Clarke, Mod

COLOMB

Doctor
GIANCARLO MARCENARO JIMÉNEZ
Superintendente Delegado Para la Propiedad Industrial

EXPEDIENTE: Expediente No. ***05.009.447*** Solicitud de Patente a nombre de ***WIETH AND PRESIDENT AND FELLOWS OF HARVARD COLLAGE***

Estimado Doctor:

En mi calidad de apoderada de la sociedad solicitante de la patente de la referencia manifiesto a usted lo siguiente:

1. Mediante memorial radicado en ese despacho el 02 de mayo de 2008, el cual no ha sido resuelto aún, di respuesta a la acción oficial de fondo de la presente solicitud.

2. En vista de que han transcurrido casi ***1 año*** sin que se haya atendido dicha solicitud, ruego a usted se sirva dar las instrucciones del caso para que se proceda de inmediato a resolver este asunto.

Cordialmente,

DILIA RODRÍGUEZ D'ALEMAN
C.C. 41.654.882 de Bogotá
T.P. 20824 del C.S.J.
p-608

Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function

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Cytokines are important in the regulation of haematopoiesis and immune responses, and can influence lymphocyte development. Here we have identified a class I cytokine receptor that is selectively expressed in lymphoid tissues and is capable of signal transduction. The full-length receptor was expressed in BaF3 cells, which created a functional assay for ligand detection and cloning. Conditioned media from activated human $CD3^+$ T cells supported proliferation of the assay cell line. We constructed a complementary DNA expression library from activated human $CD3^+$ T cells, and identified a cytokine with a four-helix-bundle structure using functional cloning. This cytokine is most closely related to IL2 and IL15, and has been designated IL21 with the receptor designated IL21R. *In vitro* assays suggest that IL21 has a role in the proliferation and maturation of natural killer (NK) cell populations from bone marrow, in the proliferation of mature B-cell populations co-stimulated with anti-CD40, and in the proliferation of T cells co-stimulated with anti-CD3.

Although primary sequence homology among the interleukins (ILs) and haematopoietic growth factors is low, these cytokines are structurally related[1] and fold into a classic 'four-helix-bundle' structure that is representative of the family. Most cytokines bind to cells and transduce signals through either the class I or the class II cytokine receptors. The class II cytokine receptors include the receptors for IL10 and the interferons, whereas the class I cytokine receptor family includes the receptors for IL2, IL3, IL4, IL5, IL6, IL7, IL9, IL11, IL12, IL13 and IL15, as well as the haematopoietic growth factors, leptin and growth hormone[2]. Class I cytokine receptors that are capable of signal transduction through homodimerization have been used in the past to design biological activity assays to screen for sources of the cognate ligand, and to facilitate either purification or expression cloning of the ligand[3].

Here we describe a new class I cytokine receptor, IL21R. We describe the use of a proliferation assay, based on BaF3 cells[4] expressing IL21R, to screen for potential interactions with known cytokines, to identify a source of the cognate ligand, and to clone the ligand, IL21. Preliminary biological characterization indicates that IL21 may be involved in haematopoietic development and differentiation of natural killer cells in conjunction with IL15, in the activation and proliferation of B cells co-stimulated through CD40 and also in the co-stimulation of T cells.

Receptor cloning and assay development

We identified an expressed sequence tag (EST) containing a predicted signal peptide and a predicted amphipathic helix. Sequencing of a full-length clone revealed a cDNA coding for a member of the class I cytokine receptor family. This cDNA, designated *IL21R*, contains an open reading frame encoding a 538-amino-acid protein that has structural motifs common to class I cytokine receptors[2,5]. Extracellular motifs include a single cytokine recognition module, two pairs of conserved cysteine residues, and a 'WSXWS' motif. The intracellular domain contains strong intracellular signalling motifs, including classical box 1 and box 2 motifs[6–10], which indicate that this receptor is probably a signalling subunit. IL21R has highest amino-acid sequence similarity to IL2Rβ and IL4Rα. We subsequently cloned murine *IL21R* from a mouse splenocyte library, and found that it shares overall structure and functional motifs with human IL21R (Fig. 1a).

IL21R was localized to human 16p11 using the GeneBridge 4 radiation hybrid mapping panel (Research Genetics, Inc.). In addition, the *IL21R* sequence was found in genomic sequence from two overlapping bacterial artificial chromosome clones mapped to 16p11 (GenBank accession nos AC002303 and AC004525). Comparison of the *IL21R* cDNA sequence with this genomic sequence revealed that the *IL21R* gene spans about 20 kilobases (kb) of genomic DNA, and lies about 65 kb from the *IL4Rα* gene.

Mpl, the receptor for thrombopoietin (TPO), delivers a proliferative signal on homodimerization in the presence of TPO[7]. To determine whether the intracellular domain of IL21R is capable of signalling as a homodimer, we constructed a chimaeric receptor using the extracellular and transmembrane domains of Mpl fused to the intracellular domain of IL21R. This chimaeric receptor was transfected into BaF3 cells[4], which are IL3-dependent and not normally responsive to TPO. Positive control cells were transfected with full-length Mpl. Both the full-length Mpl and the chimaeric receptor transduced proliferative signals in the BaF3 cells in response to TPO (data not shown). The proliferative signal delivered by the homodimeric Mpl/IL21R chimaera in BaF3 cells suggests that a ligand for IL21R may exert a proliferative effect on cells expressing the full-length receptor, thus providing an activity assay for ligand cloning.

Ligand functional cloning

We expressed full-length IL21R in BaF3 cells, creating the cell line BaF3/IL21R. Known human and murine cytokines were tested for activity, and only those that also stimulated parental BaF3 cells supported growth of BaF3/IL21R cells. Conditioned media from

more than 100 different primary and immortalized cell types were tested for activity, and conditioned medium from PMA (phorbol 12-myristate 13-acetate)/ionomycin-activated human peripheral $CD3^+$ T cells induced proliferation of BaF3/IL21R cells. The conditioned medium was inactive on wild-type BaF3 cells, indicating specificity for cells expressing IL21R. This proliferative response was suppressed by the addition of soluble IL21R (sIL21R), indicating that sIL21R is capable of binding to the proliferative factor (ligand) in the conditioned medium.

A cDNA library was constructed from sorted $CD3^+$ human T cells that had been activated for 13 h with PMA and ionomycin. Pools of 250 clones each were transfected into baby hamster kidney (BHK) cells, and conditioned media were transferred to the BaF3/IL21R assay cell line. Proliferation was determined using an Alamar blue assay (Accumed). Each positive conditioned medium was re-assayed with and without sIL21R. Results showed that each positive pool contained a proliferative factor that was neutralized by soluble receptor. Sequencing of individual positive clones from each pool revealed that all contained inserts encoding the same polypeptide, which we have designated interleukin-21 (IL21) to reflect both its structural relationship to other interleukins and its biological activities.

Ligand structure

The sequence of human *IL21* cDNA contains an open reading frame that encodes a polypeptide precursor of 162 amino acids. The signal

SEQ N° 4

```
a  mIL21R MPRGPVAALLLLLILHGAWSCLDLTCYTDYLWTITCVLETRSPNPSILSLTWQDEYEELQD 60
   hIL21R MPRGWAAPLLLLLLQGGWGCPDLVCYTDYLQTVICILEMWNLHPSTLTLTWQDQYEELKD

   mIL21R QETFCSLHRSGHNTTHIWYTCHMRLSQFLSDEVFIVNVTDQSGNNSQECGSFVLAESIKP 120
   hIL21R EATSCSLHRSAHNATHATYTCHMDVFHFMADDIFSVNITDQSGNYSQECGSFLLAESIKP

   mIL21R APPLNVTVAFSGRYDISWDSAYDEPSNYVLRGKLQYELQYRNLRDPYAVRPVTKLISVDS 180
   hIL21R APPFNVTVTFSGQYNISWRSDYEDPAFYMLKGKLQYELQYRNRGDPWAVSPRRKLISVDS

   mIL21R RNVSLLPEEFHKDSSYQLQVRAAPQPGTSFRGTWSEWSDPVIFQTQAGEPEAGWDPHMLL 240
   hIL21R RSVSLLPLEFRKDSSYELQVRAGPMPGSSYQGTWSEWSDPVIFQTQSEELKEGWNPHLLL

   mIL21R LLAVLII-VLVFMGLKIHLPWRLWKKIWAPVPTPESFFQPLYREHSGNFKKWVNTPFTAS 299
   hIL21R LLLLVIVFIPAFWSLKTHPLWRLWKKIWA-VPSPERFFMPLYKGCSGDFKKWVGAPFTGS

   mIL21R SIELVPQSSTTTSAL-----HLSLYPAKEKKFPGLPGLEEQLECDGMSEPGHWCIIPLAA 354
   hIL21R SLELGPWSPEVPSTLEVYSCHPPRSPAKRLQLTELQEPAELVESDGVPKPSFW---PTAQ

   mIL21R GQAVSAYSEERDRPYGLVSIDTVTVGDAEGLCVWPCSCEDDGYPAMNLDAGRESGPNSED 414
   hIL21R NSGGSAYSEERDRPYGLVSIDTVTVLDAEGPCTWPCSCEDDGYPALDLDAGLEPSPGLED

   mIL21R LLLVTDPAFLSCGCVSGSGLRLGGSPGSLLDRLRLSFAKEGDWTADPTWRTGSPGGGSES 474
   hIL21R PLLDAGTTVLSCGCVSAGSPGLGGPLGSLLDRLKPPLADGEDWAGGLPWGGRSPGGVSES

   mIL21R EAGSP-PGLDMDTFDSGFAGSDCGSPVE-------TDEGPPRSYLRQWVVRTPPPVDSGAQSS 529
   hIL21R EAGSPLAGLDMDTFDSGFVGSDCSSPVECDFTSPGDEGPPRSYLRQWVVIPPPLSSPGPQAS
```

SEQ N° 2.

```
b  hIL-21 --------MRSSPGNMERIVICLMVIFLGTLVHKSSSQGQDRHMIRMRQLIDIVDQLKNYVNDLV
   mIL-21 ---------------MERTLVCLVVIFLGTVAHKSSPQGPDRLLIRLRHLIDIVEQLKIYENDLD
   hIL-15 MRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFILGCFSAGLPKTEANWVNVISDLKKI-EDLI
   hIL-4  ------------------MGLTSQLLPPLFFLLACAGNFVHGHKCD-ITLQEIIKTLNSLTEQKT
   hIL-2  ---------------MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN

   hIL-21 PEF-----------------LPAPEDVETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKKLKR
   mIL-21 PEL-----------------LSAPQDVKGHCEHAAFACFQKAKLKPSNPGNNKTFIIDLVAQLRR
   hIL-15 QSMHIDAT------------LYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIIL
   hIL-4  LCTELTVTDI----------FAASKNTTE----KETFCRAATVLRQFYSHHEKDTRCLGATAQQF
   hIL-2  NYKN------PKLTRMLTFKFYMPKKATE---LKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDL

   hIL-21 KPPSTNAGRRQKHRL------TCPSCDSYEKK--PPKEFLERFKSLLQKMIHQHLSSRTHGSEDS
   mIL-21 RLPARRGGKKQKHIA------KCPSCDSYEKR--TPKEFLERLKWLLQKMIHQHLS
   hIL-15 ANNSLSSNGNVTESG-------CKECEELEEK--NIKEFLQSFVHIVQMFINTS
   hIL-4  HRHKQLIRFLKRLDRNLWGLAGLNSCPVKEANQSTLENFLERLKTIMREKYSKCSS
   hIL-2  ISNINVIVLGLKGSE------TTFMCE-YADETATIVEFLNRWITFCQSIISTL
```

Figure 1 Amino-acid sequences of IL21R and IL21. **a**, Alignment of the amino-acid sequences of murine and human IL21R. Overall identity is 62%. Signal sequence is underlined. Both pairs of cysteines (shown in bold) and the WSEWS motif (underlined) are fully conserved in the mouse. Box 1 and Box 2 (shown in bold) are less conserved, but conform to the overall consensus sequences. A consensus Stat3-binding site[28], underlined and in boldface, is present near the C terminus. **b**, Alignment of human and murine IL21 with related cytokines. Identities (colon) or similarities (asterisk) between either human or murine IL21 and human IL15 are indicated. Mature N termini are indicated by open boxes. Potential *N*-linked glycosylation sites are underlined.

higher in the samples stimulated with anti-CD3 (Fig. 4a). To further define the conditions that induce *IL21* expression, we purified peripheral T cells from a different donor and stimulated with anti-CD3 either alone or in combination with anti-CD28. The combination of anti-CD3 and anti-CD28—conditions that best mimic an antigen encounter *in vivo*—provided higher induction of *IL21* expression than that seen with anti-CD3 alone in this assay (Fig. 4b). The small increase in message seen in stimulated $CD8^+$ cells in both experiments is probably due to contaminating $CD4^+$ cells (1–3%). $CD19^+$ B cells and $CD14^+$ monocytes did not express *IL21* under any stimulation condition (data not shown).

Biological activities

Because resting B lymphocytes express IL21R (see Fig. 3a), we designed assays to determine the effects of IL21 on B-cell proliferation. Although IL21 alone has no mitogenic effect on isolated human B cells (data not shown), it significantly co-stimulates proliferation induced by anti-CD40 monoclonal antibodies (Fig. 5a). In contrast, IL21 inhibits proliferation induced by the combination of plate-bound anti-IgM antibodies and IL4 (Fig. 5b). Both effects are reversed by the addition of sIL21R to the culture.

We also tested IL21 in T-cell proliferation assays and found that it co-stimulates anti-CD3-activated murine thymocytes (Fig. 6a), leading to an accelerated outgrowth of $CD8^+CD4^-$ cells (data not shown). We did not observe significant levels of proliferation of thymocytes in response to IL21 in the absence of anti-CD3. IL21 also co-stimulates anti-CD3-activated mature murine (Fig. 6b) and human (Fig. 6c) T cells. In each case, addition of sIL21R blocked this IL21-dependent proliferation (data not shown). Notably, our early experiments suggested that the proliferative effect of IL21 is subtler on human T cells than on murine T cells. We reasoned that this might be a function of the prior activation state of the human T cells, as murine T cells are less likely to be pre-activated *in vivo*. Indeed, when we separated $CD45RA^+$ (naive) and $CD45RO^+$ (memory) human T cells and assayed them for proliferative responses to IL21, we found that naive, but not memory, T cells were co-stimulated by IL21 (Fig. 6c; and data not shown). To compare the activity of IL21 to that of related cytokines on murine T cells, we performed a cross-titration of IL21 and IL2, IL7 or IL15, in the presence or absence of a suboptimal concentration of immobilized anti-CD3 monoclonal antibody (Fig. 6d). Unlike its related counterparts, IL21 has no significant proliferative effect on T cells in the absence of anti-CD3 or other stimuli (Fig. 6d; and data not shown). However, it does act in concert with IL2, IL15, and, to a lesser extent, with IL7 to enhance T-cell proliferation, either with or without anti-CD3 stimulation (Fig. 6d).

To assess the activity of IL21 on lymphoid progenitors, we co-cultured human non-adherent bone marrow progenitor cells with stem cell factor and human IL21 in combination with various other cytokines. Although IL21 alone does not induce significant proliferation of marrow progenitors, it does synergize with IL15, consistently causing at least a twofold increase in cell proliferation as compared with cultures containing stem cell factor and IL15 alone (data not shown). Flow cytometric analysis of these expanded cells for lineage-specific markers revealed expression of the NK cell marker CD56 (data not shown).

Other groups have shown that $CD3^-CD56^+$ NK cells can be generated from 21-day cultures of human $CD34^+$ haematopoietic progenitor cells (HPCs) supplemented with IL15 in the absence of stromal cells, an effect that is enhanced by the addition of Flt3L[15,16]. Given our observation that $CD56^+$ NK cells grow out of bone marrow cultures stimulated with IL15 and IL21, we undertook an analysis of the effect of IL21 on the development of NK cells from HPCs. We isolated $CD34^+$ human HPCs (95% pure) and cultured them either with Flt3L with and without IL21, or with Flt3L plus IL15 with and without IL21. Cells were collected on day 15 to assess their NK markers by flow cytometry (Fig. 7a) and also their capacity to lyse MHC^- K562 cells in a standard ^{51}Cr-release assay (Fig. 7b). As previously reported[16], the combination of IL15 and Flt3L alone induced the outgrowth of $CD56^+$ cells. In contrast, IL21 and Flt3L alone did not significantly expand the $CD56^+$ fraction. In cultures exposed to the combination of Flt3L, IL15 and IL21, however, there was a significant increase in total cell numbers and the proportion of cells that were $CD56^+CD16^{bright}$, when compared to cultures supplemented with Flt3L and IL15 alone. The cells growing out of these Flt3L, IL15, IL21 cultures were differentiated NK cells, as they were large and granular and expressed both CD56 and CD16, but did not express CD3 (Fig. 7a; and data not shown). Furthermore, these cells exhibited significantly higher effector function at day 15 than those cells grown with only IL15 and Flt3L (Fig. 7b). After 21 days in culture, however, the cells cultured in the presence of Flt3L and IL15 (without IL21) had gained lytic activity, but remained $CD16^{low/-}$, consistent with previous reports[15,16] (data not shown).

We also tested whether IL21 can act on mature NK cells. We isolated $CD56^+$ cells from fresh human peripheral blood mononuclear cell (PBMCs) (Fig. 7c), and cultured them for 1–2 days with and without 30 ng ml^{-1} IL2, IL15 or IL21. The cells cultured with IL21 exhibited enhanced lytic activity on K562 target cells, although the effect of IL21 was never quite as pronounced as that of IL2 or IL15 (Fig. 7d). Treatment of NK cells with combinations of IL21 and

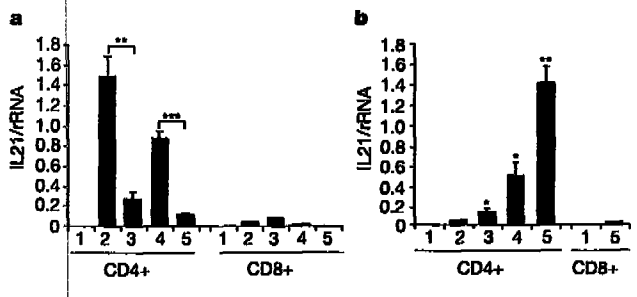

Figure 4 Quantitative RT–PCR analysis of *IL21* expression in lymphocyte subsets. **a**, Stimulation conditions are resting (lane 1); 3.5 h anti-CD3 (lane 2) or PMA plus ionomycin (lane 3); 16 h anti-CD3 (lane 4) or PMA plus ionomycin (lane 5). **b**, Stimulation conditions are resting (lane 1); 2 h anti-CD3 (lane 2); and anti-CD3 plus anti-CD28 for 2 h (lane 3), 4 h (lane 4) or 16 h (lane 5). Vertical bars represent the ratio of *IL21* message in each sample to that of 18S ribosomal RNA in the same sample. Values plotted represent the mean (± s.d.) obtained from triplicate assays. Asterisk, $P < 0.05$; two asterisks, $P < 0.005$; three asterisks, $P < 0.001$.

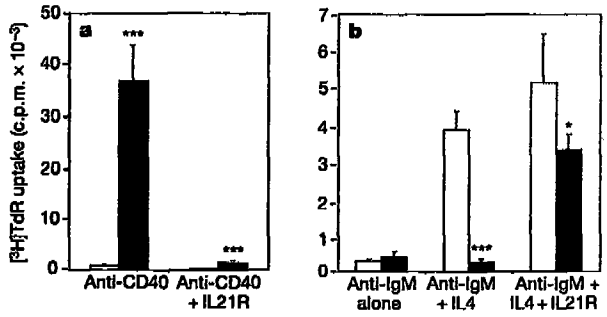

Figure 5 Effect of IL21 on the proliferative responses of human B cells. B cells were purified from peripheral blood of normal human donors and cultured either in the absence (open bars) or in the presence (filled bars) of 10 ng ml^{-1} IL21. **a**, Anti-CD40 and 25 μg ml^{-1} sIL21R were used as additional stimuli as indicated. **b**, Combinations of immobilized anti-IgM, IL4 and 25 μg ml^{-1} sIL21R were used as additional stimuli as indicated. Values plotted represent the mean value (± s.d.) obtained from triplicate cultures. Similar results were obtained in four independent experiments. Asterisk, $P < 0.05$; two asterisks, $P < 0.005$; three asterisks, $P < 0.001$.

US006307024B1

(12) **United States Patent**
Novak et al.

(10) **Patent No.: US 6,307,024 B1**
(45) **Date of Patent: Oct. 23, 2001**

(54) **CYTOKINE ZALPHA11 LIGAND**

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(73) Assignee: **ZymoGenetics, Inc.**, Seattle, WA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/522,217**

(22) Filed: **Mar. 9, 2000**

Related U.S. Application Data

(60) Provisional application No. 60/123,547, filed on Mar. 9, 1999, provisional application No. 60/123,904, filed on Mar. 11, 1999, and provisional application No. 60/142,013, filed on Jul. 1, 1999.

(51) **Int. Cl.**[7] **C07K 14/00**; C12P 21/06; C12P 21/04; A61K 39/395

(52) **U.S. Cl.** **530/351**; 530/350; 435/69.1; 435/69.7; 424/143.1; 424/145.1

(58) **Field of Search** 530/380, 351; 435/69.1, 69.7; 424/143.1, 145.1

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* cited by examiner

Primary Examiner—Jeffrey Stucker
Assistant Examiner—Jegatheesan Seharaseyon
(74) *Attorney, Agent, or Firm*—Deborah A. Sawislak

(57) **ABSTRACT**

The present invention relates to zalpha11 Ligand polynucleotide and polypeptide molecules. The zalpha11 Ligand is a novel cytokine. The polypeptides may be used within methods for stimulating the proliferation and/or development of hematopoietic cells in vitro and in vivo. The present invention also includes methods for producing the protein, uses therefor and antibodies thereto.

23 Claims, 5 Drawing Sheets

```
          1          15 16          30 31          45 46          60 61          75 76          90
hIL-2     ---------------  ------APTSSSTKK TQLQLEHLLLDLQMILNGINNYKN------ ---PKLTRMLTFKFY MPKKATE---LKHLQ
hIL-15    -----------AGIH VFILGCFSAGLPKTE ANWVNVISDLKKI-EDLIQSMHIDAT---- -------------LY TESDVHPSCKVTAMK
zal1-Lig  ---------------  --sSSQGQDRHMIRM RQLIDIVDQLKNYVNDLVPEF--------- -------------LP APEDVETNCEWSAFS
hIL-4     ---------------  ----------HKCD- ITLQEIIKTLNSLTEQKTLCTELTVTDI-- -------------FA ASKNTTE----KETF
mIL-4     ---------------  --------HIHGCDK NHLREIIGILNEVTGEGTPCTEMDVPNV-- -------------LT ATKNTTE----SELV
hGM-CSF   ---------------  ---SPSPSTQPWEHV NAIQEARRLLNLSRDTAAEMNETV------ -------------E VISEMFD---LQEPT
mGM-CSF   ---------------  -----IIVTRPWKHV EAIKEALNLLDDMPVTL---NEEV------ -------------E VVSNEFS---FKKLT

          91         105 106         120 121         135 136         150 151         165 166         180
hIL-2     CLEEELKPLEEVLNL AQSKNFHLRPRDLIS NINVIVLGLKGSE----------------- --------TTFMCE- YADETATIVEFLNRW
hIL-15    CFLLELQVISLESGD ASIHDTVENLIILAN NSLSSNGNVTESG----------------- ---------CKECEE LEEK--NIKEFLQSF
zal1-Lig  CFQKAQLKSANTGNN ERIINVSIKKLKRKP PSTNAGRRQKHRL----------------- --------TCPSCDS YEKK--PPKEFLERF
hIL-4     CRAATVLRQFYSHHE KDTRCLGATAQQFHR HKQLIRFLKRLDRNLWGLAGL--------- ----------NSCPV KEANQSTLENFLERL
mIL-4     CRASKVLRIFYLKHG K-TPCLKKNSSVLME LQRLFRAFRCLDS----------------- ---------SISCTM NESKSTSLKDFLESL
hGM-CSF   CLQTRLELYKQGLRG SLTKLKGPLTMMASH YKQHCPPTPE------------------- ----------TSCA- --TQIITFESFKENL
mGM-CSF   CVQTRLKIFEQGLRG NFTKLKGALNMTASY YQTYCPPTPE------------------- ----------TDCE- --TQVTTYADFIDSL

hIL-2     ITFCQSIISTL    132
hIL-15    VHIVQMFINTS1   134
zal1-Lig  KSLLQKMIHQHLSSR THGSEDS    136
hIL-4     KTIMREKYSKCSS    129
mIL-4     KSIMQMDYS    120
hGM-CSF   KDFLLVIPFDC--WE    119
mGM-CSF   KTFLTDIPFEC--KK PSQK    118
```

FIG. 1

162 – 100%.
136 – x = 83,95%.

230

232

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES

Abogado
Dilia María Rodríguez D'Aleman

ASUNTO	Radicación	05-9447
	Trámite	002
	Evento	001
	Actuación	430
	Oficio	
	Folios	010 1373

Patente de Invención ☐ Patente de Modelo de Utilidad ☐

Vía Nacional ☐

Vía PCT (fase nacional) ☐ Cap.I ☐ Cap. II ☐

No. Sol. Internacional PCT/US2003/021975
Fecha de Presentación Internal. 05/07/2003

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

1. Documentos estudiados

Descripción:	Folios: 36 a 123 como se radicó inicialmente.
Reivindicaciones:	Folios: 124 a 128 como se radicó inicialmente. Folios: 222 a 225 modificadas el 02/05/2008
Figuras:	Folios: 31 a 35 como se radicó inicialmente.
Prioridad:	15/07/2002, Estado Unidos, US60/396,160

2. Objeto de la invención

Título de la solicitud: "Métodos y composiciones para modular el desarrollo y la función de las células T Helper (T_h)".

El objeto de la presente solicitud de patente de invención consiste en composiciones para modular el desarrollo y la función de las células T Helper, utilizando agonistas de la IL-21 o anticuerpos del receptor de IL-21.

El problema planteado en esta solicitud es el papel inmunológico múltiple de las células T helper y las citoquinas en la promoción de respuesta inmunes específicas y en el desencadenamiento de ciertas patologías.

- La secuencia peptídica completa de IL-21, que posee una homología de 100% con la secuencia SEQ ID N° 2 de la solicitud. Además indica las regiones conservadas con el mismo péptido de origen murino (ver figura 1);

- Secuencia peptídica completa del receptor de IL-21 (IL-21R), indicando regiones conservadas con el mismo péptido de origen murino. Esta secuencia presenta un 100% homología con la secuencia SEQ ID N° 4 de la solicitud en estudio (ver fig 1);

- Menciona los sitios importantes de las secuencias IL-21 e IL-21R tales como motivos conservados y la secuencia señal del receptor de la IL-21;

- Divulga la actividad de la IL-21 en promover la expansión de las células T helper (ver pág 62);

- Establece otras actividades biológicas de la IL-21, sobre las células B y sobre las células NK (pág 60 y 62);

Con esta información, para el técnico versado en la materia era evidente la importancia y relevancia que tenía la IL-21 en la estimulación de respuesta del sistema inmune dirigida a determinadas poblaciones de células, incluidas las células T helper. Además tenía la información completa de la secuencia peptídica tanto del ligando (IL-21) como del receptor (IL-21R) y por tanto, podía realizar preparaciones que incluyeran estos péptidos.

La única diferencia entre D1 y la solicitud en estudio es que esta última presenta la molécula IL-21 e IL-21R, cada una en una composición que contiene uno o más portadores farmacéuticamente aceptados.

Sin embargo, tal diferencia corresponde a un desarrollo obvio que puede ser realizado por cualquier técnico versado en la materia, puesto que el desarrollo de la composición es realizado de la manera estándar. Lo cual es evidente de la lectura del capítulo descriptivo y reivindicatorio, donde se mencionan los portadores farmacéuticos de una manera general, sin indicar de cual se trata, mostrando que este no es un elemento relevante para las composiciones farmacéuticas preparadas.

Por otra parte, el documento US 6,307,024 (D2) también divulga:

- la secuencia del polipéptido IL-21, desde el aminoácido 26 hasta el 162, con una identidad de secuencia en esta región del 100% (ver fig 1);

- El polipéptido divulgado también se une al receptor IL-21 (ver resumen y reivindicaciones);

- Divulga la actividad biológica de la IL-21, indicando que esta molécula estimula la proliferación de células NK, células B y células T (ver resumen, ejemplos y reivindicaciones);

- Divulga anticuerpos que se unen al IL-21 R (ver resumen y ejemplo 47);

Con esta información, el técnico versado en la materia también podía llegar a la invención presentada en la solicitud, incluyendo las composiciones farmacéuticas con IL-21 y anticuerpos anti IL-21R.

La diferencia entre D2 y la solicitud en estudio, es que la secuencia polipeptídica divulgada en D2 carece del la región N-terminal de la molécula (aminoácidos 1-25) y por ello comparte el 83.5% de homología con la longitud total de la IL-21 (SEQ N° 2 de la solicitud). Sin embargo, la secuencia completa de la IL-21 era conocida en el estado de la técnica, tal como se puede corroborar con la información divulgada en D1. Por ello, la utilización de la secuencia completa para el desarrollo de la composición carece de nivel inventivo.

Por tanto, ante la divulgación de los documentos D1 y D2, independientemente o en combinación, la invención de la solicitud carece de nivel inventivo e inclumple con el artículo 18 de la D486.

8. Conclusión:

Los requisitos de patentabilidad de la presente solicitud se encuentran afectados así:

N°	Documento N°	Reivindicaciones afectadas	Requisito que afecta
D1	Parrish-Novak J. *et.al.*, NATURE, VOL 408: 57-63.	1-19	**Nivel Inventivo**
D2	US 6,307,024	1-19	**Nivel Inventivo**

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de 60 días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.

ALIX CARMENZA CÉSPEDES DE VERGEL
Directora de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha 05 FEB. 2010

CONCEPTO TÉCNICO No.	EXAMINADOR TÉCNICO
133	Código No.202052