

Señores

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

DIVISION DE NUEVAS CREACIONES

E. \_\_\_\_\_ S. \_\_\_\_\_

RECEIVED SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



NO. 96 129582-00002-0000

RECEIVED DIVISION DE NUEVAS CREACIONES  
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO  
BOGOTA - COLOMBIA  
FOLIOS 3

Ref.: Expediente No. 06.129.582

Solicitud de Patente de Invención Titulada: "ANTICUERPOS MONOCLONALES  
QUIMERICOS Y HUMANIZADOS CONTRA LA INTERLEUQUINA -13".

Solicitantes: GLAXO GROUP LIMITED

**SOLICITUD DE EXAMEN DE PATENTABILIDAD SEGÚN**  
**EL ARTÍCULO 44 DE LA DECISION 486, GACETA No. 583**

CARLOS R. OLARTE, mayor de edad y vecino de Bogotá, D.C., identificado tal como aparece al pie de mi firma, en mi condición de apoderado, respetuosamente me permito solicitar que se examine si la invención reúne los requisitos de patentabilidad previstos en la misma, dando cumplimiento a lo establecido en el artículo 44 de la Decisión 486 de la comisión de la Comunidad Andina. Adjunto recibo por el monto de \$420.000 pesos para cubrir la tasa correspondiente, según la Resolución 44372 del año 2007.

Atentamente,

CARLOS R. OLARTE  
C.C. 79.782.747 de Bogotá  
T.P. 74.295

Anexo: Recibo por \$420.000 pesos  
Sustitución

LNIB P2-000-0002780-000207

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 08 - 11,165  
FECHA : FEBRERO 11 DE 2008

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



NIT : 800176089-2

Superintendencia de Industria y Comercio  
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\*\*\*\*\* CONSIGNACION \*\*\*\*\*

| DEPOSITANTE    | TIPO PAGO    | BANCO           | CUENTA    | No. PAGO | FECHA PGO  | Vr. PAGO     |
|----------------|--------------|-----------------|-----------|----------|------------|--------------|
| OLARTE RAISBEK | CONSIGNACION | BANCO DE BOGOTA | 062754387 | 1483388  | 11/02/2008 | 5,606,000.00 |

\*\*\*\*\* CONCEPTO \*\*\*\*\*

| RENTISTICO                | CONCEPTO                             | TOTAL CONCEPTO |
|---------------------------|--------------------------------------|----------------|
| 1 50005-01-01 SOLICITUDES | 85 EXAMEN DE PATENTABILIDAD DE INVEN | 420,000.00     |
|                           | TOTAL :                              | \$ 420,000.00  |

SON: CUATROCIENTOS VEINTE MIL PESOS

RESPONSABLE :

RECIBO DE CAJA APLICADO AL EXPEDIENTE No.

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ASS

Señores

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

DIVISION DE NUEVAS CREACIONES

E. \_\_\_\_\_ S. \_\_\_\_\_ D. \_\_\_\_\_

Ref.: Expediente No. 06.129.582

Solicitud de Patente de Invención Titulada: "ANTICUERPOS MONOCLONALES  
QUIMERICOS Y HUMANIZADOS CONTRA LA INTERLEUQUINA -13".

Solicitantes: GLAXO GROUP LIMITED

**SUSTITUCIÓN DE PODER**

J. IAN RAISBECK LLINAS, mayor de edad y vecino de Bogotá D.C., identificado como aparece al pie de mi firma, en mi carácter de apoderado de la sociedad de la referencia, respetuosamente me permito manifestar que **SUSTITUYO** el poder a mi conferido por la mencionada sociedad, en el doctor **CARLOS R. OLARTE**, mayor de edad, domiciliado en Bogotá D.C., identificado con la Cédula de Ciudadanía No. 79.782.747 de Bogotá y portador de la Tarjeta Profesional No. 74.295 del Consejo Superior de la Judicatura, para que sea él quien en adelante asuma el trámite de la presente acción.

El apoderado sustituto conserva las mismas facultades del principal.

Atentamente,

J. IAN RAISBECK

C.C. No. 79.376.996 de Bogotá

T.P. 135.799 del C. S. de la J.

**DIRECCIÓN DE NUEVAS CREACIONES**

RADICACIÓN EXPEDIENTE No. 06-129582

EL EXTRACTO DE LA PRESENTE SOLICITUD DE **PATENTE DE INVENCION**

FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No **583** DE

FECHA **18 DE DICIEMBRE DE 2007** EL TÉRMINO HÁBIL SEÑALADO POR LA

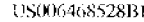
LEY EXPIRA EL **14 DE MARZO DE 2008**

NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial, deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud caiga en abandono, de acuerdo con lo establecido en el Artículo 44 de la Decisión 486/2000.

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

SI \_\_\_\_\_

NO       X



US 6,468,528 B1

In another aspect, the invention provides methods for inhibiting  $\text{IL-13}$  dependent proliferation of Hodgkin and Reed Sternberg (H/RS) cell types comprising the step of contacting the H/RS cell types with a compound that inhibits biological activity of  $\text{IL-13}$  receptors expressed on the H/RS cell types. In a preferred embodiment, methods are provided wherein the compound that inhibits  $\text{IL-13}$  receptor biological

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sion. In a preferred method, the inhibitor is an antisense polynucleotide that specifically binds a polynucleotide encoding IL-13 or an antibody that specifically recognizes an IL-13 polypeptide.

The invention also comprehends methods for treating Hodgkin's Disease comprising the step of administering to an individual in need thereof a therapeutically effective amount of a compound that inhibits IL-13 receptor biological activity. In another set, the invention provides methods for treating Hodgkin's Disease comprising the step of administering to an individual in need thereof a therapeutically effective amount of a compound that inhibits IL-13 receptor expression.

Inhibitors of the invention can be administered in combination with a pharmaceutically acceptable (i.e., sterile and non-toxic) liquid, semisolid, or solid diluents that serve as pharmaceutical vehicles, excipients, or media. Any diluent known in the art may be used. Exemplary diluents include, but are not limited to, polyoxyethylene sorbitan monolaurate, magnesium stearate, methyl- and propylhydroxybenzoate, talc, alginates, starches, lactose, saccharose, dextrose, sorbitol, trimethyl, gum acacia, calcium phosphate, mineral oil, cocoa butter, and oil of theobroma.

The inhibitor compositions can be packaged in forms convenient for delivery. The compositions can be enclosed within a capsule, sachet, cachet, gelatin, paper, or other container. These delivery forms are preferred when compatibility with entry of the composition into the recipient organism and, particularly, when the composition is being delivered in unit dose form. The dosage units can be packaged, e.g., in tablets, capsules, suppositories or cachets.

The inhibitor compositions may be introduced into the subject by any conventional method including, e.g., by intravenous, intradermal, intramuscular, intramammary, intraperitoneal, or subcutaneous injection; by oral, sublingual, nasal, anal, vaginal, or transdermal delivery; or by surgical implantation, e.g., embedded under the skin capsule or in the cornea. The treatment may consist of a single dose or a plurality of doses over a period of time. Optimal dosages may be readily determined by routine methods.

In another aspect, the invention contemplates use of an inhibitor of IL-13 expression in the production of a medication for treatment of IL-13 dependent Hodgkin and Reed Sternberg cell proliferation. As another aspect, use of an inhibitor of IL-13 receptor biological activity in the production of a medication for treatment of IL-13 dependent Hodgkin and Reed Sternberg cell proliferation is also provided. The invention also embraces use of an inhibitor of IL-13 receptor expression or the production of a medication for treatment of IL-13 dependent Hodgkin and Reed Sternberg cell proliferation. In still another aspect, the invention comprehends use of an inhibitor of IL-13 biological activity in the production of a medication for treatment of IL-13 dependent Hodgkin and Reed Sternberg cell proliferation.

While the invention is exemplified with respect to expression and biological activity of IL-13 and its cognate receptor, the invention also comprehends materials and methods to modulate expression and biological activity of other components (and their receptors where applicable) demonstrated herein to have high expression levels in HRS cells, including, for example, IL-5, ornithine decarboxylase (ODC), ICAM-3, monokine (C-PA), IgE, IL-1 receptor II, insulin-like growth factor (IGF) II, NF-IL-3A (IRP), Notch2, GM-CSF, interferon regulatory factors (IRF) 1, 5 and 6, nitric oxide synthase (NOS) 3, and the IL-13 receptor delta chain. The invention also comprehends materials and methods to modulate

late expression and biological activity of components in IL-13 pathways that participate, directly or indirectly, in HRS cell proliferation.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention is exemplified in the following examples. Example 1 describes identification of genes differentially expressed in HRS cells. Example 2 describes Northern analysis and ELISA to confirm results observed in Example 1. Example 3 provides even more confirmatory evidence of differential gene expression using *in situ* hybridization. Example 4 demonstrates that neutralization of IL-13 activity modulates HRS cell proliferation.

#### EXAMPLE 1

##### Gene Expression in HD Cells

In a first series of experiments, assays were carried out to compare overall gene expression in a HD-derived cell type compared to a normal cell line in an attempt to identify genes that are over expressed in the HD cells. Microarray analysis has recently been developed [Schema, et al., *Science* 270:1674-1678 (1995); Schema, et al., *Proc Natl Acad Sci USA* 93:10614-10619, (1996); Shalon, et al., *Genome Res* 6:639-645 (1996)] as a very efficient means of studying the differential expression of many genes simultaneously. Gene expression in two different samples is compared using competitive hybridization of two probes labeled with different fluorescent dyes.

In the first experiments, cDNA segments from genes relevant to inflammation and neoplasia were used to examine gene expression patterns in HD-derived cell lines. Microarray expression patterns in the myeloid, clonal cell lines L428 and KMH2 [Drexler, *Leukemia and Lymphoma* 9:1-25 (1993)], which were cultured from Hodgkin tissues and are regarded as HD cell lines, were compared with microarray expression patterns in control LCL-GK cells, an Epstein Barr virus (EBV) infected lymphoblastoid B cell line from a healthy individual. Additional control cells included EBV-negative, non-Hodgkin lymphoma (NHL) cell lines L43, L47, and L432. The cell lines were grown in IMDM medium supplemented with penicillin, streptomycin, and 10% fetal calf serum.

From each cell type, poly(A)<sup>+</sup> mRNA was prepared from total RNA using Oligotex-dT resin (Qiagen, Crawfordsville, Ind.) according to the manufacturer's suggested protocol. Each mRNA sample was converted into fluorescein-labeled cDNA probes using the microarray (GEM) probe labeling kit (Syntex, Palo Alto, Calif., USA), also according to manufacturer's suggested protocol. The microarray used in these experiments contained 950 human genes involved in inflammation and neoplasia (Syntex, Palo Alto, Calif., USA). Two microarrays were independently probed with 200 ng of a 1:1 mixture of fluorescein-labeled cDNA from HD-derived L428 cells and control LCL-GK cells on microarray 1 (M1) or from HD-derived KMH2 cells and LCL-GK cells on microarray 2 (M2). Known concentrations of mRNA synthesized from inter-ORI regions from *S. cerevisiae* were used as quantitation standards. The hybridization was performed as previously described [Schema, et al., 1995, *Science* 270:1674-1678 (1995)].

Of the 950 genes displayed on the microchip, those showing greater than three-fold difference in expression in both HD-derived cell lines were IL-13, IL-5, ornithine decarboxylase (ODC), ICAM-3, monokine (C-PA), IGF, IL-1 receptor II, insulin-like growth factor (IGF) II, NF-IL-3A (IRP), Notch2, GM-CSF,

receptor II, insulin-like growth factor (IGF) II, NF-IL-3A (IRP), Notch2, GM-CSF, interferon regulatory factors (IRF) 1, 5 and 6, nitric oxide synthase (NOS) 3, and the IL-13 receptor delta chain. IL-13 expression in HD-derived L428 and KMH2 cells was increased over that in control LCL-GK cells 26.7- and 17.1-fold, respectively, while IL-5 expression was increased by 11.2- and 18.5-fold, respectively. In addition, mRNA for the IL-13 receptor was found to be expressed in both HD-derived cell lines tested.

These results clearly demonstrate that HD-derived cell lines and HRS tumor cells express elevated levels of IL-13. Because IL-13 is known to promote IgE class switching, the elevation of IgE in HRS cells and in the serum of HD patients could be explained if IL-13 secreted by HRS cells affects both the HRS cells themselves and bystander B cells.

As discussed above, the microarray hybridization showed that the expression of NF-IL-3 and Notch2 was upregulated by more than 3 fold in NHL-derived cell lines. The basic leucine zipper (b-ZIP) transcription factor NF-IL-3 acts downstream of IL-13 and has been shown to prevent apoptosis after forced expression in an IL-13 dependent pro-B cell line [Kushima, et al., *Proc Natl Acad Sci USA* 94:2609-2614 (1997)]. The relevance of NF-IL-3 in HD is unknown.

Notch 2 is a transmembrane receptor that has been shown to be involved in cell fate decisions [Robey, *Curr Opin Genet Dev* 7:581-7 (1997)]. The human Notch2 gene has been mapped to chromosome 1 at position 1p34 [Fonatsch, et al., *Genomics* 23:253-58 (1994)].

Chromosome 1 has also been linked to the HD phenotype, since structural rearrangements of chromosome 1 are frequently observed in HD [Thangaveedu, et al., *Hematol Oncol Clin North Am* 3:221-36 (1984)].

Interestingly, the gene for CD30, which was first identified in the HD-derived cell line L428 [Schwarz, et al., *Nature* 299:65-67 (1982)] and is considered a marker for the disease, is also located on chromosome 1 at 1p34 [Fonatsch, et al., *Genomics* 14:825-6 (1992)]. CD30 is highly expressed in lymphoblastoid and HRS cells and has been shown to promote a Th2 phenotype [De Pre, et al., *J Hep Med* 182:1655-61 (1995)]. The possible roles of NF-IL-3 and Notch2, and their relationship to IL-13 and the CD30 antigen in the pathogenesis of HD lymphoma merit further investigation.

#### EXAMPLE 2

##### Northern Analysis and Cytokine Production

To evaluate the significance of the results from Example 1, Northern blots and ELISA were used to examine expression of IL-13, IL-5, GM-CSF, NF-IL-3, Notch2, and monokine in the HD-derived EBV-negative cell lines L428, KMH2, and HDLM2 [Drexler, *Leukemia and Lymphoma* 9:1-25 (1993)]. Control cell types included lymphoblastoid EBV-infected B cell lines LCL-GK and LCL-HO, and the three EBV-negative, non-Hodgkin lymphoma (NHL)-derived cell lines with either B cell (L43, L47) or T cell (L432) phenotype.

In the Northern blot analysis, total RNA was isolated using Trizol reagent (Gibco BRL, Gaithersburg, Md.) according to the manufacturer's suggested protocol and 15 µg of total RNA was separated on a 1% formaldehyde agarose gel. Samples were run in 0.02 M MOPS [4[N-methylpiperidine]propanesulfonic acid], pH 7.0, 8 mM sodium acetate, and 1 mM EDTA. The RNA was blotted overnight in

10xSSC onto a Hybond N+ nylon membrane (Amersham Pharmacia Biotech, Arlington Heights, Ill., USA) and cross-linked to the membrane using UV irradiation using standard techniques. Hybridization was carried out at 65° C. using c[<sup>32</sup>P]dATP-labeled oligonucleotides specific for the human Notch2, NF-IL-3, monokine, IL-13, and human β-actin genes. Probe labeling was carried out using a Multiprime DNA labeling system (Amersham Pharmacia Biotech, Arlington Heights, Ill., USA) according to the manufacturer's suggested protocol. Membranes were washed once at 65° C. for 30 minutes in 2xSSC containing 0.1% SDS, and then at 65° C. for 30 minutes in 0.2xSSC containing 0.1% SDS.

In the ELISA, cell culture supernatant (10<sup>5</sup> cells equivalently) from each cell type was recovered 48 hours after medium exchange and assayed for IL-5, IL-13, and GM-CSF production using Quantikine kits (R&D Systems, Minneapolis, Minn., USA) specific for each cytokine, according to the manufacturer's suggested protocol.

Results indicated that Notch2, monokine, and NF-IL-3 were up-regulated in LCL or NHL cell lines, as well as in the HD-derived cell lines, but overexpression of IL-13, IL-5 and GM-CSF was restricted to the HD-derived cell lines. IL-5 and GM-CSF expression and secretion could be demonstrated only in L428 and KMH2 cells, but mRNA expression and secretion of IL-13 could be detected in all three HD-derived cell lines. HDLM2 cells secreted a moderate amount of IL-13 (27 pg/ml), while L428 and KMH2 cells secreted significantly higher levels of (4800 pg/ml and 6100 pg/ml, respectively).

#### EXAMPLE 3

##### In Situ Hybridization

To confirm that IL-13 was expressed by the rare HRS tumor cells, *in situ* hybridization was carried out with sense and antisense RNA probes for IL-13 using lymph node biopsies from four untreated patients, newly diagnosed with classical nodular sclerosis HD. A benign reactive lymph node biopsy was used as a control.

For *in situ* hybridization, paraffin sections were mounted and fixed according to standard protocols. A human cDNA probe specific for IL-13 was generated by PCR using RT-PCR and IL-13-specific primers set out in SEQ ID NOS 1 and 2. PCR was carried out using standard conditions well known and routinely practiced in the art.

5'GTGACACCGGTGTCATGCTCTACT SEQ ID NO: 1

3'CTCAGTGTGAACCGTCCCTCGGAASEQ ID NO: 2

The 388 bp amplification product was cloned into the pCRIT vector (TA cloning kit, Invitrogen, Carlsbad, Calif., USA). Sense and antisense probes were synthesized from the linearized vector with SP6 or T7 polymerase, labeled with <sup>32</sup>P-UTP, and processed as previously described [Thi, et al., *Nat Genet* 3:211-216 (1993)]. The sections were counterstained with toluidine blue using a standard protocol.

The results confirmed strong and specific IL-13 expression by morphologically identifiable HRS cells in all patients, in contrast to background levels of IL-13 expression in non-HRS cells and cells from the control biopsy.

#### EXAMPLE 4

##### Analysis of Cell Proliferation after Neutralization of IL-13

To investigate the effects of IL-13 and IL-5 on the proliferation of HRS cells, L428, KMH2 and HDLM2 cells

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(19) United States

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Shealey et al.

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(54) ASTHMA-RELATED ANTI-IL-13  
IMMUNOGLOBULIN DERIVED PROTEINS,  
COMPOSITIONS, METHODS AND USES

## Related U.S. Application Data

(60) Provisional application No. 60/370,371, filed on Apr. 5, 2002

## Publication Classification

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A61K 31/56  
(52) U.S. Cl. .... 424/85.1; 424/131.1; 514/171;  
514/2(76) Inventors David Shealey, Downingtown, PA  
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(71) Appl. No. 10/408,694

(72) Filed Apr. 7, 2003

## (57) ABSTRACT

The present invention relates to therapeutic methods involving the use of at least one asthma related anti-IL-13 human Ig derived protein, as well as such proteins and isolated nucleic acids that encode at least one asthma related Ig derived protein, vectors, host cells, transgenic animals or plants, and methods of making and using thereof, including pharmaceutical compositions, methods and devices.

US 2003/023555 A1

Dec. 25, 2003

ASTHMA-RELATED ANTI-IL-13  
IMMUNOGLOBULIN DERIVED PROTEINS,  
COMPOSITIONS, METHODS AND USES

## RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional patent Appl. No. 60/370,371, filed Apr. 5, 2002, which is entirely incorporated herein by reference.

## BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention

[0003] The present invention relates to therapeutic methods involving the use of asthma related anti interleukin 13 (IL-13) immunoglobulin (Ig) derived proteins, as well as such proteins, asthma related Ig derived protein encoding and complementary nucleic acids, vectors, host cells, transgenic animals and plants, and methods of making and using thereof, including pharmaceutical compositions, methods and devices.

[0004] 2. Related Art

[0005] Asthma is a chronic inflammation of the bronchial tubes (airways) that causes swelling and narrowing (constriction) of the airways. The result is difficulty breathing. The bronchial narrowing is usually either totally or at least partially reversible with treatments. Bronchial tubes that are chronically inflamed may become overly sensitive to allergens (specific triggers) or irritants (non-specific triggers). The airways may become "twitchy" and remain in a state of heightened sensitivity. This is called "Bronchial Hyperreactivity" (BHR). It is likely that there is a spectrum of bronchial hyperreactivity in all individuals. However, it is clear that asthmatics and allergic individuals without apparent asthma have a greater degree of bronchial hyperreactivity than non-asthmatic and non-allergic people. In sensitive individuals, the bronchial tubes are more likely to swell and constrict when exposed to triggers such as allergens, tobacco smoke, or exercise. Amongst asthmatics, some may have mild BHR and no symptoms while others may have severe BHR and chronic symptoms.

[0006] Asthma causes a narrowing of the breathing airways, which interferes with the normal movement of air in and out of the lungs. Asthma involves only the bronchial tubes and does not affect the air sacs or the lung tissue. The narrowing that occurs in asthma is caused by three major factors, inflammation, bronchospasm, and hyper reactivity. The first and most important factor causing narrowing of the bronchial tubes is inflammation. The bronchial tubes become red, irritated, and swollen. The inflammation occurs in response to an allergen or irritant and results from the action of chemical mediators (histamine, leukotrienes, and others). The inflamed tissues produce an excess amount of "sticky" mucus into the tubes. The mucus can clump together and form "plugs" that can clog the smaller airways. Eosinophils and other cells, which accumulate at the site, cause tissue damage.

[0007] These damaged cells are shed into the airways, thereby contributing to the narrowing.

[0008] The muscles around the bronchial tubes tighten during an attack of asthma. This muscle constriction of the airways is called bronchospasm. Bronchospasm causes the

airway to narrow further. Chemical mediators and nerves in the bronchial tubes cause the muscles to constrict. In patients with asthma, the chronically inflamed and constricted airways become highly sensitive, or reactive, to triggers such as allergens, irritants, and infections. Exposure to these triggers may result in progressively more inflammation and narrowing. The combination of these three factors results in difficulty with breathing out, or exhaling. As a result, the air needs to be forcefully exhaled to overcome the narrowing, thereby causing the typical "wheezing" sound. People with asthma also frequently "cough" in an attempt to expel the thick mucus plugs. Reducing the flow of air may result in less oxygen passing into the blood stream and if very severe, carbon dioxide may dangerously accumulate in the blood.

[0009] Inflammation, or swelling, is a normal response of the body to injury or infection. The bloodflow increases to the affected site and cells rush in and ward off the offending problem. The healing process has begun. Usually, when the healing is complete, the inflammation subsides. Sometimes, the healing process causes scarring. The central issue in asthma, however, is that the inflammation does not resolve completely on its own. In the short term, this results in recurrent "attacks" of asthma. In the long term, it may lead to permanent thickening of the bronchial walls, called airway "remodeling." If this occurs, the narrowing of the bronchial tubes may become irreversible and poorly responsive to medications. Therefore, the goals of asthma treatment are: (1) in the short term, to control airway inflammation in order to reduce the reactivity of the airways; and (2) in the long term, to prevent airway remodeling.

[0010] Asthma symptoms may be activated or aggravated by many agents. Not all asthmatics react to the same triggers. Additionally, the effect that each trigger has on the lungs varies from one individual to another. In general, the severity of your asthma depends on how many agents activate your symptoms and how sensitive your lungs are to them. Most of these triggers can also worsen nasal or eye symptoms. Triggers fall into two categories, allergens ("specific") and non-allergens—mostly irritants (non-"specific"). Once your bronchial tubes (nose and eyes) become inflamed from an allergic exposure, a re-exposure to the offending allergens will often activate symptoms. These "reactive" bronchial tubes might also respond to other triggers, such as exercise, infections, and other irritants. About 80% of children and 50% of adults with asthma also have allergies. Irritants include respiratory infections, such as those caused by viral "colds," bronchitis, and sinusitis; drugs, such as aspirin, other NSAIDs (nonsteroidal anti-inflammatory drugs), and Beta Blockers (used to treat blood pressure and other heart conditions); tobacco smoke; outdoor factors, such as smog, weather changes, and diesel fumes; indoor factors, such as paint, detergents, deodorants, chemicals, and perfumes; nighttime GERD (gastro-esophageal reflux disorder); exercise, especially under cold dry conditions; work-related factors, such as chemicals, dusts, gases, and metals; and emotional factors, such as laughing, crying, yelling, and distress; hormonal factors, such as in premenstrual syndrome.

[0011] Asthma is often referred to as being "extrinsic" or "intrinsic." A better understanding of the nature of asthma can help explain the differences between them. Extrinsic, or allergic asthma, is more common (90% of all cases) and typically develops in childhood. Eighty percent of children

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## DESCRIPTION OF THE INVENTION

**[0030]** The present invention provides therapeutic methods comprising administering an isolated, recombinant and/or synthetic asthma related Ig derived protein. Such Ig derived proteins of the present invention comprise specific Ig derived protein sequences, domains, fragments and specified variants thereof, and methods of making and using thereof, including therapeutic compositions, methods and devices.

**[0031]** As used herein, "asthma related Ig derived protein," and the like, *modulates*, *affects*, *antagonizes*, *decreases*, *blocks*, *inhibits*, *abrogates*, *enhances*, *agonizes*, or *interferes* with at least one asthma related protein activity, binding of asthma related protein receptor activity or binding *in vitro*, *in situ* and/or preferably *in vivo*. For example, a suitable asthma related Ig derived protein, specified portion or variant of the present invention can bind at least one asthma related protein or receptor and includes asthma related Ig derived proteins, antigen-binding fragments thereof, and specified portions, variants or domains thereof that bind specifically to asthma related. A suitable asthma related Ig derived protein, specified portion, or variant can also *modulates*, *affects*, *antagonizes*, *decreases*, *blocks*, *inhibits*, *abrogates*, *enhances*, *agonizes*, or *interferes* with at least one asthma related protein RNA, DNA or protein synthesis, asthma related protein release, asthma related protein or receptor signaling, nucleic acid asthma related protein cleavage, asthma protein related activity asthma related protein production and/or synthesis, e.g., as described herein or as known in the art. In a preferred embodiment, the asthma related protein is human interleukin 13 (IL 13).

**[0032]** Asthma related Ig derived proteins useful in the methods and compositions of the present invention are characterized by high affinity binding to asthma related proteins, and optionally and preferably having low toxicity. In particular, an Ig derived protein, specified fragment or variant of the invention, where the individual components, such as the variable region, constant region and/or framework, individually and/or collectively, optionally and preferably possess low immunogenicity, is useful in the present invention. The Ig derived proteins that can be used in the invention are optionally characterized by their ability to treat patients for extended periods with good to excellent alleviation of symptoms and low toxicity. Low immunogenicity and/or high affinity, as well as other suitable properties, may contribute to the therapeutic results achieved. "Low immunogenicity" is defined herein as raising significant IAHHA, IACA or IAMA responses in less than about 75%, or preferably less than about 50% of the patients treated and/or raising low titres in the patient treated (less than about 300, preferably less than about 100 measured with a double antigen enzyme immunoassay) (Elliott et al., *Lancet* 344:1125-1127 (1994), each of the above references entirely incorporated herein by reference.)

**[0033]** Utility

**[0034]** The isolated nucleic acids of the present invention can be used for production of at least one asthma related Ig derived protein; can be used to effect in an cell, tissue, organ or animal (including mammals and humans), to modulate, treat, alleviate, help prevent the incidence of, or reduce the symptoms of, at least one asthma related pathology, disease, condition or syndrome.

**[0035]** Such a method can comprise administering an effective amount of a composition or a pharmaceutical composition comprising at least one asthma related Ig derived protein or specified portion or variant to a cell, tissue, organ, animal or patient in need of such modulation, treatment, alleviation, prevention, or reduction in symptoms, effects or mechanisms. The effective amount can comprise an amount of about 0.001 to 100 mg/kg per single or multiple administration, or to achieve a serum concentration of 0.01-5000  $\mu$ g/ml serum concentration per single or multiple administration, or any effective range or value therein, as done and determined using known methods, as described herein or known in the relevant arts.

**[0036]** Citations

**[0037]** All publications or patents cited herein are entirely incorporated herein by reference as they show the state of the art at the time of the present invention and/or to provide description and enablement of the present invention. Publications refer to any scientific or patent publications, or any other information available in any media format, including all recorded, electronic or printed formats. The following references are entirely incorporated herein by reference: Ausubel, et al., ed., *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., NY, N.Y. (1987-2003); Sambrook, et al., *Molecular Cloning: A Laboratory Manual*, 2<sup>nd</sup> Edition, Cold Spring Harbor, N.Y. (1989); Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor, N.Y. (1989); Colligan, et al., eds., *Current Protocols in Immunology*, John Wiley & Sons, Inc., NY (1994-2003); Colligan et al., *Current Protocols in Protein Science*, John Wiley & Sons, NY, N.Y. (1997-2003).

**[0038]** Immunoglobulin (Ig) Derived Proteins of the Present Invention

**[0039]** The term "immunoglobulin derived protein" or "Ig derived protein" is intended to encompass Ig derived proteins, digestion fragments, specified portions and variants thereof, including Ig derived protein mimetics or comprising portions of Ig derived proteins that mimic the structure and/or function of an antibody or specified fragment or portion thereof, including single chain Ig derived proteins and fragments thereof, and is also intended to encompass proteins that contain mimetics to therapeutic proteins, antibodies, and digestion fragments, specified portions and variants thereof, wherein the protein comprises at least one functional asthma related protein ligand binding region (LBR) that optionally replaces at least one complementarity determining region (CDR) of the antibody from which the Ig derived protein, portion or variant is derived. Such asthma related Ig derived proteins, specified portions or variants include those that mimic the structure and/or function of at least one asthma related protein antagonist, such as an asthma related protein antibody or receptor or ligand protein, or fragment or analog. Functional fragments include antigen-binding fragments that bind to asthma related proteins or fragments thereof. For example, Ig derived protein fragments capable of binding to human asthma related proteins or fragments thereof, including, but not limited to Fab (e.g., by pepsin digestion), Fab' (e.g., by pepsin digestion and partial reduction) and Fab'' (e.g., by pepsin digestion), F<sub>ab</sub>c (e.g., by plasmin digestion), pFc' (e.g., by pepsin or plasmin digestion), Fd (e.g., by pepsin digestion, partial reduction and reaggregation), Fv or scFv (e.g., by molecular biology techniques) fragments, are encompassed by the invention (see, e.g., Colligan, Immunology, supra).

**[0040]** Such fragments can be produced by enzymatic cleavage, synthetic or recombinant techniques, as known in the art and/or as described herein. Ig derived proteins can also be produced in a variety of truncated forms using Ig derived protein genes in which one or more stop codons have been introduced upstream of the natural stop site. For example, a chimeric gene encoding a T(fab') heavy chain portion can be designed to include DNA sequences encoding the CH<sub>1</sub> domain and/or hinge region of the heavy chain. The various portions of Ig derived proteins can be joined together chemically by conventional techniques, or can be prepared as a contiguous protein using genetic engineering techniques. For example, a nucleic acid encoding the variable and constant regions of a human Ig derived protein chain can be expressed to produce a contiguous protein. See, e.g., Colligan, *Current Protocols in Immunology*, supra, sections 2.8 and 2.10, for fragmentation, and Lachier et al., U.S. Pat. No. 4,946,778 and Bird, R. L., et al., *Science*, 242: 423-426 (1988), regarding single chain Ig derived proteins, each of which publications are entirely incorporated herein by reference.

**[0041]** As used herein, the term "human Ig derived protein" refers to an Ig derived protein in which substantially every part of the protein (e.g., CDR, LBR, framework, C<sub>1</sub>, C<sub>2</sub> domains (e.g., C<sub>1</sub>1, C<sub>1</sub>2, C<sub>1</sub>3), hinge, (V<sub>H</sub>, V<sub>L</sub>)) is substantially non-immunogenic, with only minor sequence changes or variations. Such changes or variations optionally and preferably retain or reduce the immunogenicity in humans relative to non-modified human Ig derived proteins. Thus, a human Ig derived protein is distinct from a chimeric or humanized Ig. It is pointed out that a human Ig derived protein can be produced by a non-human animal or prokaryotic or eukaryotic cell that is capable of expressing functionally rearranged human immunoglobulin (e.g., heavy chain and/or light chain) genes. Further, when a human Ig derived protein is a single chain Ig derived protein, it can comprise a linker peptide that is not found in native human Ig derived proteins. For example, an Fv can comprise a linker peptide, such as two to about eight glycine or other amino acid residues, which connects the variable region of the heavy chain and the variable region of the light chain. Such linker peptides are considered to be of human origin. Asthma related Ig derived proteins that comprise at least one asthma related protein ligand or receptor thereof can be designed against an appropriate ligand, such as isolated and/or asthma related protein, or a portion thereof (including synthetic molecules, such as synthetic peptides). Preparation of such asthma related Ig derived proteins are performed using known techniques to identify and characterize ligand binding regions or sequences of at least one asthma related protein or portion thereof.

**[0042]** Ig derived proteins that are specific for an asthma related protein can be raised against an appropriate immunogenic antigen, such as isolated and/or asthma related protein or a portion thereof (including synthetic molecules, such as synthetic peptides). Preparation of immunogenic antigens, and monoclonal Ig derived protein production can be performed using any suitable technique. A variety of methods have been described (see e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor, N.Y. (1989); Colligan, et al., eds., *Current Protocols in Immunology*, John Wiley & Sons, Inc., NY (1994-2003); Colligan et al., *Current Protocols in Protein Science*, John Wiley & Sons, NY, N.Y. (1997-2003); Kohler et al., *Nature*, 256:

495-497 (1975) and *Eur. J. Immunol.* 6: 511-519 (1976); Milstein et al., *Nature* 266: 550-552 (1977); Koprowski et al., U.S. Pat. No. 4,172,124; *Current Protocols in Molecular Biology*, Ausubel, L. M., et al., eds., (John Wiley & Sons: New York, N.Y.), Chapter 11, (1994-2003), each of which is entirely incorporated herein by reference. Generally, a hybridoma is produced by fusing a suitable immortal cell line (e.g., a myeloma cell line such as, but not limited to, Sp2/0, Sp2/0-AG14, NS0, NS1, NS2, A18-1, J. 5,  $\times$ 243, P3X63Ag8.653, Sp2 SA3, Sp2 MA1, Sp2 S81, Sp2 SA5, U937, MIA 144, ACTIV, MO143, DA 1, JURKAT, WEHI, K-562, COS, RAJI, NIH 3F3, HL-60, MIA 144, NAMIWA, NEURO 2A, or the like, or heteromyelomas, fusion products thereof, or any cell or fusion cell derived therefrom, or any other suitable cell line as known in the art, see, e.g., [www.atcc.org](http://www.atcc.org), [www.fhitec.com](http://www.fhitec.com), and the like, each of which is entirely incorporated herein by reference with Ig derived protein producing cells, such as, but not limited to, isolated or cloned spleen cells, or any other cells expressing heavy or light chain constant or variable or framework or CDR sequences, either as endogenous or heterologous nucleic acid, as recombinant or endogenous, viral, bacterial, algal, prokaryotic, amphibian, insect, reptile, fish, mammalian, rodent, equine, ovine, goat, sheep, primate, eukaryotic, genomic DNA, cDNA, tDNA, mitochondrial DNA or RNA, chloroplast DNA or RNA, hnRNA, mRNA, tRNA, single, double or triple stranded, hybridized, and the like, or any combination thereof. See, e.g., Ausubel, supra, and Colligan, Immunology, supra, chapter 2, each entirely incorporated herein by reference.

**[0043]** Ig derived protein producing cells can be obtained from the peripheral blood or, preferably the spleen or lymph nodes, of mice, rats, rabbits, primates, such as humans, or other suitable animals that have been immunized with the antigen of interest, including boosting with antigen or a nucleic acid encoding such antigen. Any other suitable host cell can also be used for expressing heterologous or endogenous nucleic acid encoding an Ig derived protein, specified fragment or variant thereof, of the present invention. The fused cells (e.g., hybridoma) or recombinant cells can be isolated using selective culture conditions or other suitable known methods, and cloned by limiting dilution or cell sorting, or other known methods. Cells which produce Ig derived proteins with the desired specificity can be selected by a suitable assay (e.g., ELISA). See, e.g., Ausubel, et al., ed., *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., NY, N.Y. (1987-2003); Sambrook, et al., *Molecular Cloning: A Laboratory Manual*, 2<sup>nd</sup> Edition, Cold Spring Harbor, N.Y. (1989); Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor, N.Y. (1989); Colligan, et al., eds., *Current Protocols in Immunology*, John Wiley & Sons, Inc., NY (1994-2003); Colligan et al., *Current Protocols in Protein Science*, John Wiley & Sons, NY, N.Y. (1997-2003), each which is entirely incorporated herein by reference.

**[0044]** Other suitable methods of producing or isolating antibodies of the requisite specificity can be used, including, but not limited to, methods that select recombinant antibody from a peptide or protein library (e.g., but not limited to, a bacteriophage, ribosome, oligonucleotide, RNA, cDNA, or the like, display library, e.g., as available from Cambridge antibody Technologies, Cambridge, UK; MorphoSys, Martinsried/Planegg, DE; Biorvation, Aberdeen, Scotland, UK; Bioinvent, Lund, Sweden; Dyax Corp., Fozom, Ally-

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can be produced using suitable methods, for example, fusion of a human Ig derived protein producing cell and a heteromyceloma or immortalization of an activated human B cell via infection with Epstein Barr virus (Niedbala et al., *Hybridoma*, 17(3):299-304 (1998); Zanella et al., *J Immunol Methods*, 156(2):205-215 (1992); Gustafsson et al., *Hum Ig derived proteins Hybridomas*, 2(1):26-32 (1991)). Preferably, the human anti-human asthma related proteins or fragments or specified portions or variants is generated by immortalization of a transgenic animal (e.g., mouse, rat, hamster, non human primate, and the like) capable of producing a repertoire of human Ig derived proteins, as described herein and/or as known in the art. Cells that produce a human antihuman asthma related Ig derived protein can be isolated from such animals and immortalized using suitable methods, such as the methods described herein.

[0054] Transgenic mice that can produce a repertoire of human Ig derived proteins that bind to human antigens can be produced by known methods (e.g., but not limited to, U.S. Pat. Nos. 5,770,128; 5,809,895; 5,815,806; 5,625,126; 5,625,825; 5,633,125; 5,646,015 and 5,789,650 issued to Lomborg et al.; Jakobovits et al. WO 98/50433; Jakobovits et al. WO 98/28893; Lomborg et al. WO 98/24884; Lomborg et al. WO 97/13857; Lomborg et al. WO 97/28385; Kuchelapate et al. WO 96/30095; Kuchelapate et al. EP 0460137 B1; Kuchelapate et al. EP 0710712 A1; Srinani et al. U.S. Pat. No. 5,545,807; Bruggemann et al. WO 90/04036; Bruggemann et al. EP 0438473 A1; Lomborg et al. EP 0814259 A2; Lomborg et al. GB 2,272,480 A; Lomborg et al. *Nature* 368:856-859 (1994); Taylor et al., *Int. Immunol.* 5(4):579-591 (1993); Green et al., *Nature Genetics* 3:13-21 (1994); Meuck et al., *Nature Genetics* 15:146-150 (1997); Taylor et al., *Nucleic Acids Research* 20(2):6287-6298 (1992); Tuallion et al., *Proc. Natl. Acad. Sci. USA* 90(8):3720-3721 (1993); Lomborg et al., *Int. Rev. Immunol.* 13(1):65-93 (1995) and Fishwild et al., *Nat. Biotech. J.* 14(7):845-851 (1996), which are each entirely incorporated herein by reference). Generally, these mice comprise at least one transgene comprising DNA from at least one human immunoglobulin locus that is functionally rearranged, or which can undergo functional rearrangement. The endogenous immunoglobulin locus in such mice can be disrupted or deleted to eliminate the capacity of the animal to produce Ig derived proteins encoded by endogenous genes.

[0055] The term "functionally rearranged," as used herein refers to a segment of DNA from an immunoglobulin locus that has undergone V(D)J recombination, thereby producing an immunoglobulin gene that encodes an immunoglobulin chain (e.g., heavy chain, light chain, or any portion thereof). A functionally rearranged immunoglobulin gene can be directly or indirectly identified using suitable methods, such as, for example, nucleotide sequencing, hybridization (e.g., Southern blotting, Northern blotting) using probes that can anneal to coding joints between gene segments or enzymatic amplification of immunoglobulin genes (e.g., polymerase chain reaction) with primers that can anneal to coding joints between gene segments. Whether a cell produces an Ig derived protein comprising a particular variable region or a variable region comprising a particular sequence (e.g., at least one CDR sequence) can also be determined using suitable methods. In one example, mRNA can be isolated from an Ig derived protein producing cell (e.g., a hybridoma or recombinant cell or other suitable source) and used to

produce cDNA encoding the Ig derived protein thereof. The cDNA can be cloned and sequenced or can be amplified (e.g., by polymerase chain reaction or other known and suitable methods) using a first primer that anneals specifically to a portion of the variable region of interest (e.g., CDR, coding joint) and a second primer that anneals specifically to non-variable region sequences (e.g., C<sub>H1</sub>, V<sub>H</sub>).

[0056] Screening Ig derived protein or specified portion or variants for specific binding to similar proteins or fragments can be conveniently achieved using peptide display libraries. This method involves the screening of large collections of peptides for individual members having the desired function or structure. Ig derived protein screening of peptide display libraries is well known in the art. The displayed peptide sequences can be from 3 to 5000 or more amino acids in length, frequently from 5-100 amino acids long, and often from about 8 to 25 amino acids long. In addition to direct chemical/synthetic methods for generating peptide libraries, several recombinant DNA methods have been described. One type involves the display of a peptide sequence on the surface of a bacteriophage or cell. Each bacteriophage or cell contains the nucleotide sequence encoding the particular displayed peptide sequence. Such methods are described in PCT Patent Publication Nos. 91/17271, 91/18980, 91/19878, and 93/18778. Other systems for generating libraries of peptides have aspects of both *in vitro* chemical synthesis and recombinant methods. See, PCT Patent Publication Nos. 92/05258, 92/04843, and 96/19256. See also, U.S. Pat. Nos. 5,658,754, and 5,643,768. Peptide display libraries, vector, and screening kits are commercially available from such suppliers as Invitrogen (Carlsbad, Calif.), and Cambridge Ig derived protein Technologies (Cambridge, UK). See, e.g., U.S. Pat. Nos. 4,704,692, 4,939,666, 4,946,778, 5,260,203, 5,155,030, 5,518,889, 5,534,621, 5,656,730, 5,763,733, 5,767,700, 5,856,456, assigned to Linzom; U.S. Pat. Nos. 5,223,409, 5,403,484, 5,571,698, 5,837,500, assigned to Dyax; U.S. Pat. Nos. 5,427,908, 5,580,717, assigned to Allmax; U.S. Pat. No. 5,885,793, assigned to Cambridge Ig derived protein Technologies; U.S. Pat. No. 5,750,373, assigned to Genentech; U.S. Pat. Nos. 5,618,920, 5,595,898, 5,576,195, 5,698,435, 5,693,493, 5,698,417, assigned to Noma; Colligan, supra; Ansel, supra; or Sambrook, supra, each of the above patents and publications entirely incorporated herein by reference.

[0057] Ig derived proteins, specified portions and variants of the present invention can also be prepared using at least one asthma related Ig derived protein encoding nucleic acid to provide transgenic animals or mammals, such as goats, cows, horses, sheep, and the like, that produce such Ig derived proteins or specified portions or variants in their milk. Such animals can be provided using known methods. See, e.g., but not limited to, U.S. Pat. Nos. 5,827,690; 5,849,992; 4,873,316; 5,849,992; 5,994,616; 5,565,362; 5,304,489, and the like, each of which is entirely incorporated herein by reference.

[0058] Ig derived proteins, specified portions and variants of the present invention can additionally be prepared using at least one asthma related Ig derived protein encoding nucleic acid to provide transgenic plants and cultured plant cells (e.g., but not limited to tobacco and maize) that produce such Ig derived proteins, specified portions or variants in the plant parts or in cells cultured therefrom. As a non-limiting example, transgenic tobacco leaves expressing recombinant proteins have been successfully used to provide large amounts of recombinant proteins, e.g., using

an inducible promoter. See, e.g., Cramer et al., *Curr. Top. Microbiol. Immunol.* 230/95:118 (1999) and references cited therein. Also, transgenic maize have been used to express mammalian proteins at commercial production levels, with biological activities equivalent to those produced in other recombinant systems or purified from natural sources. See, e.g., Hood et al., *Adv. Exp. Med. Biol.* 464:127-147 (1999) and references cited therein. Ig derived proteins have also been produced in large amounts from transgenic plant seeds including Ig derived protein fragments, such as single chain Ig derived proteins (scFv's), including tobacco seeds and potato tubers. See, e.g., Conrad et al., *Plant Mol. Biol.* 38:101-109 (1998) and references cited therein. Thus, Ig derived proteins, specified portions and variants of the present invention can also be produced using transgenic plants, according to known methods. See also, e.g., Fischer et al., *Biochem. Appl. Biochem.* 30:99-108 (Oct., 1999); Ma et al., *Trends Biotechnol.* 13:522-7 (1995); Ma et al., *Plant Physiol.* 109:341-6 (1995); Whitcham et al., *Biochem. Soc. Trans.* 22:919-944 (1994); and references cited therein. See, also generally for plant expression of Ig derived proteins, but not limited to, each of the above references is entirely incorporated herein by reference.

[0059] The Ig derived proteins of the invention can bind human asthma related proteins or fragments with a wide range of affinities ( $K_D$ ). In a preferred embodiment, at least one human mAb of the present invention can optionally bind human asthma related proteins or fragments with high affinity. For example, a human mAb can bind human asthma related proteins or fragments with a  $K_D$  equal to or less than about  $10^{-6}$  M or  $10^{-7}$  M or, more preferably, with a  $K_D$  equal to or less than about  $0.1-9.9$  (or any range or value therein)  $\times 10^{-10}$  M,  $10^{-11}$ ,  $10^{-12}$ ,  $10^{-13}$  or any range or value therein.

[0060] The affinity or avidity of an Ig derived protein for an antigen can be determined experimentally using any suitable method. (See, for example, Berzofsky, et al., "Ig derived protein-Antigen Interactions," In: *Fundamental Immunology*, Paul, W. L., Ed., Raven Press, New York, N.Y. (1984); Kuby, *Immunology*, W. H. Freeman and Company, New York, N.Y. (1992); and methods described herein). The measured affinity of a particular Ig derived protein/antigen interaction can vary if measured under different conditions (e.g., salt concentration, pH). Thus, measurements of affinity and other antigen-binding parameters (e.g.,  $K_{1/2}$ ,  $K_D$ ,  $K_A$ ) are preferably made with standardized solutions of Ig derived protein and antigen, and a standardized buffer, such as the buffer described herein.

[0061] Nucleic Acid Molecules

[0062] Using the information provided herein, such as the nucleotide sequences encoding at least 90-100% of the contiguous amino acids of at least one of asthma related Ig derived protein of the present invention, specified fragments, variants or consensus sequences thereof, or a deposited vector comprising at least one of these sequences, a nucleic acid molecule of the present invention encoding at least one asthma related Ig derived protein or specified portion or variant, can be obtained using methods described herein or as known in the art.

[0063] Nucleic acid molecules of the present invention can be in the form of RNA, such as mRNA, hnRNA, tRNA or any other form, or in the form of DNA, including, but not limited to, cDNA and genomic DNA obtained by cloning or

produced synthetically, or any combinations thereof. The DNA can be triple stranded, double stranded or single stranded, or any combination thereof. Any portion of at least one strand of the DNA or RNA can be the coding strand, also known as the sense strand, or it can be the non-coding strand, also referred to as the anti-sense strand.

[0064] Isolated nucleic acid molecules of the present invention can include nucleic acid molecules comprising an open reading frame (ORF), optionally with one or more introns, e.g., but not limited to, at least one specified portion of at least one CDR, as CDR1, CDR2 and/or CDR3 of at least one heavy chain or light chain, respectively; nucleic acid molecules comprising the coding sequence for an asthma related Ig derived protein or specified portion or variant; and nucleic acid molecules which comprise a nucleotide sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode at least one asthma related Ig derived protein as described herein and/or as known in the art. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate nucleic acid variants that code for specific asthma related Ig derived protein or specified portion or variants of the present invention. See, e.g., Ansel, et al., supra, and such nucleic acid variants are included in the present invention.

[0065] In another aspect, the invention provides isolated nucleic acid molecules encoding (a) asthma related Ig derived protein having an amino acid sequence, as encoded by the nucleic acid contained in the plasmid deposited as designated clone names, and ATCC Deposit Nos. \_\_\_\_\_, respectively, deposited on \_\_\_\_\_.

[0066] As indicated herein, nucleic acid molecules of the present invention which comprise a nucleic acid encoding an asthma related Ig derived protein can include, but are not limited to, those encoding the amino acid sequence of an Ig derived protein fragment, by itself, the coding sequence for the entire Ig derived protein or a portion thereof; the coding sequence for an Ig derived protein, fragment or portion, as well as additional sequences, such as the coding sequence of at least one signal leader or fusion peptide, with or without the aforementioned additional coding sequences, such as at least one intron, together with additional, non-coding sequences, including but not limited to, non coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals (for example, ribosome binding and stability of mRNA); an additional coding sequence that codes for additional amino acids, such as those that provide additional functionalities. Thus, the sequence encoding an Ig derived protein can be fused to a marker sequence, such as a sequence encoding a peptide that facilitates purification of the fused Ig derived protein comprising an Ig derived protein, fragment or portion.

[0067] Construction of Nucleic Acids

[0068] The isolated nucleic acids of the present invention can be made using: (a) recombinant methods; (b) synthetic techniques; (c) purification techniques; or combinations thereof, as well known in the art.

[0069] The nucleic acids can conveniently comprise sequences in addition to a polynucleotide of the present invention. For example, a multi-cloning site comprising one

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an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antispasmodic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, a cytokine, or a cytokine antagonist.

5. A method according to claim 1, wherein said asthma related condition is selected from at least one of asthma, bronchial inflammation, excess bronchial mucus or plugs, lung tissue damage, eosinophil accumulation, broncho-spasm, narrowing of breathing airways, airway hypersensitivity, airway remodeling, associated pulmonary or sinus inflammation leading to at least one of inspiratory or expiratory airway wheezing, breathlessness, chest tightness, coughing, dyspnea, burning, airway edema, excess mucus, bronchospasm, tachypnea, tachycardia, cyanosis, allergic rhinitis, infection, allergy, atopic dermatitis, bronchial abnormalities, Churg-Strauss syndrome, gastroesophageal reflux disease, hay fever, and allergies.

6. A method according to claim 1, wherein said asthma related Ig derived protein is selected from an antibody, an antibody fragment, an antibody protein fusion, a soluble receptor and a receptor fusion protein.

7. A method according to claim 1, wherein said asthma related Ig derived protein comprises at least one H-13 binding region.

8. A method according to claim 7, wherein said H-13 binding region comprises at least one complementarity determining region (CDR).

9. A method according to claim 1, wherein said asthma related Ig derived protein comprises at least a portion of at least one human heavy chain variable region or at least one light chain variable region.

10. A method according to claim 1, wherein said asthma related Ig derived protein is a substantially human Ig derived protein.

11. A method according to claim 1, wherein said asthma related Ig derived protein binds said asthma related protein with an affinity of at least one selected from at least  $10^{-10}$  M, at least  $10^{-11}$  M, at least  $10^{-12}$  M, or at least  $10^{-13}$  M.

12. A method according to claim 1, wherein said asthma related Ig derived protein substantially neutralizes at least one activity of at least one asthma related protein.

13. A method according to claim 1, wherein said asthma related therapeutic is selected from at least one compound or protein that acts on one or more of at least one of beta-2 agonists, anticholinergics, corticosteroids, glucocorticosteroids, anti-allergens, anti-inflammatories, bronchodilators, expectorants, allergy medications, cromolyn sodium, albuterol, Ventolin™, Proventil™, beclomethasone dipropionate inhaler, Vancort™, budesonide inhaler, Pulmicort Turbuhaler™, Pulmicort Respules™, fluticasone and salme-

terol oral inhaler, Advair™ Diskus, fluticasone propionate oral inhaler, Flovent™, hydrocortisone oral, Hydrocortone™, Cortel™, ipratropium bromide inhaler, Atrovent™, montelukast, Singulair™, prednisone, Deltasone™, Liquid Pred™, salmeterol, Serevent™, terbutaline, Brethine™, Bricanyl™, theophylline, Theo-Dur™, Resphal™, Slo-Bid™, Theo-24™, Theolair™, Uniphyll™, Slo-Phyllin™, triamcinolone acetate inhaler, Azmacort™, methylxanthate (MX), interleukin antagonists such as IL-4, IL-5, IL-12 antibodies, receptor proteins or antagonists, and antagonist fusion proteins, IgL antibodies and antagonists, CD4 antagonists, antileukotrienes, platelet activating factor, thromboxane antagonists, trypsin inhibitors, NK2 receptor antagonists, pranoprofen, theophylline, or disodium chromoglycate (DSCG).

14. A pharmaceutical composition, comprising a pharmaceutically effective amount of at least one asthma related Ig derived protein and a pharmaceutically acceptable carrier or diluent, wherein said asthma related Ig derived protein exhibits at least one biological activity of interleukin-13 (IL-13) *in vitro* or *in vivo* and specifically binds at least 1-3 amino acids of at least one selected from the group consisting of (a) 1-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-110, 110-120, 120-130, 130-140, 140-150, or 14-145 of SEQ ID NO:1.

15. A pharmaceutical composition according to claim 14, wherein said asthma related Ig derived protein is selected from an antibody, an antibody fragment, an antibody protein fusion, a soluble receptor and a receptor fusion protein.

16. A pharmaceutical composition according to claim 1, wherein said asthma related Ig derived protein comprises at least one H-13 binding region.

17. A pharmaceutical composition according to claim 16, wherein said H-13 binding region comprises at least one complementarity determining region (CDR).

18. A pharmaceutical composition according to claim 14, wherein said asthma related Ig derived protein comprises at least a portion of at least one human heavy chain variable region or at least one light chain variable region.

19. A pharmaceutical composition according to claim 14, wherein said asthma related Ig derived protein is a substantially human Ig derived protein.

20. A pharmaceutical composition according to claim 14, wherein said asthma related Ig derived protein binds said asthma related protein with an affinity of at least one selected from at least  $10^{-10}$  M, at least  $10^{-11}$  M, at least  $10^{-12}$  M, or at least  $10^{-13}$  M.

21. A pharmaceutical composition according to claim 14, wherein said asthma related Ig derived protein substantially neutralizes at least one activity of at least one asthma related protein.

\* \* \* \* \*

AGA

**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO**  
**DIRECCIÓN DE NUEVAS CREACIONES**

2020  
Bogotá, D.C.,

Abogado  
**CARLOS R. OLARTE**

|        |            |           |
|--------|------------|-----------|
| ASUNTO | Radicación | 06-129582 |
|        | Trámite    | 011       |
|        | Evento     | 378       |
|        | Actuación  | 519       |
|        | Oficio     |           |
|        | Folios     | 010       |

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|-------------------------|--------------------------|---------------------------------|----------------------------------|
| Patente de Invención    | <input type="checkbox"/> | Patente de Modelo de Utilidad   | <input type="checkbox"/>         |
| Vía Nacional            | <input type="checkbox"/> |                                 |                                  |
| Vía PCT (fase nacional) | <input type="checkbox"/> | Cap. I <input type="checkbox"/> | Cap. II <input type="checkbox"/> |

No. Solicitud Internacional PCT/GB2005/002581  
Fecha de Presentación Internacional: 30 Junio 2005

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 comunica:

**1. Documentos estudiados**

|                   |                  |                   |
|-------------------|------------------|-------------------|
| Descripción:      | Folios 215 a 359 | 27 Diciembre 2006 |
| Reivindicaciones: | Folios 382 a 393 | 27 Diciembre 2006 |
| Secuencias:       | Folios 360 a 381 | 27 Diciembre 2006 |
| Figuras:          | Folios 395 a 423 | 27 Diciembre 2006 |
| Prioridad:        | GB 0414799.7     | 01 Julio 2004     |
|                   | GB 0423675.8     | 25 Octubre 2004   |

**2. Objeto de la invención**

Título de la solicitud: "Anticuerpos monoclonales quiméricos y humanizados contra la interleuquina -13 "

El problema planteado en esta solicitud consiste en la necesidad de agentes terapéuticos específicos para el tratamiento del asma y enfermedades relacionadas como es la fibrosis quística.

Para resolver este problema técnico la solicitud en estudio proporciona anticuerpos monoclonales quiméricos y humanizados que se unen específicamente a la interleuquina 13 útiles para el tratamiento del asma.

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C07K16/00

### 3. Excepción a la Patentabilidad (Art 20 D 486 literal (d))

El objeto de las reivindicaciones 49 a 58 referentes aun procedimiento para el tratamiento de un paciente humano, no es patentable de acuerdo con el Art citado.

### 4. Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)

El uso reclamado en la reivindicación 59 no es patentable, de acuerdo con el Art 14.

### 5. De la Solicitud de Patente

#### Reivindicaciones (Art 30 D 486, Art 22 PCT)

- El capítulo reivindicatorio no es conciso porque contiene un excesivo número de reivindicaciones independientes (13), lo cual hace difícil definir cuál es el objeto que se busca proteger.
- El anticuerpo reclamado en las reivindicaciones 1 a 3, 5 a 8, 36, 37 y 61 a es caracterizado por su capacidad de unirse, modular, neutralizar hIL-13 ó el epítipo de SEQ ID NO.9 lo cual corresponde a la función o al resultado a alcanzar con dicho anticuerpo, puesto que la reivindicación incluiría no sólo la solución propuesta por el solicitante, sino todas las alternativas presentes o futuras que lleguen a ese resultado.  
Debido a que un anticuerpo puede definirse por su estructura es decir las secuencias variables de las cadenas ligera y pesada que lo conforman. Se sugiere al solicitante modificar dichas reivindicaciones y sustituir dicha función o resultado a alcanzar por las características esenciales, estructurales o funcionales, que contribuyen a la obtención de ese efecto.
- Las reivindicaciones independientes 1 a 3 y 33 a 35 que se refieren a un anticuerpo deben caracterizarlo a partir de su estructura completa, mencionando las secuencias que lo conforman en la cadena ligera y cadena pesada, o las secuencias variables de las cadenas ligera y pesada o las secuencias de los 6 CDR's que conforman ambas cadenas. La referencia a estructuras parciales o fragmentos del anticuerpo no son válidas puesto que no es una estructura activa, probada ni definida, asimismo la referencia de la actividad o función de esta.
- Aunque en el capítulo descriptivo se hace referencia a la importancia de que el anticuerpo se una a un residuo específico, el hecho de caracterizar el anticuerpo por una sola CDR como es la CDRH3 no define de manera adecuada la totalidad del anticuerpo, es así que las reivindicaciones que hacen referencia únicamente a esta CDR deben ser modificadas incluyendo las restantes 5CDR que conforman el anticuerpo funcional (reivindicaciones 3, 33, 34, 64)
- Las reivindicaciones 64 y 65 hacen referencia a la misma materia que la reivindicación 4, se sugiere hacer las aclaraciones pertinentes.
- La reivindicación 10 hace referencia a que el anticuerpo puede ser de rata, ratón, primate o humano, sin embargo según lo divulgado en el capítulo descriptivo solo se encuentran soportados los anticuerpos murinos (ratón) y humanizados.

- Las reivindicaciones 9, 12, 13, 33, 34 contienen los términos "anticuerpo intacto", "comprende una región constante humana", "región marco de la cadena pesada aceptora humana", "región marco de la cadena liviana aceptora humana" los cuales son imprecisos, por lo cual no permite distinguir la invención del estado de la técnica. Se sugiere sustituirlos por un término preciso o establecer las secuencias de las regiones a las que hace referencia.
- Cuando la reivindicación se refiere a que el anticuerpo comprende una región constante de isotipo IgG se debe especificar a que región se refiere. Se debe definir de manera inequívoca la región mediante la secuencia de aminoácidos presente. (Ver reivindicaciones 13, 14 y 24)
- La composición farmacéutica mencionada en la reivindicación 47 debe caracterizarse a partir de los elementos y proporciones de los mismos que la componen, de acuerdo con la información sustentada en la descripción.
- Las reivindicaciones 33 y 34 caracterizan el anticuerpo reclamado por una serie de sustituciones de residuos de aminoácidos realizadas sobre una CDR denominada CDRH3 la cual se une a un epítipo específico, sin embargo no es claro a que secuencia ni que posiciones son las que se sustituyen, por lo cual no se define de manera inequívoca el anticuerpo, puesto que cualquier secuencia puede presentar sustituciones en diferentes posiciones y esta puede no corresponder a lo reclamado.

#### Descripción (Art 28 D 486)

- Dado que esta solicitud se refiere a secuencias de nucleótidos o aminoácidos, deben presentarse las listas en forma separada de la descripción, bajo el título: "Listado de secuencias" y anexarlas en formato digital.

#### 6. Determinación del Estado de la Técnica

El Estado de la Técnica se determinó con base en la fecha de presentación de la solicitud de prioridad (01 julio 2004) y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

| Item | Documento      | Título                                                                                    | Fecha de Publicación |
|------|----------------|-------------------------------------------------------------------------------------------|----------------------|
| D1   | US6468528      | Materials and methods to inhibit Hodgkin and Reed Sternberg cell growth                   | 22 Octubre 2002      |
| D2   | US2003/0235555 | Asthma-related anti-IL-13 immunoglobulin derived proteins, compositions, methods and uses | 25 Diciembre 2003    |

**D1** (folios 456 a 457) divulga revela métodos para inhibir el crecimiento de células cancerígenas que comprenden la administración de un anticuerpo humanizado o humano que se liga específicamente a hIL-13 y modula la interacción entre hIL-13 y el receptor de hIL-13.

URL: [http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en\\_gb&FT=D&date=20021022&CC=US&NR=6468528B1&KC=B1](http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_gb&FT=D&date=20021022&CC=US&NR=6468528B1&KC=B1)

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D2 (folios 458 a 462) divulga métodos terapéuticos que implican el uso una proteína relacionada con el asma anti-IL-13 humana, incluyendo composiciones farmacéuticas  
URL:[http://worldwide.espacenet.com/publicationDetails/biblio?CC=US&NR=2003235555&KC=&FT=E&locale=en\\_EP](http://worldwide.espacenet.com/publicationDetails/biblio?CC=US&NR=2003235555&KC=&FT=E&locale=en_EP)

7. Examen de Patentabilidad (Art 14 D486)

Novedad (Art 16 D486)

La **reivindicación 1** consiste en un anticuerpo terapéutico o fragmento de unión al antígeno del mismo que se une específicamente a hIL-13 y neutraliza su actividad

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

| Características <b>esenciales</b>                                 | D1                                                         | D2                                                         |
|-------------------------------------------------------------------|------------------------------------------------------------|------------------------------------------------------------|
| anticuerpo terapéutico o fragmento de unión al antígeno del mismo | Anticuerpo o fragmento de unión                            | Anticuerpo o fragmento de unión                            |
| se une específicamente a hIL-13 y neutraliza su actividad         | Se une específicamente a hIL-13 y neutraliza su actividad. | Se une específicamente a hIL-13 y neutraliza su actividad. |

Frente a D1

Las características esenciales del anticuerpo reclamado en la reivindicación 1 habían sido divulgadas previamente en D1, el cual revela métodos para inhibir el crecimiento de células cancerígenas que comprenden la administración de un anticuerpo humanizado o humano que se liga específicamente a hIL-13 y modula la interacción entre hIL-13 y el receptor de hIL-13 de manera específica (folio 456, columna 2, líneas 37 a 43; columna 5, líneas 10 a 20; folio 457, reivindicación 2 y 3); asimismo, hace referencia a composiciones que contienen dicho anticuerpo (folio 457, columna 7, líneas 15 a 30).

En consecuencia, la reivindicación 1 no es nueva ó carece de novedad, según lo divulgado en el documento D1.

Las reivindicaciones dependientes 2 a 3, 5 a 11, 37, 47, 61 a 63 no mencionan alguna característica que haga nueva a la composición de la reivindicación 1 de acuerdo a lo divulgado en D1, por lo cual se considera que no son nuevas.

Frente a D2

Las características esenciales del anticuerpo de la reivindicación 1 habían sido divulgadas previamente en D1, el cual da a conocer anticuerpos anti-IL-13 que se unen específicamente a la proteína interleuquina 13 de SEQ ID NO. 1 (folio 458, párrafo 0022, folio 461, secuencia 1) útiles como agentes terapéuticos para el tratamiento del asma (folio 458, párrafo 0018), asimismo se revelan ácidos nucleícos, células huésped de tipo CHO y vectores relacionados con dichos anticuerpos (folio 458, párrafo 0018, 0028; folio 461, párrafo 0213). Dichos anticuerpos son derivados de IgG (folio 459, párrafo 0041; reivindicación 10).  
Por otro lado, los anticuerpos presentan con una constante de afinidad de menos de 10<sup>-9</sup> M. (folio 460, párrafo 0059, reivindicación 11).

D2 relaciona composiciones que contienen dicho anticuerpo y artículos o kits para su administración (folio 458, párrafo 0025-0026, folio 461, párrafo 0199 a 0206)

En consecuencia, la reivindicación 1 no es nueva, según lo divulgado en el documento D2.

Las reivindicaciones dependientes 2 a 3, 5 a 11, 13, 14, 35, 37, 38, 41 a 48, 61 a 63 no mencionan alguna característica que haga nueva a la composición de la reivindicación 1 de acuerdo a lo divulgado en D2, por lo cual se considera que no son nuevas

## 8. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

| Item | Documento      | Reivindicaciones afectadas                          | Requisito que afecta |
|------|----------------|-----------------------------------------------------|----------------------|
| D1   | US6468528      | 1 a 3, 5 a 11, 37, 47, 61 a 63                      | Novedad              |
| D2   | US2003/0235555 | 1 a 3, 5 a 11, 13, 14, 35, 37, 38, 41 a 48, 61 a 63 | Novedad              |

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 sesenta (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 No. 10 de 2001.

  
**JOSÉ LUIS SALAZAR LÓPEZ**  
Director de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha

Elaborado por: Ana Delia Pinzón García  
Revisado Por: Jacqueline Alfonso *JE*