al. (12) and in Patents US-A-4 683 195, 4 683 202, 4 889 818.

For the PCR, a double strand DNA can be used which is denatured or a cDNA obtained from RNA using reverse transcriptase as mentioned above.

The polymerization agent is a DNA polymerase, in

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particular, Taq polymerase.

Generally the amplification cycle is repeated 25 to 40 times.

The probes which are used for detecting the amplified sequences can be obtained by labelling the oligonucleotides with a radio-active isotope, which leads to detection by autoradiography, or by conjugation with an enzyme such as peroxidase (ECL Amersham system), alkaline phosphatase or β-galactosidase (Tropix Ozyme system), which leads to detection by chemiluminescence.

The following example illustrates the implementation of the detection process according to the invention.

The peripheral lymphocytes of a healthy individual were prepared by density gradient centrifugation. The total DNA was extracted according to a one-stage method by extraction with guanidium isothiocyanate, phenol and chloroform (Chomczynski, 11). The complementary DNA was synthesized in a final volume of 20 µl at 42°C for one hour using 1 to 5 µg of total RNA, the reverse transcriptase and the CαB primer (1.25 µM).

The material obtained was then heated at 95°C for 3 minutes before being subjected to an amplification according to the PCR technique using in parallel each of the specific Vα primers corresponding to sequences SEQ ID No. 26 to 54 and the CαB primer specific to the Cα region (SEQ ID No. 56). This amplification was carried out in a final volume of 10 µl per tube containing 50 mM of KCl, 10 mM of tris-HCl pH 8.3, 1.5 mM of $MgCl_2$, 0.1% (weight/volume) of gelatine, 200 µM of dNTP, 0.25 units of Taq polymerase and 0.25 µM of each primer. A control amplification was carried out in each tube from 25 mN of a DNA fragment of β-actin of 877 base pairs prepared by PCR and Act 1 and Act 2 primers (SEQ ID No. 58 and 59) specific to actin. 30 amplification cycles were carried out followed by a final elongation stage of 5 minutes at 72°C. Each cycle included a denaturation stage at 94°C for one minute, a hybridization stage at 65°C for one minute and an elongation period at 72°C for one minute.

The products obtained were separated by electrophoresis

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on 2% agarose gel, transferred onto nylon membranes in an alkaline buffer and hybridized simultaneously with the Cα C oligonucleotide probes (SEQ ID No. 57) and Act 3 (SEQ ID No. 60) labelled with ^{32}P by the polynucleotidyl T4 kinase enzyme. The hybridization was carried out at 42°C for 16 hours in a buffer containing 6 X SSC, 0.5% SDS, 5 X Denhardt's, 0.05% NaH_2PO_4 and 100µg/ml of denatured salmon sperm DNA. The membranes were then washed with SSC 6 X, 20 mM NaH_2PO_4, twice at ambient temperature for 5 minutes and once at 50°C for 30 minutes then autoradiographed.

The results obtained are shown in Figure 4.

The actin control (band of 877 base pairs) allows the amplification to be verified in all wells. A specific signal appears below this band the size of which corresponds to the size of corresponding amplified fragments, each fragment having a length corresponding to the distance between the locus of the specific Vα oligonucleotide and the Cα primer.

With the individual tested, Figure 4 shows the preferential expression of certain genetic segments defined relative to the others. For example, the Vα 27, 28 and 29 sub-families are less well represented than the Vα 2, 3 and 6 sub-families.

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LIST OF SEQUENCES

I - GENERAL INFORMATION

(1) APPLICANT: Company called ROUSSEL-UCLAF

(2) TITLE OF THE INVENTION:

Nucleotide sequences coding for the variable regions of α chains of human T lymphocyte receptors, corresponding peptide segments and the diagnostic and therapeutic uses.

(3) NUMBER OF SEQUENCES: 62

II - INFORMATION FOR SEQ ID No. 1

CHARACTERISTICS OF THE SEQUENCES

TYPE: nucleotide and its corresponding protein

LENGTH: 371 base pairs

NUMBER OF STRANDS: single

CONFIGURATION: linear

TYPE OF MOLECULE: cDNA for mRNA

ORIGIN

ORGANISM: human

CELL LINE: human T lymphocytes

CHARACTERISTICS

NAME OF DNA: IGR a 02

SEQUENCE: Vα w24

DESCRIPTION OF THE SEQUENCE

```
                AGTCAACTTC TGGGAGCAGT CTCTGCAGAA TAAAA ATG AAA AAG CAT     47
                                                       Met Lys Lys His
                                                       1

CTG ACG ACC TTC TTG GTG ATT TTG TGG CTT TAT TTT TAT AGG GGG AAT            95
Leu Thr Thr Phe Leu Val Ile Leu Trp Leu Tyr Phe Tyr Arg Gly Asn
5                   10                  15                  20

GGC AAA AAC CAA GTG GAG CAG AGT CCT CAG TCC CTG ATC ATC CTG GAG           143
Gly Lys Asn Gln Val Glu Gln Ser Pro Gln Ser Leu Ile Ile Leu Glu
                25                  30                  35

GGA AAG AAC TGC ACT CTT CAA TGC AAT TAT ACA GTG AGC CCC TTC AGC           191
Gly Lys Asn Cys Thr Leu Gln Cys Asn Tyr Thr Val Ser Pro Phe Ser
            40                  45                  50

AAC TTA AGG TGG TAT AAG CAA GAT ACT GGG AGA GGT CCT GTT TCC CTG           239
Asn Leu Arg Trp Tyr Lys Gln Asp Thr Gly Arg Gly Pro Val Ser Leu
        55                  60                  65

ACA ATC ATG ACT TTC AGT GAG AAC ACA AAG TCG AAC GGA AGA TAT ACA           287
Thr Ile Met Thr Phe Ser Glu Asn Thr Lys Ser Asn Gly Arg Tyr Thr
    70                  75                  80

GCA ACT CTG GAT GCA GAC ACA AAG CAA AGC TCT CTG CAC ATC ACA GCC           335
Ala Thr Leu Asp Ala Asp Thr Lys Gln Ser Ser Leu His Ile Thr Ala
85                  90                  95                  100

TCC CAG CTC AGC GAT TCA GCC TCC TAC ATC TGT GTG                           371
Ser Gln Leu Ser Asp Ser Ala Ser Tyr Ile Cys Val
                105                 110
```

III - INFORMATION FOR SEQ ID No. 2

CHARACTERISTICS OF THE SEQUENCES

TYPE: nucleotide and its corresponding protein

LENGTH: 400 base pairs

NUMBER OF STRANDS: single

CONFIGURATION: linear

TYPE OF MOLECULE: cDNA for mRNA

ORIGIN

ORGANISM: human

CELL LINE: human T lymphocytes

CHARACTERISTICS

NAME OF DNA: IGR a 03

SEQUENCE: V_{α} w25

DESCRIPTION OF THE SEQUENCE

```
                                GACTCTAAGC CCAAGAGAGT TTCTTGAAGC AAAAAAAAAA    40

AAAACCCATT CAGGAAATAA TTCTTTGCTG ATAAGG ATG CTC CTT GAA CAT TTA               94
                                        Met Leu Leu Glu His Leu
                                        1               5

TTA ATA ATC TTG TGG ATG CAG CTG ACA TGG GTC AGT GGT CAA CAG CTG              142
Leu Ile Ile Leu Trp Met Gln Leu Thr Trp Val Ser Gly Gln Gln Leu
            10                  15                  20

AAT CAG AGT CCT CAA TCT ATG TTT ATC CAG GAA GGA GAA GAT GTC TCC              190
Asn Gln Ser Pro Gln Ser Met Phe Ile Gln Glu Gly Glu Asp Val Ser
        25                  30                  35

ATG AAC TGC ACT TCT TCA AGC ATA TTT AAC ACC TGG CTA TGG TAC AAG              238
Met Asn Cys Thr Ser Ser Ser Ile Phe Asn Thr Trp Leu Trp Tyr Lys
    40                  45                  50

CAG GAC CCT GGG GAA GGT CCT GTC CTC TTG ATA GCC TTA TAT AAG GCT              286
Gln Asp Pro Gly Glu Gly Pro Val Leu Leu Ile Ala Leu Tyr Lys Ala
55                  60                  65                  70

GGT GAA TTG ACC TCA AAT GGA AGA CTG ACT GCT CAG TTT GGT ATA ACC              334
Gly Glu Leu Thr Ser Asn Gly Arg Leu Thr Ala Gln Phe Gly Ile Thr
                75                  80                  85

AGA AAG GAC AGC TTC CTG AAT ATC TCA GCA TCC ATA CCT AGT GAT GTA              382
Arg Lys Asp Ser Phe Leu Asn Ile Ser Ala Ser Ile Pro Ser Asp Val
            90                  95                  100

GGC ATC TAC TTC TGT GCT                                                     400
Gly Ile Tyr Phe Cys Ala
        105
```

IV - INFORMATION FOR SEQ ID No. 3

CHARACTERISTICS OF THE SEQUENCES

TYPE: nucleotide and its corresponding protein

LENGTH: 339 base pairs

NUMBER OF STRANDS: single

CONFIGURATION: linear

TYPE OF MOLECULE: cDNA for mRNA

ORIGIN

ORGANISM: human

CELL LINE: human T lymphocytes

CHARACTERISTICS

NAME OF DNA: IGR a 04

SEQUENCE: Vα w26

DESCRIPTION OF THE SEQUENCE

```
  AGCTAAGGG ATG GAG ACT GTT CTG CAA GTA CTC CTA GGG ATA TTG GGG     48
            Met Glu Thr Val Leu Gln Val Leu Leu Gly Ile Leu Gly
            1               5                  10

TTC CAA GCA GCC TGG GTC AGT AGC CAA GAA CTG GAG CAG AGT CCT CAG     96
Phe Gln Ala Ala Trp Val Ser Ser Gln Glu Leu Glu Gln Ser Pro Gln
    15                  20                  25

TCC TTG ATC GTC CAA GAG GGA AAG AAT CTC ACC ATA AAC TGC ACG TCA    144
Ser Leu Ile Val Gln Glu Gly Lys Asn Leu Thr Ile Asn Cys Thr Ser
30                  35                  40                  45

TCA AAG ACG TTA TAT GGC TTA TAC TGG TAT AAG CAA AAG TAT GGT GAA    192
Ser Lys Thr Leu Tyr Gly Leu Tyr Trp Tyr Lys Gln Lys Tyr Gly Glu
                50                  55                  60

GGT CTT ATC TTC TTG ATG ATG CTA CAG AAA GGT GGG GAA GAG AAA AGT    240
Gly Leu Ile Phe Leu Met Met Leu Gln Lys Gly Gly Glu Glu Lys Ser
            65                  70                  75

CAT GAA AAG ATA ACT GCC AAG TTG GAT GAG AAA AAG CAG CAA AGT TCC    288
His Glu Lys Ile Thr Ala Lys Leu Asp Glu Lys Lys Gln Gln Ser Ser
        80                  85                  90

CTG CAT ATC ACA GCC TCC CAG CCC AGC CAT GCA GGC ATC TAC CTC TGT    336
Leu His Ile Thr Ala Ser Gln Pro Ser His Ala Gly Ile Tyr Leu Cys
    95                  100                 105

GGA                                                                339
Gly
110
```

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V - INFORMATION FOR SEQ ID No. 4

CHARACTERISTICS OF THE SEQUENCES

TYPE: nucleotide and its corresponding protein

LENGTH: 335 base pairs

NUMBER OF STRANDS: single

CONFIGURATION: linear

TYPE OF MOLECULE: cDNA for mRNA

ORIGIN

ORGANISM: human

CELL LINE: human T lymphocytes

CHARACTERISTICS

NAME OF DNA: IGR a 05

SEQUENCE: Vα w27

DESCRIPTION OF THE SEQUENCE

```
                AGAAAAAAAA AATGAAGAAG CTACTAGCAA TGATCCTGTG GCTTCAACTA     50

                 GACCGGTTAA GTGGAGAGCT GAAAGTG GAA CAA AAC CCT CTG TTC     95
                                           Glu Gln Asn Pro Leu Phe
                                           1               5

CTG AGC ATG CAG GAG GGA AAA AAC TAT ACC ATC TAC TGC AAT TAT TCA    143
Leu Ser Met Gln Glu Gly Lys Asn Tyr Thr Ile Tyr Cys Asn Tyr Ser
            10                  15                  20

ACC ACT TCA GAC AGA CTG TAT TGG TAC AGG CAG GAT CCT GGG AAA AGT    191
Thr Thr Ser Asp Arg Leu Tyr Trp Tyr Arg Gln Asp Pro Gly Lys Ser
        25                  30                  35

CTG GAA TCT CTG TTT GTG TTG CTA TCA AAT GGA GCA GTG AAG CAG GAG    239
Leu Glu Ser Leu Phe Val Leu Leu Ser Asn Gly Ala Val Lys Gln Glu
    40                  45                  50

GGA CGA TTA ATG GCC TCA CTT GAT ACC AAA GCC CGT CTC AGC ACC CTC    287
Gly Arg Leu Met Ala Ser Leu Asp Thr Lys Ala Arg Leu Ser Thr Leu
55                  60                  65                  70

CAC ATC ACA GCT GCC GTG CAT GAC CTC TCT GCC ACC TAC TTC TGT GCC    335
His Ile Thr Ala Ala Val His Asp Leu Ser Ala Thr Tyr Phe Cys Ala
                75                  80                  85
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VI - INFORMATION FOR SEQ ID No. 5

CHARACTERISTICS OF THE SEQUENCES

TYPE: nucleotide and its corresponding protein

LENGTH: 361 base pairs

NUMBER OF STRANDS: single

CONFIGURATION: linear

TYPE OF MOLECULE: cDNA for mRNA

ORIGIN

ORGANISM: human

CELL LINE: human T lymphocytes

CHARACTERISTICS

NAME OF DNA: IGR a 07

SEQUENCE: Vα w29

DESCRIPTION OF THE SEQUENCE

```
                GAAGCTGACT GGATATTCTG GCAGGCCAAG G ATG GAG ACT CTC CTG     46
                                                   Met Glu Thr Leu Leu
                                                   1               5

AAA GTG CCT TCA GGC ACC TTG TTG TGG CAG TTG ACC TGG GTG GGA AGC     94
Lys Val Pro Ser Gly Thr Leu Leu Trp Gln Leu Thr Trp Val Gly Ser
                10                  15                  20

CAA CAA CCA GTG CAG AGT CCT CAA GCC GTG ATC CTC CGA GAA GGG GAA    142
Gln Gln Pro Val Gln Ser Pro Gln Ala Val Ile Leu Arg Glu Gly Glu
            25                  30                  35

GAT GCT GTC ACC AAC TGC AGT TCC TCC AAG GCT TTA TAT TCT GTA CAC    190
Asp Ala Val Thr Asn Cys Ser Ser Ser Lys Ala Leu Tyr Ser Val His
        40                  45                  50

TGG TAC AGG CAG AAG CAT GGT GAA GCA CCC GTC TTC CTG ATG ATA TTA    238
Trp Tyr Arg Gln Lys His Gly Glu Ala Pro Val Phe Leu Met Ile Leu
    55                  60                  65

CTG AAG GGT GGA GAA CAG ATG CGT CGT GAA AAA ATA TCT GCT TCA TTT    286
Leu Lys Gly Gly Glu Gln Met Arg Arg Glu Lys Ile Ser Ala Ser Phe
70                  75                  80                  85

AAT GAA AAA AAG CAG CAA AGC TCC CTG TAC CTT ACG GCC TCC CAG CTC    334
Asn Glu Lys Lys Gln Gln Ser Ser Leu Tyr Leu Thr Ala Ser Gln Leu
                90                  95                  100

AGT TAC TCA GGA ACC TAC TTC TGC GGG                                361
Ser Tyr Ser Gly Thr Tyr Phe Cys Gly
            105                 110
```



VII - INFORMATION FOR SEQ ID No. 6

CHARACTERISTICS OF THE SEQUENCES

TYPE: nucleotide and its corresponding protein

LENGTH: 569 base pairs

NUMBER OF STRANDS: single

CONFIGURATION: linear

TYPE OF MOLECULE: cDNA for mRNA

ORIGIN

ORGANISM: human

CELL LINE: human T lymphocytes

CHARACTERISTICS

NAME OF DNA: IGR a 08

SEQUENCE: Vα 1

DESCRIPTION OF THE SEQUENCE

```
                                                                  TCAGTTTCTT  10

CTTCCTGCAG CTGGTTGAGT TCTTTCCAGA CAAAGACAAG TGACAAGAAT TAGAGGTTTA  70

AAAAGCAACC AGATTCATCT CAGCAGCTTT TGTAGTTTTA AATAAGCAAG GAGTTTCTCC 130

AGCGAAACTT CCTCACACCT CTTGGTCTTG GTCTCTTCAG ACACTTTCCT TCCTGTTCTC 190

TGGAGATCTT GCAGAAAAGA GCCTGCAGTG TTTCCCTTGC TCAGCC ATG CTC CTG     245
                                                     Met Leu Leu
                                                      1

GAG CTT ATC CCA CTG CTG GGG ATA CAT TTT GTC CTG AGA ACT GCC AGA     293
Glu Leu Ile Pro Leu Leu Gly Ile His Phe Val Leu Arg Thr Ala Arg
    5                  10                  15

GCC CAG TCA GTG ACC CAG CCT GAC ATC CAC ATC ACT GTC TCT GAA GGA    341
Ala Gln Ser Val Thr Gln Pro Asp Ile His Ile Thr Val Ser Glu Gly
20                  25                  30                  35

GCC TCA CTG GAG TTG AGA TGT AAC TAT TCC TAT GGG GCA ACA CCT TAT    389
Ala Ser Leu Glu Leu Arg Cys Asn Tyr Ser Tyr Gly Ala Thr Pro Tyr
                40                  45                  50

CTC TTC TGG TAT GTC CAG TCC CCC GGC CAA GGC CTC CAG CTG CTC CTG    437
Leu Phe Trp Tyr Val Gln Ser Pro Gly Gln Gly Leu Gln Leu Leu Leu
            55                  60                  65

AAG TAC TTT TCA GGA GAC ACT CTG GTT CAA GGC ATT AAA GGC TTT GAG    485
Lys Tyr Phe Ser Gly Asp Thr Leu Val Gln Gly Ile Lys Gly Phe Glu
        70                  75                  80

GCT GAA TTT AAG AGG AGT CAA TCT TCC TTC AAC CTG AGG AAA CCC TCT    533
Ala Glu Phe Lys Arg Ser Gln Ser Ser Phe Asn Leu Arg Lys Pro Ser
    85                  90                  95

GTG CAT TGG AGT GAT GCT GCT GAG TAC TTC TGT GCT                    569
Val His Trp Ser Asp Ala Ala Glu Tyr Phe Cys Ala
100                 105                 110
```



VIII - INFORMATION FOR SEQ ID No. 7

CHARACTERISTICS OF THE SEQUENCES

TYPE: nucleotide and its corresponding protein

LENGTH: 330 base pairs

NUMBER OF STRANDS: single

CONFIGURATION: linear

TYPE OF MOLECULE: cDNA for mRNA

CONSENSUS SEQUENCE

ORIGIN

ORGANISM: human

CELL LINE: human T lymphocytes

CHARACTERISTICS

NAME OF DNA: IGR a 09

SEQUENCE: Vα 2

DESCRIPTION OF THE SEQUENCE

```
AAA TCC TTG AGA GTT TTA CTA GTG ATC CTG TGG CTT CAG CTG AGC CGG    48
Lys Ser Leu Arg Val Leu Leu Val Ile Leu Trp Leu Gln Leu Ser Arg
1               5                  10                  15

GTT TGG AGC CAA CAG AAG GAG GTG GAG CAG AAT TCT GGA CCC CTC AGT    96
Val Trp Ser Gln Gln Lys Glu Val Glu Gln Asn Ser Gly Pro Leu Ser
            20                  25                  30

GTT CCA GAG GGA GCC ATT GCC TCT CTC AAC TGC ACT TAC AGT GAC CGA   144
Val Pro Glu Gly Ala Ile Ala Ser Leu Asn Cys Thr Tyr Ser Asp Arg
        35                  40                  45

GGT TCC CAG TCC TTC TTC TGG TAC AGA CAA TAT TCT GGG AAA AGC CCT   192
Gly Ser Gln Ser Phe Phe Trp Tyr Arg Gln Tyr Ser Gly Lys Ser Pro
    50                  55                  60

GAG TTG ATA ATG TCC ATA TAC TCC AAT GGT GAC AAA GAA GAT GGA AGG   240
Glu Leu Ile Met Ser Ile Tyr Ser Asn Gly Asp Lys Glu Asp Gly Arg
65                  70                  75                  80

TTT ACA GCA CAG CTC AAT AAA GCC AGC CAG TAT GTT TCT CTG CTC ATC   288
Phe Thr Ala Gln Leu Asn Lys Ala Ser Gln Tyr Val Ser Leu Leu Ile
                85                  90                  95

AGA GAC TCC CAG CCC AGT GAT TCA GCC ACC TAC CTC TGT GCC           330
Arg Asp Ser Gln Pro Ser Asp Ser Ala Thr Tyr Leu Cys Ala
            100                 105                 110
```



IX - INFORMATION FOR SEQ ID No. 8

CHARACTERISTICS OF THE SEQUENCES

TYPE: nucleotide and its corresponding protein

LENGTH: 400 base pairs

NUMBER OF STRANDS: single

CONFIGURATION: linear

TYPE OF MOLECULE: cDNA for mRNA

CONSENSUS SEQUENCE

ORIGIN

ORGANISM: human

CELL LINE: human T lymphocytes

CHARACTERISTICS

NAME OF DNA: IGR a 10

SEQUENCE: Vα 5

DESCRIPTION OF THE SEQUENCE

```
GCCAAACAGA ATGGCTTTTT GGCTGAGAAG GCTGGGTCTA CATTTCAGGC     50

CACATTTGGG GAGACGA ATG GAG TCA TCC CTG GGA GGT GTT TTG      94
                       Met Glu Ser Ser Leu Gly Gly Val Leu
                       1               5

CTG ATT TTG TGG CTT CAA GTG GAC TGG GTG AAG AGC CAA AAG ATA GAA   142
Leu Ile Leu Trp Leu Gln Val Asp Trp Val Lys Ser Gln Lys Ile Glu
10                  15                  20                  25

CAG AAT TCC GAG GCC CTG AAC ATT CAG GAG GGT AAA ACG GCC ACC CTG   190
Gln Asn Ser Glu Ala Leu Asn Ile Gln Glu Gly Lys Thr Ala Thr Leu
                30                  35                  40

ACC TGC AAC TAT ACA AAC TAT TCT CCA GCA TAC TTA CAG TGG TAC CGA   238
Thr Cys Asn Tyr Thr Asn Tyr Ser Pro Ala Tyr Leu Gln Trp Tyr Arg
            45                  50                  55

CAA GAT CCA GGA AGA GGC CCT GTT TTC TTG CTA CTC ATA CGT GAA AAT   286
Gln Asp Pro Gly Arg Gly Pro Val Phe Leu Leu Leu Ile Arg Glu Asn
        60                  65                  70

GAG AAA GAA AAA AGG AAA GAA AGA CTG AAG GTC ACC TTT GAT ACC ACC   334
Glu Lys Glu Lys Arg Lys Glu Arg Leu Lys Val Thr Phe Asp Thr Thr
    75                  80                  85

CTT AAA CAG AGT TTG TTT CAT ATC ACA GCC TCC CAG CCT GCA GAC TCA   382
Leu Lys Gln Ser Leu Phe His Ile Thr Ala Ser Gln Pro Ala Asp Ser
90                  95                  100                 105

GCT ACC TAC CTC TGT GCT                                          400
Ala Thr Tyr Leu Cys Ala
                110
```

X - INFORMATION FOR SEQ ID No. 9

CHARACTERISTICS OF THE SEQUENCES

TYPE: nucleotide and its corresponding protein

LENGTH: 386 base pairs

NUMBER OF STRANDS: single

CONFIGURATION: linear

TYPE OF MOLECULE: cDNA for mRNA

CONSENSUS SEQUENCE

ORIGIN

ORGANISM: human

CELL LINE: human T lymphocytes

CHARACTERISTICS

NAME OF DNA: IGR a 11

SEQUENCE: Vα 7

DESCRIPTION OF THE SEQUENCE

```
                GCCTTCTGCA GACTCCAATG GCTCAGGAAC TGGGAATGCA GTGCCAGGCT     50

            CGTGGTATCC TGCAGCAG ATG TGG GGA GTT TTC CTT CTT TAT GTT      95
                                Met Trp Gly Val Phe Leu Leu Tyr Val
                                1               5

TCC ATG AAG ATG GGA GGC ACT ACA GGA CAA AAC ATT GAC CAG CCC ACT     143
Ser Met Lys Met Gly Gly Thr Thr Gly Gln Asn Ile Asp Gln Pro Thr
10                  15                  20                  25

GAG ATG ACA GCT ACG GAA GGT GCC ATT GTC CAG ATC AAC TGC ACG TAC     191
Glu Met Thr Ala Thr Glu Gly Ala Ile Val Gln Ile Asn Cys Thr Tyr
                30                  35                  40

CAG ACA TCT GGG TTC AAC GGG CTG TTC TGG TAC CAG CAA CAT GCT GGC     239
Gln Thr Ser Gly Phe Asn Gly Leu Phe Trp Tyr Gln Gln His Ala Gly
            45                  50                  55

GAA GCA CCC ACA TTT CTG TCT TAC AAT GTT CTG GAT GGT TTG GAG GAG     287
Glu Ala Pro Thr Phe Leu Ser Tyr Asn Val Leu Asp Gly Leu Glu Glu
        60                  65                  70

AAA GGT CGT TTT TCT TCA TTC CTT AGT CGG TCT AAA GGG TAC AGT TAC     335
Lys Gly Arg Phe Ser Ser Phe Leu Ser Arg Ser Lys Gly Tyr Ser Tyr
    75                  80                  85

CTC CTT TTG AAG GAG CTC CAG ATG AAA GAC TCT GCC TCT TAC CTC TGT     383
Leu Leu Leu Lys Glu Leu Gln Met Lys Asp Ser Ala Ser Tyr Leu Cys
90                  95                  100                 105

GCT                                                                 386
Ala
```



XI - INFORMATION FOR SEQ ID No. 10

CHARACTERISTICS OF THE SEQUENCES

TYPE: nucleotide and its corresponding protein

LENGTH: 383 base pairs

NUMBER OF STRANDS: single

CONFIGURATION: linear

TYPE OF MOLECULE: cDNA for mRNA

ORIGIN

ORGANISM: human

CELL LINE: human T lymphocytes

CHARACTERISTICS

NAME OF DNA: IGR a 12

SEQUENCE: Vα 22

DESCRIPTION OF THE SEQUENCE

```
                TGTGACTTCT TCATGTTAAG GATCAAGACC ATTATTTGGG TAACACACTA     50

        AAG ATG AAC TAT TCT CCA GGC TTA GTA TCT CTG ATA CTC TTA CTG      95
            Met Asn Tyr Ser Pro Gly Leu Val Ser Leu Ile Leu Leu Leu
            1               5                   10

CTT GGA AGA ACC CGT GGA GAT TCA GTG ACC CAG ATG GAA GGG CCA GTG     143
Leu Gly Arg Thr Arg Gly Asp Ser Val Thr Gln Met Glu Gly Pro Val
15                  20                  25                  30

ACT CTC TCA GAA GAG GCC TTC CTG ACT ATA AAC TGC ACG TAC ACA GCC     191
Thr Leu Ser Glu Glu Ala Phe Leu Thr Ile Asn Cys Thr Tyr Thr Ala
                35                  40                  45

ACA GGA TAC CCT TCC CTT TTC TGG TAT GTC CAA TAT CCT GGA GAA GGT     239
Thr Gly Tyr Pro Ser Leu Phe Trp Tyr Val Gln Tyr Pro Gly Glu Gly
            50                  55                  60

CTA CAG CTC CTC CTG AAA GCC ACG AAG GCT GAT GAC AAG GGA AGC AAC     287
Leu Gln Leu Leu Leu Lys Ala Thr Lys Ala Asp Asp Lys Gly Ser Asn
        65                  70                  75

AAA GGT TTT GAA GCC ACA TAC CGT AAA GAA ACC ACT TCT TTC CAC TTG     335
Lys Gly Phe Glu Ala Thr Tyr Arg Lys Glu Thr Thr Ser Phe His Leu
    80                  85                  90

GAG AAA GGC TCA GTT CAA GTG TCA GAC TCA GCG GTG TAC TTC TGT GCT     383
Glu Lys Gly Ser Val Gln Val Ser Asp Ser Ala Val Tyr Phe Cys Ala
95                  100                 105                 110
```

XII - INFORMATION FOR SEQ ID No. 11

CHARACTERISTICS OF THE SEQUENCES

TYPE: nucleotide and its corresponding protein

LENGTH: 364 base pairs

NUMBER OF STRANDS: single

CONFIGURATION: linear

TYPE OF MOLECULE: cDNA for mRNA

ORIGIN

ORGANISM: human

CELL LINE: human T lymphocytes

CHARACTERISTICS

NAME OF DNA: IGR a 13

SEQUENCE: Vα 16

DESCRIPTION OF THE SEQUENCE

```
                AATCCCGCCC GCCGTGAGCT TAGCTGGAGC C ATG GCC TCT GCA CCC     46
                                                   Met Ala Ser Ala Pro
                                                   1               5

ATC TCG ATG CTT GCG ATG CTC TTC ACA TTG AGT GGG CTG AGA GCT CAG     94
Ile Ser Met Leu Ala Met Leu Phe Thr Leu Ser Gly Leu Arg Ala Gln
                10                  15                  20

TCA GTG GCT CAG CCG GAA GAT CAG GTC AAC GTT GCT GAA GGG AAT CCT    142
Ser Val Ala Gln Pro Glu Asp Gln Val Asn Val Ala Glu Gly Asn Pro
            25                  30                  35

CTC ACT GTG AAA TGC ACC TAT TCA GTC TCT GGA AAC CCT TAT CTT TTT    190
Leu Thr Val Lys Cys Thr Tyr Ser Val Ser Gly Asn Pro Tyr Leu Phe
        40                  45                  50

TGG TAT GTT CAA TAC CCC AAC CGA GGC CTC CAG TTC CTT CTG AAA TAC    238
Trp Tyr Val Gln Tyr Pro Asn Arg Gly Leu Gln Phe Leu Leu Lys Tyr
    55                  60                  65

ATC ACA GGG GAT AAC CTG GTT AAA GGC AGC TAT GGC TTT GAA GCT GAA    286
Ile Thr Gly Asp Asn Leu Val Lys Gly Ser Tyr Gly Phe Glu Ala Glu
70                  75                  80                  85

TTT AAC AAG AGC CAA ACC TCC TTC CAC CTG AAG AAA CCA TCT GCC CTT    334
Phe Asn Lys Ser Gln Thr Ser Phe His Leu Lys Lys Pro Ser Ala Leu
                90                  95                  100

GTG AGC GAC TCC GCT TTG TAC TTC TGT GCT                            364
Val Ser Asp Ser Ala Leu Tyr Phe Cys Ala
            105                 110
```



XIII - INFORMATION FOR SEQ ID No. 12

CHARACTERISTICS OF THE SEQUENCES

TYPE: nucleotide and its corresponding protein

LENGTH: 264 base pairs

NUMBER OF STRANDS: single

CONFIGURATION: linear

TYPE OF MOLECULE: cDNA for mRNA

ORIGIN

ORGANISM: human

CELL LINE: human T lymphocytes

CHARACTERISTICS

NAME OF DNA: Ja 01

SEQUENCE: Jα

DESCRIPTION OF THE SEQUENCE

```
CCTTCAAGGA AAATTAAGGC AAATAGAATT GGGCTGGGGA GTTGCTACTT ATTAGTATTC  60

CTCCCACGTT CTAACCTAAT TATAAGGAGG TTGTTTTGGC CATGGGCAGT CATCTCAGGT 120

TTTGTTTTCC TGCTTTCCTC CCTAACCTCC ACCTGTCTTC CTAGAGGCCT GAGTCAAGGT 180

TATTGCAATA GCACTAAAGA CTGTGT AAC ACC AAT GCA GGC AAA TCA ACC TTT  233
                              Asn Thr Asn Ala Gly Lys Ser Thr Phe
                              1               5

 GGG GAT GGG ACT ACG CTC ACT GTG AAG CCA                          264
 Gly Asp Gly Thr Thr Leu Thr Val Lys Pro
 10                  15
```



XIV - INFORMATION FOR SEQ ID No. 13

CHARACTERISTICS OF THE SEQUENCES

TYPE: nucleotide and its corresponding protein

LENGTH: 277 base pairs

NUMBER OF STRANDS: single

CONFIGURATION: linear

TYPE OF MOLECULE: cDNA for mRNA

ORIGIN

ORGANISM: human

CELL LINE: human T lymphocytes

CHARACTERISTICS

NAME OF DNA: Ja 02

SEQUENCE: Jα

DESCRIPTION OF THE SEQUENCE

```
AAGGACACAG ACTGCCTGCA TGAAGGCTGG AGCTGGGCCC AGGATGAGGA AAGGCCTCAG   60

GAAGGAAGGG CTGACACGAA ATAAGGAATA CCATGGCATT CATGAGATGT GCGTCTGAAT  120

CCTCTCTCTT GCCTGAGAAG CTTTAGCTTC CACCTTGAGA CACAAAACAT GTGGTTATGA  180

AGAGATGACA AGGTTTTTGT AAAAGAATGA GCCATTGTGG ATA GGC TTT GGG AAT    234
                                           Ile Gly Phe Gly Asn
                                          1               5

GTG CTG CAT TGC GGG TCC GGC ACT CAA GTG ATT GTT TTA CCA             277
Val Leu His Cys Gly Ser Gly Thr Gln Val Ile Val Leu Pro
            10                  15
```

XVI - INFORMATION FOR SEQ ID No. 15

CHARACTERISTICS OF THE SEQUENCES

TYPE: nucleotide and its corresponding protein

LENGTH: 60 base pairs

NUMBER OF STRANDS: single

CONFIGURATION: linear

TYPE OF MOLECULE: cDNA for mRNA

ORIGIN

ORGANISM: human

CELL LINE: human T lymphocytes

CHARACTERISTICS

NAME OF DNA: Ja 04

SEQUENCE: Jα

DESCRIPTION OF THE SEQUENCE

```
TA GAT ACT GGA GGC TTC AAA ACT ATC TTT GGA GCA GGA ACA AGA CTA    47
   Asp Thr Gly Gly Phe Lys Thr Ile Phe Gly Ala Gly Thr Arg Leu
   1               5                  10                  15

TTT GTT AAA GCA A                                              60
Phe Val Lys Ala
```

XVII - INFORMATION FOR SEQ ID No. 16

CHARACTERISTICS OF THE SEQUENCES

TYPE: nucleotide and its corresponding protein

LENGTH: 59 base pairs

NUMBER OF STRANDS: single

CONFIGURATION: linear

TYPE OF MOLECULE: cDNA for mRNA

ORIGIN

ORGANISM: human

CELL LINE: human T lymphocytes

CHARACTERISTICS

NAME OF DNA: Ja 05

SEQUENCE: Jα

DESCRIPTION OF THE SEQUENCE

```
C CTA ACT GGG GCA AAC AAC GTC TTC TTT GGG ACT GGA ACG AGA CTC    46
  Leu Thr Gly Ala Asn Asn Val Phe Phe Gly Thr Gly Thr Arg Leu
  1               5                  10                  15

ACC GTT CTT CCC T                                            59
Thr Val Leu Pro
```

XVIII - INFORMATION FOR SEQ ID No. 17

CHARACTERISTICS OF THE SEQUENCES

TYPE: nucleotide and its corresponding protein

LENGTH: 60 base pairs

NUMBER OF STRANDS: single

CONFIGURATION: linear

TYPE OF MOLECULE: cDNA for mRNA

ORIGIN

ORGANISM: human

CELL LINE: human T lymphocytes

CHARACTERISTICS

NAME OF DNA: Ja 06

SEQUENCE: Jα

DESCRIPTION OF THE SEQUENCE

```
AT GGA GGA AGC CAA GGA AAT CTC ATC TTT GGA AAA GGC ACT AAA CTC    47
   Gly Gly Ser Gln Gly Asn Leu Ile Phe Gly Lys Gly Thr Lys Leu
   1               5                  10                  15

TCT GTT AAA CCA A                                              60
Ser Val Lys Pro
```

XIX - INFORMATION FOR SEQ ID No. 18

CHARACTERISTICS OF THE SEQUENCES

TYPE: nucleotide and its corresponding protein

LENGTH: 56 base pairs

NUMBER OF STRANDS: single

CONFIGURATION: linear

TYPE OF MOLECULE: cDNA for mRNA

ORIGIN

ORGANISM: human

CELL LINE: human T lymphocytes

CHARACTERISTICS

NAME OF DNA: Ja 07

SEQUENCE: Jα

DESCRIPTION OF THE SEQUENCE

```
GGA GCC AAT AGT AAG CTG ACA TTT GGA AAA GGA ATA ACT CTG AGT GTT    48
Gly Ala Asn Ser Lys Leu Thr Phe Gly Lys Gly Ile Thr Leu Ser Val
1               5                  10                  15

                -                                                  56
AGA CCA GA
Arg Pro
```

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XX - INFORMATION FOR SEQ ID No. 19

CHARACTERISTICS OF THE SEQUENCES

TYPE: nucleotide and its corresponding protein

LENGTH: 57 base pairs

NUMBER OF STRANDS: single

CONFIGURATION: linear

TYPE OF MOLECULE: cDNA for mRNA

ORIGIN

ORGANISM: human

CELL LINE: human T lymphocytes

CHARACTERISTICS

NAME OF DNA: Ja 08

SEQUENCE: Jα

DESCRIPTION OF THE SEQUENCE

```
CT GGT GGC TAC AAT AAG CTG ATT TTT GGA GCA GGG ACC AGG CTG GCT    47
   Gly Gly Tyr Asn Lys Leu Ile Phe Gly Ala Gly Thr Arg Leu Ala
   1               5                  10                  15

GTA CAC CCA T                                                   57
Val His Pro
```

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XXI - INFORMATION FOR SEQ ID No. 20

CHARACTERISTICS OF THE SEQUENCES

TYPE: nucleotide and its corresponding protein

LENGTH: 50 base pairs

NUMBER OF STRANDS: single

CONFIGURATION: linear

TYPE OF MOLECULE: cDNA for mRNA

ORIGIN

ORGANISM: human

CELL LINE: human T lymphocytes

CHARACTERISTICS

NAME OF DNA: Ja 09

SEQUENCE: Jα

DESCRIPTION OF THE SEQUENCE

```
T GGA AAC AAG CTG GTC TTT GGC GCA GGA ACC ATT CTG AGA GTC AAG    46
  Gly Asn Lys Leu Val Phe Gly Ala Gly Thr Ile Leu Arg Val Lys
  1               5                  10                  15

TCC T                                                          50
Ser
```

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XXII - INFORMATION FOR SEQ ID No. 21 to 25

TYPE: OLIGONUCLEOTIDES

DESCRIPTION OF THE SEQUENCES

No. 21 A (5'-GTTGCTCCAGGCCACAGCACTG)

No. 22 poly C (5'-GCATGCGCGCGGCCGCGGAGG-14C)

No. 23 B (5'-GTCCATAGACCTCATGTCCAGCACAG)

No. 24 C (5'-ATACACATCAGAATTCTTACTTTG)

No. 25 D (5'-GTCACTGGATTTAGAGTCT)

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XXIII - INFORMATION FOR SEQ ID No. 26 TO 63
TYPE OLIGONUCLEOTIDES
DESCRIPTION OF SEQUENCES

SEQ ID N°	Sequence	Type	Clone	Position
26	5'-GGCATTAACGGTTTTGAGGCTGGA-3'	Vα1	AB22	235
27	5'-CAGTGTTCCAGAGGGAGCCATTGC-3'	Vα2	IGRa09	93*
28	5'-CCGGGCAGCAGACACTGCTTCTTA-3'	Vα3	HAP05	297
29	5'-TTGGTATCGACAGCTTCCCTCCCA-3'	Vα4	HAP08	153
30	5'-CGGCCACCCTGACCTGCAACTATA-3'	Vα5	IGRa10	113
31	5'-TCCGCCAACCTTGTCATCTCCGCT-3'	Vα6	HAP01	287
32	5'-GCAACATGCTGGCGGAGCACCCAC-3'	Vα7	IGRa11	159
33	5'-CATTCGTTCAAATGTGGGCAAAAG-3'	Vα8	HAP41	204
34	5'-CCAGTACTCCAGACAACGCCTGCA-3'	Vα9	HAVP36	168
35	5'-CACTGCGGCCCAGCCTGGTGATAC-3'	Vα10	HAP58	282
36	5'-CGCTGCTCATCCTCCAGGTGCGGG-3'	Vα11	AB19	254*
37	5'-TCGTCGGAACTCTTTTGATGAGCA-3'	Vα12	V12MA483	213
38	5'-TTCATCAAAACCCTTGGGGACAGC-3'	Vα13	HAVT15	152*
39	5'-CCCAGCAGGCAGATGATTCTCGTT-3'	Vα14	HAVT20	181
40	5'-TTGCAGACACCGAGACTGGGGACT-3'	Vα15	HAVT31	278
41	5'-TCAACGTTGCTGAAGGGAATCCTC-3'	Vα16	IGRa13	89
42	5'-TGGGAAAGGCCGTGCATTATTGAT-3'	Vα17	AB11	204
43	5'-CAGCACCAATTTCACCTGCAGCTT-3'	Vα18	AB21	114
44	5'-ACACTGGCTGCAACAGCATCCAGG-3'	Vα19	AC24	162
45	5'-TCCCTGTTTATCCCTGCCGACAGA-3'	Vα20	AE212	232
46	5'-AGCAAAATTCACCATCCCTGAGCG-3'	Vα21	AF211	92
47	5'-CCTGAAAGCCACGAAGGCTGATGA-3'	Vα22	IGRa12	197
48	5'-TGCCTCGCTGGATAAATCATCAGG-3'	VαW23	IGRa01	246
49	5'-CTGGATGCAGACACAAAGCAGAGC-3'	VαW24	IGRa02	259
50	5'-TGGCTACGGTACAAGCCGGACCCT-3'	VαW25	IGRa03	148
51	5'-AGCGCAGCCATGCAGGCATGTACC-3'	VαW26	IGRa04	299
52	5'-AAGCCCGTCTCAGCACCCTCCACA-3'	VαW27	IGRa05	268*
53	5'-TGGTTGTGCACGAGCGAGACACTG-3'	VαW28	IGRa06	95
54	5'-GAAGGGTGGAGAACAGATGCGTCG-3'	VαW29	IGRa07	210
55	5'-ATACACATCAGAATTCTTACTTTG-3'	CαA		129



SEQ ID N°	Sequence	Type	Clone	Position
56	5'-GTTGCTCCAGGCCGCGGCACTGTT-3'	CαB		201
57	5'-GTCACTGGATTTAGAGTCT-3'	CαC		57
58	5'-ATTTGCGGTGGACGATGGAGGGGC-3'	Act 1	ß-actin	1161
59	5'-GGCATCGTCACCAACTGGGACGAC-3'	Act 2	ß-actin	261
60	5'-ACCACCACGGCGGAGCGGG-3'	Act 3	ß-actin	642
61	5'-CGGGCTGCTCCTTGAGGGGCTGCG-3'	CβF		135
62	5'-CCCACCCGAGGTCGCTGTG-3'	CβK		20
63	5'-TCTGCTTCTGATGGCTCAA-3'	CβC		58

CLAIMS

1. Nucleotide sequences coding for the variable regions of α chains of human T lymphocyte receptors, corresponding to cDNAs containing nucleotide sequences chosen from any one of:

a - Vα segments corresponding to one of the sequences SEQ ID No. 1 to 11, and

b - Jα segments corresponding to one of the sequences SEQ ID No. 12, 13 and 15 to 20 and the sequences which differ from them by one or more nucleotides.

2. Sequences according to claim 1 coding for the variable regions of α chains of human T lymphocyte receptors, corresponding to the cDNAs containing nucleotide sequences chosen from any one of the Vα segments corresponding to one of the sequences SEQ ID 1 to 10, and the sequences which differ from them by one or more nucleotides.

3. Sequences according to claim 1 coding for the variable regions of α chains of human T lymphocyte receptors, corresponding to the cDNAs containing nucleotide sequences chosen from any one of the Jα segments corresponding to one of the sequences SEQ ID No. 12, 13 and 15 to 20, and the sequences which differ from them by one or more nucleotides.

4. Nucleotide sequences coding for the variable regions of α chains of human T lymphocyte receptors, corresponding to the cDNAs corresponding to ~~all or part of~~ the nucleotide sequences chosen from any one of the Vα segments corresponding to one of the sequences SEQ ID No. 2 to 5, and the sequences which differ from them by one or two nucleotides.

5. Nucleotide sequences coding for the variable regions of α chains of human T lymphocyte receptors, corresponding to the cDNAs corresponding to ~~all or part of~~ the nucleotide sequences chosen from any one of the Vα segments corresponding to one of the sequences 1 to 200 of SEQ ID No. 1

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1 to 467 of SEQ ID No. 6
1 to 77 of SEQ ID No. 7
1 to 151 of SEQ ID No. 8
291 to 386 of SEQ ID No. 9
1 to 260 of SEQ ID No. 10

and the sequences which differ from them by one or two nucleotides.

6. Nucleotide sequences coding for the variable regions of α chains of human T lymphocyte receptors, corresponding to the cDNAs corresponding to ~~all or part of~~ the nucleotide sequence corresponding to SEQ ID No. 11 and which contains nucleotide 108.

7. Nucleotide sequences coding for the variable regions of α chains of human T lymphocyte receptors, corresponding to the cDNAs corresponding to ~~all or part of~~ the nucleotide sequences chosen from any one of the Jα segments corresponding to one of the sequences SEQ ID No. 12, 13 and 15 to 20 and the sequences which differ from them by one or more nucleotides.

8. Peptides coded by any one of the nucleotide sequences as defined in any one of claims 1 to 7, as well as the alleles and the derivatives of the latter which possess the same function.

9. Peptides constituted by or composed of one peptide sequence coded by ~~all or part of~~ the sequence 108 to 364 of SEQ ID No. 11.

10. Expression vectors containing a DNA sequence coding for one of the peptides as defined in claim 8 or claim 9.

11. Hosts transformed with a vector according to claim 10.

12. Antibodies directed against an antigenic determinant of one of the peptides defined in claim 8 or claim 9.

13. Antibody according to claim 11 which is a monoclonal antibody or a fragment of the latter.

14. Fragment Fab, Fab' or $(Fab')_2$ of a monoclonal antibody according to claim 13 and derivatives of the latter.

15. Derivatives of a monoclonal antibody or a fragment of the latter according to claim 13 or 14 to which a detectable

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marker and/or at least one therapeutic molecule is attached.

16. Hybridomas producing an antibody according to claim 13.

17. Diagnostic composition containing one or more monoclonal antibodies, fragments or derivatives of the latter as defined in any one of claims 13 to 15.

18. Therapeutic composition containing one or more monoclonal antibodies, fragments or derivatives of the latter as defined in one of claims 13 to 15.

19. Composition according to claim 18 containing derivatives containing a cytotoxic molecule or a radio-isotope.

20. Therapeutic composition containing at least one of the peptides defined in claim 8 or claim 9.

21. Therapeutic composition containing anti sense oligonucleotides corresponding to any one of the sequences of a Vα segment or to any one of the sequences of a Jα segment as defined in any one of claims 4 to 7.

22. Use, as primers for the amplification of DNA, of nucleotide sequences of about at least 17 nucleotides contained in any one of the sequences of a Vα segment or in any one of the sequences of a Jα segment as defined in any one of claims 4 to 7.

23. Use, as a detection probe, of nucleotide sequences of about at least 10 nucleotides contained in any one of the sequences of a Vα segment or in any one of the sequences of a Jα segment as defined in any one of claims 4 to 7.

24. Oligonucleotides, which can be used as primers for the amplification of DNA corresponding to variable regions of α chains of T-cell receptors, chosen from the sequences SEQ ID No. 26 to 54.

25. Use, as primers for the amplification of DNA corresponding to the variable regions of α chains of T-cell receptors, of oligonucleotides according to claim 24.

26. Use according to claim 25 of oligonucleotides chosen from the sequences SEQ ID No. 49, 50, 51, 52 and 54.

27. Detection process of nucleotide sequences coding for the Vα segments of T receptors or of cDNA corresponding to transcription products of the latter, in a biological sample,

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characterized in that it contains:

a) the amplification of DNA with at least one pair of primers formed by one of the oligonucleotides according to claim 24 and one oligonucleotide belonging to the Cα segment, and

b) the detection of amplified sequences with a Cα probe.

28. Process according to claim 27, characterized in that the amplification is carried out in the presence of a pair of control primers and detection is carried out using a control probe.

29. Diagnostic kit for the implementation of a process according to claim 27, characterized in that it contains:

a) at least one oligonucleotide according to claim 24,

b) a Cα primer,

c) a Cα probe.

30. Diagnostic kit for the implementation of a process according to claim 28, characterized in that it contains:

a) at least one oligonucleotide according to claim 24,

b) a Cα primer,

c) a pair of control primers,

d) a Cα probe,

e) a control probe.

31. Kit according to claim 29 or claim 30, containing:

a) the group of 29 oligonucleotides according to claim 24,

b) a Cα primer chosen ~~form~~ from the sequences corresponding to sequences SEQ ID 55 and 56,

c) a pair of control primers for the β-actin having a sequence corresponding to sequences SEQ ID No. 58 and 59 respectively,

d) a Cα probe corresponding to the sequence SEQ ID No. 57,

e) a control probe for the β-actin corresponding to the sequence SEQ ID No. 60.

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Vα1

```
                                  1
IGRa08 AGTGTTTCCCTTGCTCAGCCATGCTCCTGGAGCTTATCCCACTGCTGGGGATAC

IGRa08 ATTTTGTCCTGAGAACTGCCAGAGCCCAGTCAGTGACCCAGCCTGACATCCACA

IGRa08 TCACTGTCTCTGAAGGAGCCTCACTGGAGTTGAGATGTAACTATTCCTATGGGG

IGRa08 CAACACCTTATCTCTTCTGGTATGTCCAGTCCCCCGGCCAAGGCCTCCAGCTGC

IGRa08 TCCTGAAGTACTTTTCAGGAGACACTCTGGTTCAAGGCATTAAAGGCTTTGAGG
AE11                                                .................

IGRa08 CTGAATTTAAGAGGAGTCAATCTTCCTTCAACCTGAGGAAACCCTCTGTGCATT
AE11   ..............................T.....................

IGRa08 GGAGTGATGCTGCTGAGTACTTCTGTGCT 333
AE11   ............................ 102
```

FIG. 1A

Vα2

```
IGRa09                             AAATCCTTGAGAGTTTTACTAGTGATCCTGTGGC

IGRa09 TTCAGCTGAGCCGGGTTTGGAGCCAACAGAAGGAGGTGGAGCAGAATTCTGGAC
AF110                                                ..........

IGRa09 CCCTCAGTGTTCCAGAGGGAGCCATTGCCTCTCTCAACTGCACTTACAGTGACC
AF110  ..................................................

IGRa09 GAGGTTCCCAGTCCTTCTTCTGGTACAGACAATATTCTGGGAAAAGCCCTGAGT
AF110  ..................................................

IGRa09 TGATAATGTCCATATACTCCAATGGTGACAAAGAAGATGGAAGGTTTACAGCAC
AF110  ..................................................

IGRa09 AGCTCAATAAAGCCAGCCAGTATGTTTCTCTGCTCATCAGAGACTCCCAGCCCA
AF110  ..................................................

IGRa09 GTGATTCAGCCACCTACCTCTGTGCC 330
AF110  ........................... 252
```

FIG. 1B

Vα5

```
                                  1
IGRa10 GGCCACATTTGGGGAGACGAATGGAGTCATCCCTGGGAGGTGTTTTGCTGATTT

IGRa10 TGTGGCTTCAAGTGGACTGGGTGAAGAGCCAAAAGATAGAACAGAATTCCGAGG
HAP35                                                      ....

IGRa10 CCCTGAACATTCAGGAGGGTAAAACGGCCACCCTGACCTGCAACTATACAAACT
HAP35  ..................................................

IGRa10 ATTCTCCAGCATACTTACAGTGGTACCGACAAGATCCAGGAAGAGGCCCTGTTT
HAP35  ..................................................

IGRa10 TCTTGCTACTCATACGTGAAAATGAGAAAGAAAAAAGGAAAGAAAGACTGAAGG
HAP35  ..................................................

IGRa10 TCACCTTTGATACCACCCTTAAACAGAGTTTGTTTCATATCACAGCCTCCCAGC
HAP35  ..................................................

IGRa10 CTGCAGACTCAGCTACCTACCTCTGTGCT 333
HAP35  ........................... 249
```

FIG. 1C

Vα7

```
                                  1
              IGRa11 CTCGTGGTATCCTGCAG-
CAGATGTGGGGAGTTTTCCTTCTTTATGTTTCCATGA
HAP12                  ..............................

IGRa11 AGATGGGAGGCACTACAGGACAAAACATTGACCAGCCCACTGAGATGACAGCTA
HAP12  ..................................................

IGRa11 CGGAAGGTGCCATTGTCCAGATCAACTGCACGTACCAGACATCTGGGTTCAACG
HAP12  ..................................  ..............

IGRa11 GGCTGTTCTGGTACCAGCAACATGCTGGCGAAGCACCCACATTTCTGTCTTACA
HAP12  ..................................................

IGRa11 ATGTTCTGGATGGTTTGGAGGAGAAAGGTCGTTTTTCTTCATTCCTTAGTCGGT
HAP12  ........................

IGRa11 CTAAAGGGTACAGTTACCTCCTTTTGAAGGAGCTCCAGATGAAAGACTCTGCCT
HAP12

IGRa11 CTTACCTCTGTGCT 318
HAP12           222
```

FIG. 1D

Vα22

```
                                  1
IGRa12 ATTTGGGTAACACACTAAAGATGAACTATTCTCCAGGCTTAGTATCTCTGATAC

IGRa12 TCTTACTGCTTGGAAGAACCCGTGGAGATTCAGTGACCCAGATGGAAGGGCCAG

IGRa12 TGACTCTCTCAGAAGAGGCCTTCCTGACTATAAACTGCACGTACACAGCCACAG

IGRa12 GATACCCTTCCCTTTTCTGGTATGTCCAATATCCTGGAGAAGGTCTACAGCTCC

IGRa12 TCCTGAAAGCCACGAAGGCTGATGACAAGGGAAGCAACAAAGGTTTTGAAGCCA
AC9                              ..............................

IGRa12 CATACCGTAAAGAAACCACTTCTTTCCACTTGGAGAAAGGCTCAGTTCAAGTGT
AC9    ..............................:.............................

IGRa12 CAGACTCAGCGGTGTACTTCTGTGCT  330
AC9    .........................  113
```

FIG. 1E

```
                                                                  F  G     G  T
IGRJa01G GGTTATTGCAATAGCACTAAAGACTGTGTAACACCAATGCAGGCAAATCAACCTTTGGGGATGGGACTACGCTCACTGTGAAGCCA

IGRJa02G GGTTTTTGTAAAAGAATGAGCCATTGTGGATAGGCTTTGGGAATGTGCTGCATTGCGGGTCCGGCACTCAAGTGATTGTTTTACCA


IGRJa04                                TAGATACTGGAGGCTTCAAAACTATCTTTGGAGCAGGAACAAGACTATTTGTTAAAGCA

IGRJa05                                 CCTAACTGGGGCAAACAACGTCTTCTTTGGGACTGGAACGAGACTCACCGTTCTTCCC

IGRJa06                                ATGGAGGAAGCCAAGGAAATCTCATCTTTGGAAAAGGCACTAAACTCTCTGTTAAACCA

IGRJa07                                     GGAGCCAATAGTAAGCTGACATTTGGAAA[illegible]ACTCTGAGTGTTAGACCA

IGRJa08                                   CTGGTGGCTACAATAAGCTGATTTTTGGAGCA[illegible]ACCAGGCTGGCTGTACACCCA

IGRJa09                                          TGGAAACAAGCTGGTCTTTGGCGCAGGAACCATTCTGAGAGTCAAGTCC
```

FIG. 2

SEQ ID N°1_SEQ ID N°2_SEQ ID N°3_SEQ ID N°4_SEQ ID N°5_



FIG.3

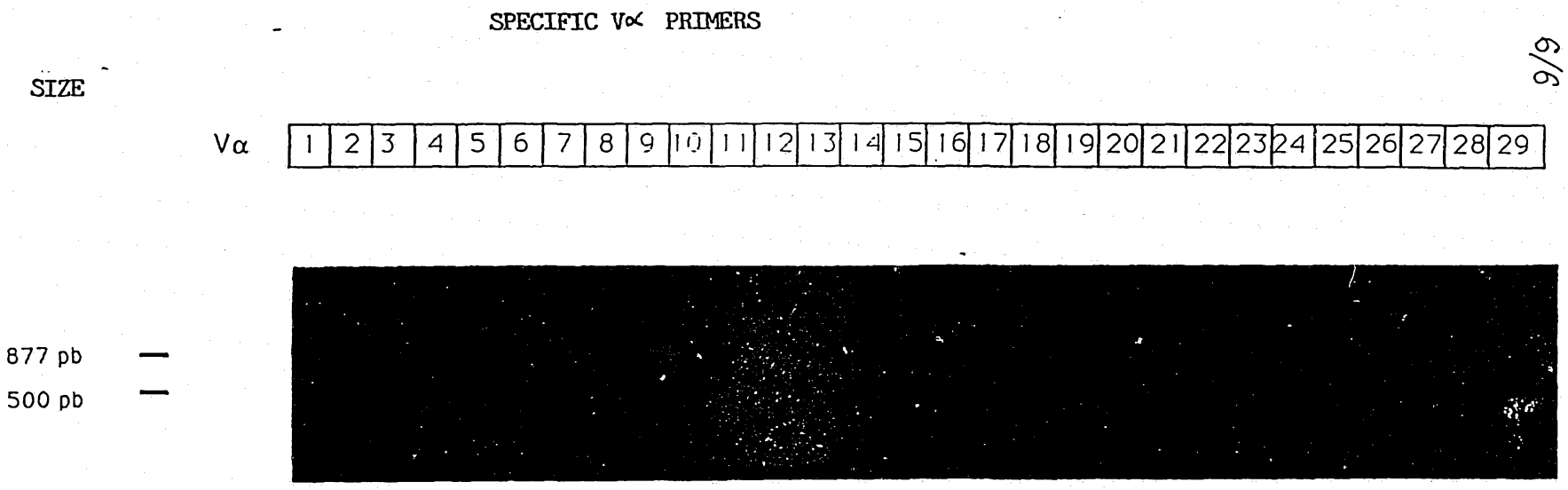
SPECIFIC Vα PRIMERS
SIZE
Vα
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
877 pb
500 pb

FIG.4

RAPPORT DE RECHERCHE INTERNATIONALE

Demande Internationale No PCT/FR 92/00111

I. CLASSEMENT DE L'INVENTION (si plusieurs symboles de classification sont applicables, les indiquer tous) 7

Selon la classification internationale des brevets (CIB) ou à la fois selon la classification nationale et la CIB

CIB 5 C12N15/12; C12N15/63; C12P21/08; A61K37/02
A61K39/395; G01N33/577; C12Q1/68; C07K15/00

II. DOMAINES SUR LESQUELS LA RECHERCHE A PORTE

Documentation minimale consultée 8

Système de classification	Symboles de classification
CIB 5	C07K

Documentation consultée autre que la documentation minimale dans la mesure où de tels documents font partie des domaines sur lesquels la recherche a porté 9

III. DOCUMENTS CONSIDERES COMME PERTINENTS 10

Catégorie °	Identification des documents cités, avec indication, si nécessaire, 12 des passages pertinents 13	No. des revendications visées 14
P,X	EUROPEAN JOURNAL OF IMMUNOLOGY vol. 21, no. 4, 9 Avril 1991, VCH VERLAGSGESELLSCHAFT, DEUTSCHLAND pages 927 - 933; Roman-Roman, S. et al.: 'Studies on the human T cell receptor alpha/beta variable region genes. I. Identification of 7 additional Valpha subfamilies and 14 J alpha gene segments.' voir le document en entier	1-29
X	EUROPEAN JOURNAL OF IMMUNOLOGY vol. 17, 1987, VCH VERLAGSGESELLSCHAFT, DEUTSCHLAND pages 375 - 383; Kimura, N. et al.: 'Sequences and repertoire of the human T cell receptor alpha and beta chain variable region genes in thymocytes.' voir le document en entier	1,2,5,8, 10-23

-/--

° Catégories spéciales de documents cités: 11

"A" document définissant l'état général de la technique, non considéré comme particulièrement pertinent

"E" document antérieur, mais publié à la date de dépôt international ou après cette date

"L" document pouvant jeter un doute sur une revendication de priorité ou cité pour déterminer la date de publication d'une autre citation ou pour une raison spéciale (telle qu'indiquée)

"O" document se référant à une divulgation orale, à un usage, à une exposition ou tous autres moyens

"P" document publié avant la date de dépôt international, mais postérieurement à la date de priorité revendiquée

"T" document ultérieur publié postérieurement à la date de dépôt international ou à la date de priorité et n'appartenenant pas à l'état de la technique pertinent, mais cité pour comprendre le principe ou la théorie constituant la base de l'invention

"X" document particulièrement pertinent; l'invention revendiquée ne peut être considérée comme nouvelle ou comme impliquant une activité inventive

"Y" document particulièrement pertinent; l'invention revendiquée ne peut être considérée comme impliquant une activité inventive lorsque le document est associé à un ou plusieurs autres documents de même nature, cette combinaison étant évidente pour une personne du métier.

"&" document qui fait partie de la même famille de brevets

IV. CERTIFICATION

Date à laquelle la recherche internationale a été effectivement achevée	Date d'expédition du présent rapport de recherche internationale
06 MAI 1992	13 MAY 1992

Administration chargée de la recherche internationale	Signature du fonctionnaire autorisé
OFFICE EUROPEEN DES BREVETS	NAUCHE S.A.

III. DOCUMENTS CONSIDERES COMME PERTINENTS [14] (SUITE DES RENSEIGNEMENTS INDIQUES SUR LA DEUXIEME FEUILLE)

Catégorie °	Identification des documents cités, [16] avec indication, si nécessaire des passages pertinents [17]	No. des revendications visées [18]
X	THE JOURNAL OF EXPERIMENTAL MEDECINE vol. 164, no. 1, 1 Juillet 1986, THE ROCKEFELLER UNIV. PRESS pages 90 - 103; Yoshika, Y. et al.: 'Sequences and repertoire of human T cell receptor alpha chain variable region genes in mature T lymphocytes.' voir le document en entier	1,2,5
X	THE JOURNAL OF EXPERIMENTAL MEDECINE vol. 164, no. 6, 1 Décembre 1986, THE ROCKEFELLER UNIV. PRESS pages 1940 - 1957; Calman, A.F. et al.: 'Expression of T cell receptor genes in human B cells.' voir le document en entier	1,3,7,8, 10,11-23
X	EMBO JOURNAL. vol. 7, no. 6, 1988, EYNSHAM, OXFORD GB pages 1661 - 1668; Baer, R. et al.: 'Complex rearrangements within the human Jdelta-Cdelta /Jalpah-Calpha locus and aberrant recombination between Jalpha segments.' voir le document en entier	1,2,5,8, 10,11-14
Y		15-23
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 86, 1989, WASHINGTON US pages 602 - 606; Russo. G. et al.: 'Molecular analysis of a t(14;14) translocation in leukemie T-cells of an ataxia telangiectasia patient.' voir le document en entier	1,2,4
Y	US,A,4 874 845 (HARUO SAITO ET AL.) 17 Octobre 1989 voir le document en entier	15-23
Y	WO,A,9 006 758 (T CELL SCIENCES, INC. (USA)) 28 Juin 1990 cité dans la demande voir les revendications	16-18
Y	EP,A,0 261 671 (PASTAN, I. ET AL.) 30 Mars 1988 voir revendication 7	18
Y	WO,A,8 703 600 (T CELL SCIENCES, INC. (USA)) 18 Juin 1987 voir le résumé	20

ANNEXE AU RAPPORT DE RECHERCHE INTERNATIONALE RELATIF A LA DEMANDE INTERNATIONALE NO.

FR 9200111
SA 57201

La présente annexe indique les membres de la famille de brevets relatifs aux documents brevets cités dans le rapport de recherche internationale visé ci-dessus.
Lesdits membres sont contenus au fichier informatique de l'Office européen des brevets à la date du
Les renseignements fournis sont donnés à titre indicatif et n'engagent pas la responsabilité de l'Office européen des brevets. 06/05/92

Document brevet cité au rapport de recherche	Date de publication	Membre(s) de la famille de brevet(s)		Date de publication
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		JP-T-	2502063	12-07-90
		WO-A-	8802401	07-04-88
		US-A-	5082927	21-01-92
WO-A-8703600	18-06-87	US-A-	4845026	04-07-89
		AU-B-	617980	12-12-91
		AU-A-	6772587	30-06-87
		EP-A-	0248887	16-12-87
		JP-T-	63501721	14-07-88

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