(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 June 2016 (30.06.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/102641 A1

(51) International Patent Classification:
A61K 38/20 (2006.01) *A61P 11/00* (2006.01)

(21) International Application Number:
PCT/EP2015/081112

(22) International Filing Date:
23 December 2015 (23.12.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
14307183.5 24 December 2014 (24.12.2014) EP

(71) Applicants: **INSERM (INSTITUT NATIONAL DE LA SANTÉ ET DE LA RECHERCHE MÉDICALE)** [FR/FR]; 101, rue de Tolbiac, 75013 Paris (FR). **CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE (CNRS)** [FR/FR]; 3, Rue Michel Ange, 75016 Paris (FR). **INSTITUT PASTEUR DE LILLE** [FR/FR]; 1, rue du Professeur Calmette, 59000 Lille (FR). **UNIVERSITÉ DE LILLE 1 SCIENCES ET TECHNOLOGIES** [FR/FR]; Cité Scientifique, 59650 Villeneuve d'Ascq (FR). **UNIVERSITÉ DE DROIT ET DE LA SANTÉ DE LILLE 2** [FR/FR]; 42 rue Paul Duez, 59800 Lille (FR).

(72) Inventors: **GOSSET, Philippe**; Inserm U 1019 - Cnrs Umr 8204, Institut Pasteur de Lille, 1 rue du Professeur Calmette, 59019 Lille Cedex (FR). **PICHAVANT, Muriel**; Inserm U 1019 - Cnrs Umr 8204, Institut Pasteur de Lille, 1 rue du Professeur Calmette, 59019 Lille Cedex (FR). **KONE, Bachirou**; Inserm U 1019 - Cnrs Umr 8204, Institut Pasteur de Lille, 1 rue du Professeur Calmette, 59019 Lille Cedex (FR). **PEREZ-CRUZ, Magdiel**; Inserm U 1019 - Cnrs Umr 8204, Institut Pasteur de Lille, 1 rue du Professeur Calmette, 59019 Lille Cedex (FR).

(74) Agent: **COLLIN, Matthieu**; Inserm Transfert, 7 rue Watt, 75013 Paris (FR).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2016/102641 A1

(54) Title: METHODS AND PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF ACUTE EXACERBATIONS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

(57) Abstract: The present invention relates to methods and pharmaceutical compositions for the treatment of acute exacerbation of chronic obstructive pulmonary disease. In particular, the present invention relates to an agonist of IL-23 for use in a method for the treatment of acute exacerbation of chronic obstructive pulmonary disease in a subject in need thereof.

METHODS AND PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF ACUTE EXACERBATIONS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

FIELD OF THE INVENTION:

The present invention relates to methods and pharmaceutical compositions for the treatment of acute exacerbation of chronic obstructive pulmonary disease.

BACKGROUND OF THE INVENTION:

Chronic obstructive pulmonary disease (COPD) represents a severe and increasing global health problem. By 2020, COPD will have increased from 6th (as it is currently) to the 3rd most common cause of death worldwide. In the United States, COPD is believed to account for up to 120,000 deaths per year. The clinical course of COPD is characterized by chronic disability, with intermittent, acute exacerbations which may be triggered by a variety of stimuli including exposure to pathogens, inhaled irritants (e.g., cigarette smoke), allergens, or pollutants. "Acute exacerbation" refers to worsening of a subject's COPD symptoms from his or her usual state that is beyond normal day-to-day variations, and is acute in onset. Acute exacerbations of COPD greatly affect the health and quality of life of subjects with COPD. Acute exacerbation of COPD is a key driver of the associated substantial socioeconomic costs of the disease. Multiple studies have also shown that prior exacerbation is an independent risk factor for future hospitalization for COPD. In conclusion, exacerbations of COPD are of major importance in terms of their prolonged detrimental effect on subjects, the acceleration in disease progression and the high healthcare costs. However up to now there is no method for the treatment of acute exacerbation of COPD.

SUMMARY OF THE INVENTION:

The present invention relates to methods and pharmaceutical compositions for the treatment of acute exacerbation of chronic obstructive pulmonary disease. In particular, the present invention is defined by the claims.

DETAILED DESCRIPTION OF THE INVENTION:

Acute episodes of bacterial exacerbations mark the progression of chronic obstructive pulmonary disorder (COPD). These exacerbations often result in an increased inflammation of

the respiratory tract causing decline in lung function. *Streptococcus pneumoniae* (Sp) is one of the most commonly isolated bacteria during these episodes. Mechanisms responsible for the increased susceptibility to pathogens are unknown. The inventors' aim was to characterize the cytokine response to Sp by using a mouse model of COPD. Mice were chronically exposed to cigarette smoke for 12 weeks and subsequently challenged with a sub-lethal dose of Sp. Systemic and local inflammation, immune responses, and bacterial burden were evaluated at 1, 3 and 7 days post-infection. Air mice were able to clear the bacteria within 24 hour post-infection, whereas COPD mice developed strong lung infection. COPD mice show also a defect in immune cell recruitment and activation, and in IL-23 production in response to Sp. This was associated with a defect of IL-23 production in AM and DC. These data identified IL-23 as a susceptibility factor in COPD exacerbation.

Accordingly, the present invention relates to an agonist of IL-23 for use in a method for the treatment of acute exacerbation of chronic obstructive pulmonary disease in a subject in need thereof.

As used herein the term "acute exacerbation" has its general meaning in the art and refers to worsening of a subject's COPD symptoms from his or her usual state that is beyond normal day-to-day variations, and is acute in onset. Typically, the acute exacerbation of COPD is manifested by one or more symptoms selected from worsening dyspnea, increased sputum production, increased sputum purulence, change in color of sputum, increased coughing, upper airway symptoms including colds and sore throats, increased wheezing, chest tightness, reduced exercise tolerance, fatigue, fluid retention, and acute confusion, and said method comprises reducing the frequency, severity or duration of one or more of said symptoms. Acute exacerbation may have various etiologies, but typically may be caused by viral infections, bacterial infections, or air pollution. For example, approximately 50% of acute exacerbations are due primarily to the bacteria *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* (all of them causing pneumonia). Viral pathogens associated with acute exacerbations in subjects with COPD include rhinoviruses, influenza, parainfluenza, coronavirus, adenovirus, and respiratory syncytial virus.

In some embodiments, the acute exacerbation of COPD is caused by a bacterial infection. In some embodiments, the acute exacerbation of COPD is caused by a viral infection. In some embodiments, the acute exacerbation of COPD is caused by air pollution.

In some embodiments, the subject experienced an acute exacerbation of COPD or is at risk of experiencing an acute exacerbation of COPD. In some embodiments, the subject has experienced at least one acute exacerbation of COPD in the past 24 months. In one particular embodiment, the subject has experienced at least one acute exacerbation of COPD in the past 12 months. In some embodiments, subject is a frequent exacerbator. As used herein the term "frequent exacerbator" refers to a subject who suffers from or is undergoing treatment for COPD and who experiences at least 2, and more typically 3 or more, acute exacerbations during a 12 months period.

In some embodiments, "treating" refers to treating an acute exacerbation of COPD, reducing the frequency, duration or severity of an acute exacerbation of COPD, treating one or more symptoms of acute exacerbation of COPD, reducing the frequency, duration or severity of one or more symptoms of an acute exacerbation of COPD, preventing the incidence of acute exacerbation of COPD, or preventing the incidence of one or more symptoms of acute exacerbation of COPD, in a human. The reduction in frequency, duration or severity is relative to the frequency, duration or seventy of an acute exacerbation or symptom in the same human not undergoing treatment according to the methods of the present invention. A reduction in frequency, duration or severity of acute exacerbation or one or more symptoms of acute exacerbation may be measured by clinical observation by an ordinarily skilled clinician with experience of treating COPD subjects or by subjective self evaluations by the subject undergoing treatment. Clinical observations by an ordinarily skilled clinician may include objective measures of lung function, as well as the frequency with which intervention is required to maintain the subject in his or her most stable condition, and the frequency of hospital admission and length of hospital stay required to maintain the subject in his or her most stable condition. Typically, subjective self evaluations by a subject are collected using industry-recognized and/or FDA-recognized subject reported outcome (PRO) tools. Such tools may allow the subject to evaluate specific symptoms or other subjective measures of quality of life. An example of one subject reported outcome tool is Exacerbations from Pulmonary Disease Tool (EXACT-PRO), which is currently being developed for evaluating clinical response in acute bacterial exacerbations by United BioSource Corporation along with a consortium of pharmaceutical industry sponsors in consultation with the FDA.

In some embodiments, the treatment is a prophylactic treatment. As used herein, the term “prophylactic treatment" refers to any medical or public health procedure whose purpose is to prevent a disease. As used herein, the terms "prevent", "prevention" and "preventing" refer to the reduction in the risk of acquiring or developing a given condition, or the reduction or inhibition of the recurrence or said condition in a subject who is not ill, but who has been or may be near a subject with the disease.

The term "IL-23 interleukin” has its general meaning in the art and includes naturally occurring IL-23 and function conservative variants and modified forms thereof. Interleukin-23 (IL-23) is a heterodimeric cytokine composed of the p19 subunit and the p40 subunit of IL-12. The IL-23 polypeptide can be from any source, but typically is a mammalian (e.g., human and non-human primate) IL-23, and more particularly a human IL-23. An exemplary human amino acid sequence for interleukin 23, alpha subunit p19 is provided by SEQ ID NO:1 and an exemplary human amino acid sequence for interleukin 23 subunit p40 is provided by SEQ ID NO:2.

SEQ ID NO: 1: Interleukin 23, alpha subunit p19 [Homo sapiens] (GenBank: AAH67511.1): MLGSRAVMLL LLLPWTAQGR AVPGGSSPAW TQCQQLSQKL CTLAWSAHPL VGHMDLREEG DEETTNDVPH IQCGDGCDPQ GLRDNSQFCL QRIHQGLIFY EKLLGSDIFT GEPSLLPDSP VGQLHASLLG LSQLLQPEGH HWETQQIPSL SPSQPWQRLL LRFKILRSLQ AFVAVAARVF AHGAATLSP

SEQ ID NO: 2: Interleukin 23, subunit p40 [Homo sapiens] (GenBank: AAG32620.1): MCHQQLVISW FSLVFLASPL VAIWELKKDV YVVELDWYPD APGEMVVLTC DTPEEDGITW TLDQSSEVLG SGKTLTIQVK EFGDAGQYTC HKGGEVLSHS LLLLHKKEDG IWSTDILKDQ KEPKNKTFLR CEAKNYSGRF TCWWLTTIST DLTFSVKSSR GSSDPQGVTC GAATLSAERV RGDNKEYEYS VECQEDSACP AAEESLPIEV MVDAVHKLKY ENYTSSFFIR DIIKPDPPKN LQLKPLKNSR QVEVSWEYPD TWSTPHSYFS LTFCVQVQGK SKREKKDRVF TDKTSATVIC RKNASISVRA QDRYYSSSWS EWASVPCS

“Agonist of IL-23” and “IL-23 agonist” encompasses a compound (e.g. an agonistic antibody) that specifically binds to IL-23 receptor (IL-23R) and increases the signalling properties of IL-23R. Agonist of IL-23 typically encompasses an agonistic antibody that specifically binds to the complex of IL-23R and IL-12Rbeta1.

In some embodiments, the agonist of IL-23 is IL-23 itself. Accordingly the subject is thus administered with the complex between a polypeptide consisting of an amino acid sequence having at least 70% of identity with SEQ ID NO:1 and a polypeptide consisting of an amino acid sequence having at least 70% of identity with SEQ ID NO:2. According to the invention to the invention a first amino acid sequence having at least 70% of identity with a second amino acid sequence means that the first sequence has 70; 71; 72; 73; 74; 75; 76; 77; 78; 79; 80; 81; 82; 83; 84; 85; 86; 87; 88; 89; 90; 91; 92; 93; 94; 95; 96; 97; 98; or 99, or 100% of identity with the second amino acid sequence. According to the invention, the polypeptides of the invention may be produced by conventional automated peptide synthesis methods or by recombinant expression. General principles for designing and making proteins are well known to those of skill in the art. The polypeptides of the invention may be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols as described in Stewart and Young; Tam et al., 1983; Merrifield, 1986 and Barany and Merrifield, Gross and Meienhofer, 1979. The polypeptides of the invention may also be synthesized by solid-phase technology employing an exemplary peptide synthesizer such as a Model 433A from Applied Biosystems Inc. The purity of any given protein; generated through automated peptide synthesis or through recombinant methods may be determined using reverse phase HPLC analysis. Chemical authenticity of each peptide may be established by any method well known to those of skill in the art. As an alternative to automated peptide synthesis, recombinant DNA technology may be employed wherein a nucleotide sequence which encodes a protein of choice is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression as described herein below. Recombinant methods are especially preferred for producing longer polypeptides. A variety of expression vector/host systems may be utilized to contain and express the peptide or protein coding sequence. These include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeast transformed with yeast expression vectors (Giga-Hama et al., 1999); insect cell systems infected with virus expression vectors (e.g., baculovirus, see Ghosh et al., 2002); plant cell systems transfected with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with bacterial expression vectors (e.g., Ti or pBR322 plasmid; see e.g., Babe et al., 2000); or animal cell systems. Those of skill in the art are aware of various techniques for optimizing mammalian expression of proteins, see e.g., Kaufman, 2000; Colosimo et al., 2000. Mammalian cells that are useful in recombinant protein

productions include but are not limited to VERO cells, HeLa cells, Chinese hamster ovary (CHO) cell lines, COS cells (such as COS-7), W138, BHK, HepG2, 3T3, RIN, MDCK, A549, PC12, K562 and 293 cells. Exemplary protocols for the recombinant expression of the peptide substrates or fusion polypeptides in bacteria, yeast and other invertebrates are known to those of skill in the art and a briefly described herein below. Mammalian host systems for the expression of recombinant proteins also are well known to those of skill in the art. Host cell strains may be chosen for a particular ability to process the expressed protein or produce certain post-translation modifications that will be useful in providing protein activity. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be important for correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, 293, WI38, and the like have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the introduced, foreign protein. In the recombinant production of the polypeptides of the invention, it would be necessary to employ vectors comprising polynucleotide molecules for encoding the polypeptides of the invention. Methods of preparing such vectors as well as producing host cells transformed with such vectors are well known to those skilled in the art. The polynucleotide molecules used in such an endeavor may be joined to a vector, which generally includes a selectable marker and an origin of replication, for propagation in a host. These elements of the expression constructs are well known to those of skill in the art. Generally, the expression vectors include DNA encoding the given protein being operably linked to suitable transcriptional or translational regulatory sequences, such as those derived from a mammalian, microbial, viral, or insect genes. Examples of regulatory sequences include transcriptional promoters, operators, or enhancers, mRNA ribosomal binding sites, and appropriate sequences which control transcription and translation. The terms "expression vector," "expression construct" or "expression cassette" are used interchangeably throughout this specification and are meant to include any type of genetic construct containing a nucleic acid coding for a gene product in which part or all of the nucleic acid encoding sequence is capable of being transcribed. The choice of a suitable expression vector for expression of the peptides or polypeptides of the invention will of course depend upon the specific host cell to be used, and is within the skill of the ordinary artisan. Expression requires that appropriate signals be provided in the vectors, such as enhancers/promoters from both viral and mammalian sources that may be used to drive expression of the nucleic acids of interest in host cells. Usually, the nucleic acid being expressed

is under transcriptional control of a promoter. A "promoter" refers to a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a gene. Nucleotide sequences are operably linked when the regulatory sequence functionally relates to the DNA encoding the protein of interest (e.g., IL-17, IL-23, a variant and the like). Thus, a promoter nucleotide sequence is operably linked to a given DNA sequence if the promoter nucleotide sequence directs the transcription of the sequence.

In some embodiments, the agonist of IL-23 is an agonistic antibody. For instance, the antibody disclosed herein specifically binds a target antigen, such as human IL-23R or IL-12Rbeta.1. In some embodiments, the agonist of IL-23 is selected from the group consisting of anti-IL-23R antibodies, and anti-IL12Rbeta1, and antibodies which are directed to the complex of IL-23R and IL-12Rbeta1.

An antibody that “specifically binds” (used interchangeably herein) to a target or an epitope is a term well understood in the art, and methods to determine such specific binding are also well known in the art. A molecule is said to exhibit “specific binding” if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular target antigen than it does with alternative targets. An antibody “specifically binds” to a target antigen if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. It is also understood by reading this definition that, for example, an antibody that specifically binds to a first target antigen may or may not specifically or preferentially bind to a second target antigen. As such, “specific binding” or “preferential binding” does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding.

The term "antibody" is thus used to refer to any antibody-like molecule that has an antigen binding region, and this term includes antibody fragments that comprise an antigen binding domain such as Fab', Fab, F(ab')2, single domain antibodies (DABs), TandAbs dimer, Fv, scFv (single chain Fv), dsFv, ds-scFv, Fd, linear antibodies, minibodies, diabodies, bispecific antibody fragments, bibody, tribody (scFv-Fab fusions, bispecific or trispecific, respectively); sc-diabody; kappa(lamda) bodies (scFv-CL fusions); BiTE (Bispecific T-cell Engager, scFv-scFv tandems to attract T cells); DVD-Ig (dual variable domain antibody, bispecific format); SIP (small immunoprotein, a kind of minibody); SMIP ("small modular

immunopharmaceutical" scFv-Fc dimer; DART (ds-stabilized diabody "Dual Affinity ReTargeting"); small antibody mimetics comprising one or more CDRs and the like. The techniques for preparing and using various antibody-based constructs and fragments are well known in the art (see Kabat et al., 1991, specifically incorporated herein by reference). Diabodies, in particular, are further described in EP 404, 097 and WO 93/1 1 161; whereas linear antibodies are further described in Zapata et al. (1995). Antibodies can be fragmented using conventional techniques. For example, F(ab')2 fragments can be generated by treating the antibody with pepsin. The resulting F(ab')2 fragment can be treated to reduce disulfide bridges to produce Fab' fragments. Papain digestion can lead to the formation of Fab fragments. Fab, Fab' and F(ab')2, scFv, Fv, dsFv, Fd, dAbs, TandAbs, ds-scFv, dimers, minibodies, diabodies, bispecific antibody fragments and other fragments can also be synthesized by recombinant techniques or can be chemically synthesized. Techniques for producing antibody fragments are well known and described in the art. For example, each of Beckman et al., 2006; Holliger & Hudson, 2005; Le Gall et al., 2004; Reff & Heard, 2001 ; Reiter et al., 1996; and Young et al., 1995 further describe and enable the production of effective antibody fragments.

In natural antibodies, two heavy chains are linked to each other by disulfide bonds and each heavy chain is linked to a light chain by a disulfide bond. There are two types of light chain, lambda (l) and kappa (k). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE. Each chain contains distinct sequence domains. The light chain includes two domains, a variable domain (VL) and a constant domain (CL). The heavy chain includes four domains, a variable domain (VH) and three constant domains (CH1, CH2 and CH3, collectively referred to as CH). The variable regions of both light (VL) and heavy (VH) chains determine binding recognition and specificity to the antigen. The constant region domains of the light (CL) and heavy (CH) chains confer important biological properties such as antibody chain association, secretion, trans-placental mobility, complement binding, and binding to Fc receptors (FcR). The Fv fragment is the N-terminal part of the Fab fragment of an immunoglobulin and consists of the variable portions of one light chain and one heavy chain. The specificity of the antibody resides in the structural complementarity between the antibody combining site and the antigenic determinant. Antibody combining sites are made up of residues that are primarily from the hypervariable or complementarity determining regions (CDRs). Occasionally, residues from nonhypervariable or framework regions (FR) influence the overall domain structure and hence the combining site. Complementarity Determining Regions or CDRs refer to amino acid

sequences which together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site. The light and heavy chains of an immunoglobulin each have three CDRs, designated L-CDR1, L-CDR2, L-CDR3 and H-CDR1, H-CDR2, H-CDR3, respectively. An antigen-binding site, therefore, includes six CDRs, comprising the CDR set from each of a heavy and a light chain V region. Framework Regions (FRs) refer to amino acid sequences interposed between CDRs. In addition, determination of CDR regions in an antibody is well within the skill of the art. There are at least two techniques for determining CDRs: (1) an approach based on cross-species sequence variability (i.e., Kabat et al. Sequences of Proteins of Immunological Interest, (5th ed., 1991, National Institutes of Health, Bethesda Md.)); and (2) an approach based on crystallographic studies of antigen-antibody complexes (Chothia et al. (1989) Nature 342:877; Al-lazikani et al (1997) J. Molec. Biol. 273:927-948)). As used herein, a CDR may refer to CDRs defined by either approach or by a combination of both approaches.

The term "Fab" denotes an antibody fragment having a molecular weight of about 50,000 and antigen binding activity, in which about a half of the N-terminal side of H chain and the entire L chain, among fragments obtained by treating IgG with a protease, papain, are bound together through a disulfide bond.

The term "F(ab')2" refers to an antibody fragment having a molecular weight of about 100,000 and antigen binding activity, which is slightly larger than the Fab bound via a disulfide bond of the hinge region, among fragments obtained by treating IgG with a protease, pepsin.

The term "Fab' " refers to an antibody fragment having a molecular weight of about 50,000 and antigen binding activity, which is obtained by cutting a disulfide bond of the hinge region of the F(ab')2.

A single chain Fv ("scFv") polypeptide is a covalently linked VH::VL heterodimer which is usually expressed from a gene fusion including VH and VL encoding genes linked by a peptide-encoding linker. "dsFv" is a VH::VL heterodimer stabilised by a disulfide bond. Divalent and multivalent antibody fragments can form either spontaneously by association of monovalent scFvs, or can be generated by coupling monovalent scFvs by a peptide linker, such as divalent sc(Fv)2.

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites.

Monoclonal antibodies may be generated using the method of Kohler and Milstein (Nature, 256:495, 1975). To prepare monoclonal antibodies useful in the invention, a mouse or other appropriate host animal is immunized at suitable intervals (e.g., twice-weekly, weekly, twice-monthly or monthly) with the relevant antigenic forms (e.g. IL-23R). The animal may be administered a final "boost" of antigen within one week of sacrifice. It is often desirable to use an immunologic adjuvant during immunization. Suitable immunologic adjuvants include Freund's complete adjuvant, Freund's incomplete adjuvant, alum, Ribi adjuvant, Hunter's Titermax, saponin adjuvants such as QS21 or Quil A, or CpG-containing immunostimulatory oligonucleotides. Other suitable adjuvants are well-known in the field. The animals may be immunized by subcutaneous, intraperitoneal, intramuscular, intravenous, intranasal or other routes. A given animal may be immunized with multiple forms of the antigen by multiple routes.

Briefly, the recombinant antigen (e.g. IL-23R) may be provided by expression with recombinant cell lines. For instance receptors (e.g. IL-23R) may be provided in the form of human cells expressing the receptor at their surface. Recombinant forms of the cytokine or receptor may be provided using any previously described method. Following the immunization regimen, lymphocytes are isolated from the spleen, lymph node or other organ of the animal and fused with a suitable myeloma cell line using an agent such as polyethylene glycol to form a hydridoma. Following fusion, cells are placed in media permissive for growth of hybridomas but not the fusion partners using standard methods, as described (Coding, Monoclonal Antibodies: Principles and Practice: Production and Application of Monoclonal Antibodies in Cell Biology, Biochemistry and Immunology, 3rd edition, Academic Press, New York, 1996). Following culture of the hybridomas, cell supernatants are analyzed for the presence of antibodies of the desired specificity, i.e., that selectively bind the antigen. Suitable analytical techniques include ELISA, flow cytometry, immunoprecipitation, and western blotting. Other screening techniques are well-known in the field. Preferred techniques are those that confirm

binding of antibodies to conformationally intact, natively folded antigen, such as non-denaturing ELISA, flow cytometry, and immunoprecipitation.

Significantly, as is well-known in the art, only a small portion of an antibody molecule, the paratope, is involved in the binding of the antibody to its epitope (see, in general, Clark, W. R. (1986) *The Experimental Foundations of Modern Immunology* Wiley & Sons, Inc., New York; Roitt, I. (1991) *Essential Immunology,* 7th Ed., Blackwell Scientific Publications, Oxford). The Fc' and Fc regions, for example, are effectors of the complement cascade but are not involved in antigen binding. An antibody from which the pFc' region has been enzymatically cleaved, or which has been produced without the pFc' region, designated an F(ab')2 fragment, retains both of the antigen binding sites of an intact antibody. Similarly, an antibody from which the Fc region has been enzymatically cleaved, or which has been produced without the Fc region, designated an Fab fragment, retains one of the antigen binding sites of an intact antibody molecule. Proceeding further, Fab fragments consist of a covalently bound antibody light chain and a portion of the antibody heavy chain denoted Fd. The Fd fragments are the major determinant of antibody specificity (a single Fd fragment may be associated with up to ten different light chains without altering antibody specificity) and Fd fragments retain epitope-binding ability in isolation.

Within the antigen-binding portion of an antibody, as is well-known in the art, there are complementarity determining regions (CDRs), which directly interact with the epitope of the antigen, and framework regions (FRs), which maintain the tertiary structure of the paratope (see, in general, Clark, 1986; Roitt, 1991). In both the heavy chain Fd fragment and the light chain of IgG immunoglobulins, there are four framework regions (FR1 through FR4) separated respectively by three complementarity determining regions (CDR1 through CDRS). The CDRs, and in particular the CDRS regions, and more particularly the heavy chain CDRS, are largely responsible for antibody specificity.

It is now well-established in the art that the non CDR regions of a mammalian antibody may be replaced with similar regions of specific or heterospecific antibodies while retaining the epitopic specificity of the original antibody. This is most clearly manifested in the development and use of "humanized" antibodies in which non-human CDRs are covalently joined to human FR and/or Fc/pFc' regions to produce a functional antibody.

In some embodiments, the antibody is a humanized antibody. As used herein, "humanized" describes antibodies wherein some, most or all of the amino acids outside the CDR regions are replaced with corresponding amino acids derived from human immunoglobulin molecules. Methods of humanization include, but are not limited to, those described in U.S. Pat. Nos. 4,816,567, 5,225,539, 5,585,089, 5,693,761, 5,693,762 and 5,859,205, which are hereby incorporated by reference. The above U.S. Pat. Nos. 5,585,089 and 5,693,761, and WO 90/07861 also propose four possible criteria which may used in designing the humanized antibodies. The first proposal was that for an acceptor, use a framework from a particular human immunoglobulin that is unusually homologous to the donor immunoglobulin to be humanized, or use a consensus framework from many human antibodies. The second proposal was that if an amino acid in the framework of the human immunoglobulin is unusual and the donor amino acid at that position is typical for human sequences, then the donor amino acid rather than the acceptor may be selected. The third proposal was that in the positions immediately adjacent to the 3 CDRs in the humanized immunoglobulin chain, the donor amino acid rather than the acceptor amino acid may be selected. The fourth proposal was to use the donor amino acid reside at the framework positions at which the amino acid is predicted to have a side chain atom within 3A of the CDRs in a three dimensional model of the antibody and is predicted to be capable of interacting with the CDRs. The above methods are merely illustrative of some of the methods that one skilled in the art could employ to make humanized antibodies. One of ordinary skill in the art will be familiar with other methods for antibody humanization.

In some embodiments, some, most or all of the amino acids outside the CDR regions have been replaced with amino acids from human immunoglobulin molecules but where some, most or all amino acids within one or more CDR regions are unchanged. Small additions, deletions, insertions, substitutions or modifications of amino acids are permissible as long as they would not abrogate the ability of the antibody to bind a given antigen. Suitable human immunoglobulin molecules would include IgGl, IgG2, IgG3, IgG4, IgA and IgM molecules. A "humanized" antibody retains a similar antigenic specificity as the original antibody. However, using certain methods of humanization, the affinity and/or specificity of binding of the antibody may be increased using methods of "directed evolution", as described by Wu et al., /. *Mol. Biol.* 294:151, 1999, the contents of which are incorporated herein by reference.

Fully human monoclonal antibodies also can be prepared by immunizing mice transgenic for large portions of human immunoglobulin heavy and light chain loci. See, e.g., U.S. Pat. Nos. 5,591,669, 5,598,369, 5,545,806, 5,545,807, 6,150,584, and references cited therein, the contents of which are incorporated herein by reference. These animals have been genetically modified such that there is a functional deletion in the production of endogenous (e.g., murine) antibodies. The animals are further modified to contain all or a portion of the human germ-line immunoglobulin gene locus such that immunization of these animals will result in the production of fully human antibodies to the antigen of interest. Following immunization of these mice (e.g., XenoMouse (Abgenix), HuMAb mice (Medarex/GenPharm)), monoclonal antibodies can be prepared according to standard hybridoma technology. These monoclonal antibodies will have human immunoglobulin amino acid sequences and therefore will not provoke human anti-mouse antibody (KAMA) responses when administered to humans. In vitro methods also exist for producing human antibodies. These include phage display technology (U.S. Pat. Nos. 5,565,332 and 5,573,905) and in vitro stimulation of human B cells (U.S. Pat. Nos. 5,229,275 and 5,567,610). The contents of these patents are incorporated herein by reference.

Thus, as will be apparent to one of ordinary skill in the art, the present invention also provides for F(ab') 2 Fab, Fv and Fd fragments; chimeric antibodies in which the Fc and/or FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric F(ab')2 fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric Fab fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; and chimeric Fd fragment antibodies in which the FR and/or CDR1 and/or CDR2 regions have been replaced by homologous human or non-human sequences. The present invention also includes so-called single chain antibodies.

The various antibody molecules and fragments may derive from any of the commonly known immunoglobulin classes, including but not limited to IgA, secretory IgA, IgE, IgG and IgM. IgG subclasses are also well known to those in the art and include but are not limited to human IgGl, IgG2, IgG3 and IgG4.

The binding affinity of an antibody can be less than any of about 100 nM, about 50 nM, about 10 nM, about 1 nM, about 500 pM, about 100 pM, or about 50 pM to any of about 2 pM. Binding affinity can be expressed KD or dissociation constant, and an increased binding affinity corresponds to a decreased KD. One way of determining binding affinity of antibodies to is by measuring binding affinity of monofunctional Fab fragments of the antibody. To obtain monofunctional Fab fragments, an antibody (for example, IgG) can be cleaved with papain or expressed recombinantly. The affinity of a Fab fragment of an antibody can be determined by surface plasmon resonance (BIAcore3000™ surface plasmon resonance (SPR) system, BIAcore, INC, Piscaway N.J.). Kinetic association rates (kon) and dissociation rates (koff) (generally measured at 25° C.) are obtained; and equilibrium dissociation constant (KD) values are calculated as koff/kon.

Examples of anti-IL23R antibodies include, but are not limited to, those disclosed in US 7893215.

Another aspect of the invention relates to an agonist of IL-23 which is a nucleic acid molecule encoding for IL-23 for use in a method for the treatment of acute exacerbation of COPD in a subject in need thereof. Typically, said nucleic acid is a DNA or RNA molecule, which may be included in any suitable vector, such as a plasmid, cosmid, episome, artificial chromosome, phage or a viral vector as above described. So, a further object of the invention relates to a vector comprising a nucleic acid encoding for a polypeptide of the invention for use in a method for the treatment of acute exacerbation of COPD in a subject in need thereof.

By a "therapeutically effective amount" is meant a sufficient amount of the agonist of IL-23 for the treatment of acute exacerbation of COPD at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the age, body weight, general health, sex and diet of the subject; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific polypeptide employed; and like factors well known in the medical arts. For example, it is well known within the skill of the art to start doses of the compound at levels lower than those required to achieve the

desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. However, the daily dosage of the products may be varied over a wide range from 0.01 to 1,000 mg per adult per day. Preferably, the compositions contain 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100, 250 and 500 mg of the active ingredient for the symptomatic adjustment of the dosage to the subject to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the active ingredient, preferably from 1 mg to about 100 mg of the active ingredient. An effective amount of the drug is ordinarily supplied at a dosage level from 0.0002 mg/kg to about 20 mg/kg of body weight per day, especially from about 0.001 mg/kg to 7 mg/kg of body weight per day.

Typically the active ingredient of the present invention (i.e. IL-23 agonist) is combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form pharmaceutical compositions. The term "Pharmaceutically" or "pharmaceutically acceptable" refers to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetables oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin. In the pharmaceutical compositions of the present invention, the active ingredients of the invention can be administered in a unit administration form, as a mixture with conventional pharmaceutical supports. Suitable unit administration forms comprise oral-route forms such as tablets, gel capsules, powders, granules and oral suspensions or solutions, sublingual and buccal administration forms, aerosols, implants, subcutaneous, transdermal, topical, intraperitoneal, intramuscular, intravenous, subdermal, transdermal, intrathecal and intranasal administration forms and rectal administration forms.

In some embodiment, the agonist of IL-23 according to the invention is administered to the subject in combination with an anti-bacterial agent, such as antibiotics or antiviral agents. Suitable antibiotics that could be coadministered in combination with the polypeptide include, but are not limited to, at least one antibiotic selected from the group consisting of: ceftriaxone, cefotaxime, vancomycin, meropenem, cefepime, ceftazidime, cefuroxime, nafcillin, oxacillin, ampicillin, ticarcillin, ticarcillin/clavulinic acid (Timentin), ampicillin/sulbactam (Unasyn), azithromycin, trimethoprim-sulfamethoxazole, clindamycin, ciprofloxacin, levofloxacin, synercid, amoxicillin, amoxicillin/clavulinic acid (Augmentin), cefuroxime,trimethoprim/sulfamethoxazole, azithromycin, clindamycin, dicloxacillin, ciprofloxacin, levofloxacin, cefixime, cefpodoxime, loracarbef, cefadroxil, cefabutin, cefdinir, and cephradine. Example of antiviral agents include but are not limited to acyclovir, famciclovir, valaciclovir, ganciclovir, cidofovir; amantadine, rimantadine; ribavirin; zanamavir and/or oseltamavir; a protease inhibitor, such as indinavir, nelfinavir, ritonavir and/or saquinavir; a nucleoside reverse transcriptase inhibitor, such as didanosine, lamivudine, stavudine, zalcitabine, zidovudine; a non-nucleoside reverse transcriptase inhibitor, such as nevirapine, efavirenz.

Combination treatment may also include respiratory stimulants. Corticosteroids may be beneficial in acute exacerbations of COPD. Examples of corticosteroids that can be used in combination with the polypeptide (or the nucleic acid encoding thereof) are prednisolone, methylprednisolone, dexamethasone, naflocort, deflazacort, halopredone acetate, budesonide, beclomethasone dipropionate, hydrocortisone, triamcinolone acetonide, fluocinolone acetonide, fluocinonide, clocortolone pivalate, methylprednisolone aceponate, dexamethasone palmitoate, tipredane, hydrocortisone aceponate, prednicarbate, alclometasone dipropionate, halometasone, methylprednisolone suleptanate, mometasone furoate, rimexolone, prednisolone farnesylate, ciclesonide, deprodone propionate, fluticasone propionate, halobetasol propionate, loteprednol etabonate, betamethasone butyrate propionate, flunisolide, prednisone, dexamethasone sodium phosphate, triamcinolone, betamethasone 17-valerate, betamethasone, betamethasone dipropionate, hydrocortisone acetate, hydrocortisone sodium succinate, prednisolone sodium phosphate and hydrocortisone probutate. Particularly preferred corticosteroids under the present invention are: dexamethasone, budesonide, beclomethasone, triamcinolone, mometasone, ciclesonide, fluticasone, flunisolide, dexamethasone sodium phosphate and esters thereof as well as 6α,9α -difluoro-17α-[(2-furanylcarbonyl)oxy]-11β-

hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carbothioic acid (S)-fluoromethyl ester. Still more preferred corticosteroids under the present invention are: budesonide, beclomethasone dipropionate, mometasone furoate, ciclesonide, triamcinolone, triamcinolone acetonide, triamcinolone hexaacetonide and fluticasone propionate optionally in the form of their racemates, their enantiomers, their diastereomers and mixtures thereof, and optionally their pharmacologically-compatible acid addition salts. Even more preferred are budesonide, beclomethasone dipropionate, mometasone furoate, ciclesonide and fluticasone propionate. The most preferred corticosteroids of the present invention are budesonide and beclomethasone dipropionate.

Bronchodilator dosages may be increased during acute exacerbations to decrease acute bronchospasm. Examples of bronchodilators include but are not limited to β2-agonists (e.g. salbutamol, bitolterol mesylate, formoterol, isoproterenol, levalbuterol, metaproterenol, salmeterol, terbutaline, and fenoterol), anticholinergic (e.g. tiotropium or ipratropium), methylxanthined, and phosphodiesterase inhibitors.

In some embodiments, the agonist of IL-23 of the invention is administered to the subject in combination with a vaccine which contains an antigen or antigenic composition capable of eliciting an immune response against a virus or a bacterium. Typically, the vaccine composition is used to eliciting an immune response against at least one bacterium selected from the group consisting of *Streptococcus pneumoniae, Staphylococcus aureus, Burkholderis ssp., Streptococcus agalactiae, Haemophilus influenzae, Haemophilus parainfluenzae, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Moraxella catarrhalis, Chlamydophila pneumoniae, Mycoplasma pneumoniae, Legionella pneumophila, Serratia marcescens, Mycobacterium tuberculosis, Bordetella pertussis*. In particular, the vaccine composition is directed against *Streptococcus pneumonia* or *Haemophilus influenza.* More particularly, the vaccine composition is directed against Non-typeable Haemophilus influenzae (NTHi). Typically, vaccine composition typically contains whole killed or inactivated (eg., attenuated) bacteria isolate(s). However, soluble or particulate antigen comprising or consisting of outer cell membrane and/or surface antigens can be suitable as well, or instead of, whole killed organisms. In one or more embodiments, the outer cellular membrane fraction or membrane protein(s) of the selected isolate(s) is used. For instance, NTHi OMP P6 is a highly conserved 16-kDa lipoprotein (Nelson, 1988) which is a target of human bactericidal antibody and induces protection both in humans and in animal models. In chronic pulmonary obstructive

disease (COPD), OMP P6 has been shown to evoke a lymphocyte proliferative response that is associated with relative protection from NTHi infection (Abe, 2002). Accordingly, OMP P6 or any other suitable outer membrane NTHi proteins, polypeptides (eg., P2, P4 and P26) or antigenic fragments of such proteins or polypeptides can find application for a NTHi vaccine. Soluble and/or particulate antigen can be prepared by disrupting killed or viable selected isolate(s). A fraction for use in the vaccine can then be prepared by centrifugation, filtration and/or other appropriate techniques known in the art. Any method which achieves the required level of cellular disruption can be employed including sonication or dissolution utilizing appropriate surfactants and agitation, and combination of such techniques. When sonication is employed, the isolate can be subjected to a number of sonication steps in order to obtain the required degree of cellular disruption or generation of soluble and/or particulate matter of a specific size or size range. In some embodiments, the vaccine composition comprises an adjuvant, in a particular TLR agonist. In one embodiment, the TLR agonist is selected from the group consisting of TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, or TLR13 agonists.

In certain embodiments, oxygen requirements may increase and supplemental oxygen may be provided.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURES:

Figure 1: COPD patients have a defective response to *S. pneumoniae*. Levels of IL-1β, IL-6 and IL-23 was quantified by ELISA in supernatants from mononuclear cells from healthy non-smoker subjects (control), healthy smokers and COPD patients. Results were expressed as mean ± SEM.

Figure 2: COPD mice infected by *S. pneumoniae* exhibited a defective expression of mRNA encoding for cytokines in pulmonary APC. Mice were chronically exposed to cigarette smoke over a period of 12 weeks and then intranasally challenged with 5 $x10^4$ CFU

of *Streptococcus pneumoniae* (Sp- black bars) or not (Mock- white bars). Lung tissues were collected 1 day post-infection to sort pulmonary APC sub-populations: $CD45^+$ Siglec F^+ alveolar macrophages (A) $CD45^+$ Siglec F^- Ia^+ $CD64^+$ DC (B), and $CD45^+$ Siglec F^- Ia^+ $CD64^-$ inflammatory monocytes (C). mRNA levels of IL-1β, IL-6, IL-12p40 and IL-23 were evaluated compared to GAPDH. One representative experiment out of three independent ones is represented.

Figure 3: COPD mice exhibited a defective response of pulmonary APC to *S. pneumoniae*. Mice were chronically exposed to CS over a period of 12 weeks and then intranasally challenged with $5x10^4$ CFU of *S. pneumoniae* (Sp) or not (Mock). Lung tissues were collected 24h post-infection. Expression of CD86 (MFI) was evaluated on alveolar macrophages (AM) and DC (A). $CD45^+$ Siglec F^+ AM and $CD11c^+$ Ia^+ $CD64^+$ DC were sorted by flow cytometry 24 hours post-infection. IL-1beta and IL-23 levels were evaluated by ELISA in supernatants (B). Cocultures were performed between sorted AM or DC and splenic $CD4^+$ T cells purified from *S. pneumoniae* -infected Air mice. Supernatants were collected 48 hours later and levels of IL-17 were evaluated by ELISA (C). Results are expressed as mean ± SEM. One representative experiment out of three was shown.

Figure 4: COPD mice exposed to *S. pneumoniae* exhibited a defective expression of IL-1β and IL-23 in the lung. Mice were chronically exposed to CS over a period of 12 weeks and then intranasally challenged with $5 x10^4$ CFU of *Streptococcus pneumoniae* (Sp) or not (Mock). IL-1β and IL-23 mRNA levels were analyzed in lung tissues at day 1 post-infection. Results were expressed as mean ± SEM. *: $p<0.05$ vs controls.

EXAMPLE 1:

Material & methods

Patients with COPD

Peripheral blood and induced or spontaneous sputum were collected in stable COPD patients (n = 10), in smokers (without COPD, n=13)) and in non smoker healthy controls (n =14) (CPP 2008-A00690-55) in order to evaluate *ex vivo* the Th17 response to infection with *S. pneumoniae*. Peripheral blood mononuclear cells (PBMC) were purified on Ficoll Paque

gradient and $3x10^6$ cells/ml in complete RPMI1640 were exposed to *S. pneumoniae* (MOI=2) or to a positive control, phytohemagglutinin (1 µg/ml) (PHA, Difco). After 90 min, antibiotics were added to stop bacteria growth and supernatants were collected after 24h incubation. In parallel, another batch of cells was incubated with brefeldin A (10 µg/ml, Sigma) for 4h before collection and was used for intracellular staining of cytokines in lymphocytes.

Mice

Six- to eight-week-old male wild-type (WT) C57BL/6 (H-$2D^b$) mice were purchased from Janvier (Le Genest-St-Isle, France). For *S. pneumoniae* infection, mice were maintained in a biosafety level 2 facility. All animal work conformed to the guidelines of Animal Care and Use Committee from Nord Pas-De-Calais (agreement no. AF 16/20090).

Reagents and Abs

mAbs against mouse CD3 (APC-conjugated), CD5 (FITC-conjugated), NK1.1 (PerCp-Cy5.5–conjugated), TCR-β (V450-conjugated), CD25 (APC-conjugated), CD69 (Alexa700-conjugated), CD11b (V450–conjugated), Ly-6G (APC-Cy7-conjugated), CD8 (V500-conjugated), CD4 (APC-conjugated), CD103 (PE-conjugated), CD11c (APC-conjugated), CD45 (Q-dot605-conjugated), F4/80 (PerCP-Cy5.5-conjugated), Siglec F (PE-conjugated), CD64 (APC-conjugated), CD86 (PE-conjugated), CD40 (PE-conjugated), I-Ab (FITC-conjugated), IFN-gamma (PE-conjugated), IL-17 (APC-conjugated), CD11c (PE-Cy7-conjugated), F4/80 (PerCP-Cy5.5-conjugated), CD11b (V450-conjugated) and CD103 (PE-conjugated) and isotype controls were purchased from Biolegend (Le Pont de Claix, France). mAbs against human CD were also used including anti-CD11c, CD14, CD19, CD20 (PE-CF594-conjugated), CD117, TCRγdelta (V450-conjugated), CD4, CD3 (Alexa-700 conjugated), CD8, CD127 (V500 conjugated), CD196-, CD3 (BV605 conjugated), CD25, CD86 (APC-conjugated), CD56, Vα7.2 (PerCP-Cy5.5 conjugated), TCR Vα24Jα18, CD161 (PE-Cy7 conjugated) and CD45 (APC-H7 conjugated) (BD Biosciences, Biolegend and Myltenyi Biotech) as well as the Alexa488 anti-IFN-γ, Alexa647 anti-IL-17 (BD Biosciences) and PE anti-IL-22 antibodies (e-Biosciences) and the isotype controls. 3R4F research cigarettes were purchased from University of Kentucky (USA) and used to induce COPD like symptoms (13).

***Streptoccus pneumoniae* and bacterial counts**

Mice were inoculated by the intranasal route with a clinical isolate of *S. pneumoniae* serotype 1 described elsewhere (14-16). Mice were anesthetized and administered intranasally with 5 $\times 10^4$ Colony-forming units (CFU) in 50 µl. Mice were monitored daily for illness and mortality up to 7 days. Bacterial burden in the lungs, bronchoalveolar lavages (BAL) and blood was measured by plating lung homogenates, BAL or blood samples onto chocolate plates. CFU were enumerated 24 hours later. In some experiments, COPD and Air mice received rmIL-22 (1µg/ mouse) by intranasal route 24h before Sp challenge.

Assessment of airway inflammation and remodeling

Mice were sacrificed for sampling the lung lumen by BAL. Total cell numbers per BAL was determined. A morphology-based differential cell count was conducted on cytospin preparations, after staining with Diff-Quick solution (Sigma). For histopathology, lungs were fixed by inflation and immersion in PFA and embedded in parafin. To evaluate airway inflammation, lung slices (4-µm sections) were done for H&E staining.

Pulmonary cells from air or COPD mice were prepared as previously described (19) and were analyzed by flow cytometry. To analyze cytokine profiles, pulmonary cell suspensions were incubated with phorbol 12-myristate 13-acetate (PMA; 20 ng/ml) and ionomycin (500 ng/ml) for 3 h. Cells were stained with appropriate extracellular markers, and then fixed, permeabilized (BD Cytofix/cytoperm, BD Bioscience), and incubated with PE-conjugated mAb against IL-22 (eBiosciences) and AF647-conjugated mAb against IL-17 (Biolegend), or control rat IgG1 mAb in permeabilization buffer. Cells were acquired and analyzed on a Fortessa (Becton Dickinson) cytometer, and using the FlowJo software respectively.

Cytokine production was analyzed in total lung cells. For this, 5×10^5 lung cells were seeded on 96-well plates coated anti-CD3 Abs (eBiosciences). Forty-eight hours later, supernatants were collected and analyzed for IFN-gamma, IL-17, and IL-22 concentration by ELISA (R&D Systems).

Cell sorting and cocultures

Pulmonary cells from air or COPD mice were prepared as previously described (19) and were analyzed by flow cytometry on BD Aria machine. $CD45^+$ Siglec F^+ alveolar macrophages, $CD45^+$ Siglec F^- Ia^+ $CD64^+$ DC and $CD45^+$ Siglec F^- Ia^+ $CD64^-$ inflammatory monocytes were sorted (purity > 98%). Splenic $CD4^+$ T cells were purified from Sp-infected Air mice using magnetic microbeads (Myltenyi Biotech).

Sorted AM and DC were cultured with CD4+ T cells in RPMI 10% FCS, with the ratio 1/10. Supernatants were collected 48 hours later to evaluate IL-17 and IL-22 levels.

Statistical analysis

All the experiments are repeated at least 3 times with 5 mice per group. Results are expressed as the mean ± SEM. The statistical significance of differences between experimental groups was calculated by a one-way Anova with a Bonferroni post test or an unpaired Student t test (GraphPad, San Diego, CA). The possibility to use these parametric tests was assessed by checking if the population is Gaussian and the variance is equal (Bartlett's test). Results with a p value <0.05 were considered significant.

Results

Production of Th17 cytokines in response to *S. pneumoniae* is altered in peripheral blood mononuclear cells from COPD patients

In order to evaluate the production of Th17 cytokines in response to infection in COPD patients, their secretion was measured in the supernatants of PBMC exposed to *Streptococcus pneumoniae* (serotype 1) (Sp) and PHA as a positive control. The concentrations of cytokines in resting cells were not significantly different among the three groups. Whereas both stimuli significantly increased the levels of IL-17, IL-22 and IFN-γ in non-smokers and smokers, the exposure to Sp did not significantly amplify their secretion in COPD patients. The response to PHA was also partially altered in COPD patients, mainly for IL-17 and IL-22. In order to identify the nature of the defect in COPD patients, we analyzed the intracellular cytokines in cell populations involved in the production of cytokines in response to bacteria such as conventional T cells, NK, NKT, γdeltaT, mucosal- associated invariant T (MAIT) and Lineage$^-$ (Lin$^-$) cells. Exposure to Sp for 24h in non-smokers increased the percentage of IL-17$^+$ cells mainly in Lin$^-$, NK and NKT cells whereas that of IL-22$^+$ cells was amplified in NK and NKT cells. The activation with Sp significantly increased the percentage of IFN-γ$^+$ in innate lymphocytes (mainly NK, NKT, Lin$^-$ and MAIT cells (not shown)) from non-smokers. In COPD patients, the production of the three cytokines was altered in NK, NKT and Lin$^-$ cells. Concerning smokers, the IL-17 production induced by Sp was also altered in these three cell types whereas IL-22 expression was only reduced in NK cells. No modification of the percentage of cytokine$^+$ cells was detected among the three groups of patients for γdeltaT, MAIT and CD4/CD8$^+$ T cells (data not shown).

In parallel with the evaluation of Th17 cytokines, we analyzed the secretion of immuno-modulatory cytokines implicated in the control of the Th17 response such as IL-1β, IL-6 and IL-23. In PBMC from controls (non-smoker), stimulation by Sp significantly increased the secretion of both IL-1β, IL-6 and IL-23 as well as the activation by lipopolysaccharide LPS, the positive control (Figure 1). In contrast, infection by Sp did not enhance the secretion of the 3 cytokines in PBMC from COPD patients whereas the reponse of LPS was only altered for IL-1β. The response to Sp in smokers was only decreased for IL-23 as compared to controls.

These data showed that the Sp-induced production of Th17 cytokines in non-conventional lymphocytes was altered in PBMC from COPD patients, whereas current smokers revealed an intermediate situation. This might be related to an altered production of pro-Th17 cytokines such as IL-1β and IL-23 potentially produced by antigen-presenting cells.

Intranasal challenge with *S. pneumoniae* exacerbates lung inflammation in COPD mice

We first aimed to establish a mouse model of COPD exacerbation using Sp as the trigger. Whereas Air mice survived after being challenged with $5x10^5$ CFU, all COPD mice died within a week. Using a sub-lethal dose of 5×10^4 CFU/mouse, COPD and Air mice survived, as observed during COPD patients undergoing exacerbation. Inflammation due to Sp challenge was increased in COPD mice compared to Air mice, and was mainly characterized by the recruitment of neutrophils in the BAL and the lungs. This inflammatory infiltrate was present both in peribronchial areas and alveolar spaces. Moreover, a large thickening of the alveolar walls was only observed at day 3 post-infection (pi) in COPD mice, whereas the inflammation was nearly resolved in infected Air mice. Air mice cleared the bacteria within 24h, whereas in COPD mice, pulmonary bacterial load increased until day 3 pi and then declined at day 7 pi. Of interest was the observation that Sp dissiminated in the blood of COPD mice at 3 and 7 days pi. This was not related to a decreased expression for mRNA encoding anti-microbial peptides (β-defensins 2 and 3, LL37 and S100A9).

These data suggest that COPD mice are more susceptible to Sp, exhibiting a greater inflammation and a delayed clearance of Sp, compared to Air mice.

Alteration of the cytokine response in COPD mice challenged with *S. pneumoniae*

We next investigated the cytokine response in BAL fluids, lungs and sera in infected mice. Challenge with a sub-lethal dose of Sp induced higher levels of IL-22, IL-17 and IFN-γ in the BAL and lung lysates of Air mice whereas no increase in these cytokines was observed

in COPD mice. The same profile for IL-22 was found in the serum, whereas IFN-γ and IL-17 were undetectable in all mice. In response to CD3 Abs stimulation, pulmonary cells from infected Air mice produced more IL-22, IL-17 and IFN-γ as compared with mock animals. Whilst challenge with SP in COPD mice increased the levels of IL-17 and IFN-γ produced by lung cells upon CD3 stimulation, this was not the case for IL-22.

In order to elucidate the mechanism responsible for the defect of cytokine production, we analyzed the recruitment and the activation of lymphocytes in the lung. Infection with Sp enhanced the number of NK and NKT cells within lung tissues of Air mice and their activation as attested by the increased expression of CD69. However, Sp challenge in COPD mice failed to induce a higher recruitment and activation of NKT cells. The recruitment of $CD4^+$ and $CD8^+$ T cells due to Sp exposure was majored in COPD mice, although the infection in COPD mice did not enhanced the expression of CD69, in contrast to Air mice. Other types of lymphocytes, such as gamma/delta T cells, were not affected in infected COPD and Air mice.

We next investigated the cellular sources of IL-17 and IL-22 in the lung. In infected Air mice, the percentages of IL-17^+ NK, NKT and Lin- cells, as well as conventional T cells were significantly increased after challenge with Sp. In infected COPD mice, percentages of IL-17^+ NK and NKT cells were strongly reduced whereas IL-17^+ T cells and Lin^- cells were not affected. Percentages of IL-22^+ NK, NKT and Lin^- cells were also significantly diminished in infected COPD vs Air mice ($p<0.05$). IL-22^+ T cells were also decreased in COPD mice after Sp challenge compared to Air mice (from 10 down to 0.5%, data not shown).

These data showed that Sp-induced production of Th-17 cytokines (mainly IL-22) is defective in COPD mice, through an altered activation of innate lymphocytes.

CS exposure alters the function of pulmonary APC

Chronic exposure to CS alters pulmonary APC phenotype and functions (13) which might result in a defective lymphocyte response to bacteria. We first analyzed the phenotype of APC in the lung of infected COPD and Air mice. Infection of COPD mice with a sublethal dose of Sp triggered maturation of pulmonary APC, as depicted by increased expression of CD86 and Ia MHC molecule on AM and DC in contrast to Air mice. In order to analyze their functionality, we sorted APC populations: $CD45^+$ Siglec F^+ alveolar macrophages, $CD45^+$ Siglec F^- Ia^+ $CD11c^+$ $CD64^-$ DC and $CD45^+$ Siglec F^- Ia^+ $CD64^+$ inflammatory monocytes. In response to Sp challenge, lung AM and DC from Air mice expressed higher mRNA levels of 1L-1β, IL-6 and IL-23, but not IL-12p40 (Figure 2). A defect in IL-1β expression was detected in AM of COPD mice whereas the expression of IL-23 mRNA was undetectable in lung DC.

At the protein level, IL-1β and IL-23 secretion was increased in AM and DC from infected Air mice, but not in cells from COPD mice (Figure 3). This was confirmed by the mRNA expression analysis which revealed lower mRNA levels of IL-1β and IL-23 in lung tissues of infected COPD mice as compared with infected air mice (Figure 4).

To evaluate the capacity of these sorted cells to promote the immune response, lung DC and AM were cultured with isolated splenic $CD4^+$ T cells from Sp-infected Air mice. DC sorted from Sp-infected Air mice triggered higher IL-17 production by $CD4^+$ T cells, whereas those from COPD mice failed to do so (Figure 3). IL-22 was unfortunately undetectable in all tested conditions (data not shown).

These data suggested that the defect in the Th17 response to Sp is probably linked to alteration of pulmonary DC function.

Supplementation with recombinant IL-22 partially restores a competent immune response in COPD mice to Sp.

In order to determine the implication of Th17 cytokines in the enhanced bacterial susceptibility of COPD mice, we next investigated the effect of recombinant murine IL-22 (rmIL-22) in our model. Since IL-17 was involved in COPD physiopathology, we focused on the role of IL-22 cytokine. Given intranasally before Sp infection, rmIL-22 strongly improved the clearance of the bacteria in COPD mice since CFU counts were decreased in the BAL, the lungs and the blood. In the lung, the treatment with rmIL-22 strongly reduced the inflammatory infiltrate and the thickening of the alveolar walls which was nearly undetectable, in infected COPD mice. Recombinant mIL-22 increased mRNA levels of anti-microbial peptides such as Defb2 and Defb3 in a time-dependant manner. These effects on the inflammatory cells were associated with an increased production of IL-17 and IFN-gamma by stimulated pulmonary cells with anti-CD3 Abs. Supplementation with rmIL-22 was associated to an increased recruitment of NK cells and NKT cells, and an increased expression of CD69 on NKT cells at day 1 post infection. Interestingly, rmIL-22 supplementation had no effect on neutrophil recruitment. In contrast, treatment with rmIL-22 enhanced the recruitment of AM and DC in infected COPD mice whereas the CD86 expression was only amplified in DC.

Taken together, these data suggest that IL-22 could be a key cytokine involved in the control of COPD exacerbation in mice.

Discussion

COPD is a major public health problem and will be one of the leading global causes of mortality over the coming decades. Much of the morbidity, mortality and health care costs of COPD are attributable to acute exacerbations, the commonest causes of which are bacterial respiratory infections including Sp. In this study, we develop an experimental model that accurately reflects disease physiopathology in order to promote the development of new therapies. This study identified IL-22 defect as a key factor in COPD exacerbation in mice and humans.

CS and subsequent bacterial colonization with gram-positive bacteria *Streptococcus pneumoniae* are the main factors responsible for COPD exacerbations (17, 18, 19). Innate immunity associated with recruitment of alveolar macrophages and neutrophils is crucial in the early phase of natural anti-pneumococcal host defense and particularly in the clearance of bacteria (20). In our mouse model of COPD characterized by a lung function decline and a mild inflammation, intranasal application of Sp resulted in increased lung inflammation and tissue remodeling, as observed previously with SEB and *Haemophilus influenzae* (21). The inflammatory cell recruitment associated to infection was characterized by a strong increase of neutrophil recruitment in COPD mice. Moreover, we observed some important modification in the activation of innate immune cells from COPD mice, mainly targeting innate lymphocytes and APC. Although NK, NKT and γdeltaT cells are activated in uninfected COPD mice, as shown by upregulation of CD69 expression, in the context of infection, no increase of this expression was exhibited in NK and NKT cells from COPD mice. In addition, the level of IL-17 and IL-22 was strongly reduced within these cells as well as in lin- lymphocytes (potentially including ILC). According to our protocol, the cell sources for IL-17 and IL-22 are mostly related to non-conventional lymphocytes. In naive mice, the major sources of IL-22 include cells of the innate cells (NK, NKT and ILC) and conventional T cells (24-27). The role of these cells in the protection against Sp infection has been previously confirmed in naive mice (11, 14, 15, 20, 27). The profile of inhibition was similar in PBMC from COPD patients reinforcing the pertinence of this observation. In contrast, the activation of conventional T cells as well as γdeltaT cells was not clearly modified during COPD.

Similarly, the Sp-dependent stimulation of APC is also deficient in COPD mice, particularly in AM and DC (13). This is related with a decreased expression in the lungs of infected COPD mice of IL-1β and IL-23, both cytokines being essential for the secretion of Th17 cytokines (22, 23). This could, at least partially, explain the failure in the education of

innate lymphoid cells by CS-exposed DC. Indeed, in murine models of lung infection with Sp, IL-23 plays a key role in the clearance of the bacteria and the production of Th17 cytokines by ILC (19). IL-23 is also needed for IL-17 expression by others immune cells like NKT and gamma/delta T-cells (20). Interestingly, the same defect in IL-17 and IL-22 production in response to Sp was observed in COPD patients, and was related to an altered response in Lin^-, NK and NKT cells. The production of IL-1β and IL-23 was also altered in Sp-activated mononuclear cells from COPD patients. This is also observed after *in vitro* exposure to cigarette smoke in DC, leading to a decreased ability to induce Th17 cytokine production by lymphocytes (manuscript in preparation). The reduced production of IL-17 and IL-22 in innate lymphocytes might result from a deficient response of DC to Sp during COPD.

Functionally, IL-17 and IL-22 have been reported as essential factors in anti-bacterial defenses. IL-22 is involved in chemotaxis, antimicrobial peptide expression, tissue repair and epithelial cell survival, proliferation and differentiation (10, 28-32). Recent evidence further implicates IL-22 and IL-22-induced Reg3β and Reg3γ, two C-type lectins, in the containment of Gram-negative and Gram-positive pathogens (28, 29, 32, 33). During infection, the early production of IL-22 by innate immune cells is crucial for host protective immunity against some extracellular bacteria (32, 34, 35). In our report, the lack of IL-22 was not associated with an impaired production of antimicrobial peptides. However, the bacterial load was strongly higher in COPD mice than in controls suggesting that identical levels of antimicrobial peptide expression might not be sufficient in order to control bacteria growth in COPD mice. Interestingly, local administration of rmIL-22 was able to restore Reg3β and Reg3γ, and β-defensin levels in the lungs and a competent immune response allowing Sp clearance in COPD mice. In addition, an important neutrophil influx is observed in infected COPD mice without clearance of the bacteria. This suggests that these neutrophils are not appropriately primed in order to efficiently kill the bacteria. This is probably linked to the defect of IL-17 and IL-22, a mechanism potentially participating in the susceptibility to infection. In these settings, IL-22 might play a positive role in the prevention of injury, through the induction of antimicrobial peptides, the activation of immune cells (including neutrophils) and probably in the maintenance of the epithelial barrier (11, 36). In our model, a protective role for IL-17 can not be excluded (37), since IL-17 was also defective in COPD mice and COPD patients in response to *Sp*. The balance between IL-17 and/or IL-22 expression has been found to contribute to either the proinflammatory or tissue-protective phases of an immune response, depending on the context in which it is expressed (10, 12, 25). This might result in a more efficient resolution of

infection and in the maintenance of mucosal barrier integrity (38) as we reported in Sp infected COPD animals treated with rmIL-22.

These results reveal complex interactions between mucosal immune cell subsets providing potential mechanistic insights into mechanisms of mucosal immune dysregulation during COPD exacerbation, and offer hints for development of novel therapeutic strategies to address this aspect of the disease. Since we identified in the current study that the defect in IL-22 was a key factor in COPD exacerbation, restoring a competent immune response by targeting this cytokine or the pro-Th17 cytokines including IL-23 seems promising.

REFERENCES:

Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

1. Fletcher, C., and R. Peto. 1977. The natural history of chronic airflow obstruction. *Br Med J* 1:1645-1648.

2. Soler, N., S. Ewig, A. Torres, X. Filella, J. Gonzalez, and A. Zaubet. 1999. Airway inflammation and bronchial microbial patterns in patients with stable chronic obstructive pulmonary disease. *Eur Respir J* 14:1015-1022.

3. Soler-Cataluna, J. J., M. A. Martinez-Garcia, P. Roman Sanchez, E. Salcedo, M. Navarro, and R. Ochando. 2005. Severe acute exacerbations and mortality in patients with chronic obstructive pulmonary disease. *Thorax* 60:925-931.

4. Drannik, A. G., M. A. Pouladi, C. S. Robbins, S. I. Goncharova, S. Kianpour, and M. R. Stampfli. 2004. Impact of cigarette smoke on clearance and inflammation after Pseudomonas aeruginosa infection. *Am J Respir Crit Care Med* 170:1164-1171.

5. Sethi, S., and T. F. Murphy. 2008. Infection in the pathogenesis and course of chronic obstructive pulmonary disease. *N Engl J Med* 359:2355-2365.

6. Aaron, S. D., J. B. Angel, M. Lunau, K. Wright, C. Fex, N. Le Saux, and R. E. Dales. 2001. Granulocyte inflammatory markers and airway infection during acute exacerbation of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 163:349-355.

7. Agusti, A. G., A. Noguera, J. Sauleda, E. Sala, J. Pons, and X. Busquets. 2003. Systemic effects of chronic obstructive pulmonary disease. *Eur Respir J* 21:347-360.

8. Barnes, P. J., and R. A. Stockley. 2005. COPD: current therapeutic interventions and future approaches. *Eur Respir J* 25:1084-1106.

9. Caramori, G., I. M. Adcock, A. Di Stefano, and K. F. Chung. Cytokine inhibition in the treatment of COPD. *Int J Chron Obstruct Pulmon Dis* 9:397-412.

10. Eyerich, S., K. Eyerich, A. Cavani, and C. Schmidt-Weber. IL-17 and IL-22: siblings, not twins. *Trends Immunol* 31:354-361.

11. Paget, C., S. Ivanov, J. Fontaine, J. Renneson, F. Blanc, M. Pichavant, L. Dumoutier, B. Ryffel, J. C. Renauld, P. Gosset, P. Gosset, M. Si-Tahar, C. Faveeuw, and F. Trottein. Interleukin-22 is produced by invariant natural killer T lymphocytes during influenza A virus infection: potential role in protection against lung epithelial damages. *J Biol Chem* 287:8816-8829.

12. Sonnenberg, G. F., M. G. Nair, T. J. Kirn, C. Zaph, L. A. Fouser, and D. Artis. Pathological versus protective functions of IL-22 in airway inflammation are regulated by IL-17A. *J Exp Med* 207:1293-1305.

13. Pichavant, M., G. Remy, S. Bekaert, O. Le Rouzic, G. Kervoaze, E. Vilain, N. Just, I. Tillie-Leblond, F. Trottein, D. Cataldo, and P. Gosset. Oxidative stress-mediated iNKT-cell activation is involved in COPD pathogenesis. *Mucosal Immunol* 7:568-578.

14. Marques, J. M., A. Rial, N. Munoz, F. X. Pellay, L. Van Maele, H. Leger, T. Camou, J. C. Sirard, A. Benecke, and J. A. Chabalgoity. Protection against Streptococcus pneumoniae serotype 1 acute infection shows a signature of Th17- and IFN-gamma-mediated immunity. *Immunobiology* 217:420-429.

15. Munoz, N., L. Van Maele, J. M. Marques, A. Rial, J. C. Sirard, and J. A. Chabalgoity. Mucosal administration of flagellin protects mice from Streptococcus pneumoniae lung infection. *Infect Immun* 78:4226-4233.

16. Zemlickova, H., M. I. Crisostomo, M. C. Brandileone, T. Camou, E. Castaneda, A. Corso, G. Echaniz-Aviles, M. Pasztor, and A. Tomasz. 2005. Serotypes and clonal types of penicillin-susceptible streptococcus pneumoniae causing invasive disease in children in five Latin American countries. *Microb Drug Resist* 11:195-204.

17. Gaschler, G. J., C. M. Bauer, C. C. Zavitz, and M. R. Stampfli. 2007. Animal models of chronic obstructive pulmonary disease exacerbations. *Contrib Microbiol* 14:126-141.

18. Klugman, K. P., and C. Feldman. 2001. Streptococcus pneumoniae respiratory tract infections. *Curr Opin Infect Dis* 14:173-179.

19. van der Poll, T., and S. M. Opal. 2009. Pathogenesis, treatment, and prevention of pneumococcal pneumonia. *Lancet* 374:1543-1556.

20. Clement, C. G., S. E. Evans, C. M. Evans, D. Hawke, R. Kobayashi, P. R. Reynolds, S. J. Moghaddam, B. L. Scott, E. Melicoff, R. Adachi, B. F. Dickey, and M. J. Tuvim. 2008. Stimulation of lung innate immunity protects against lethal pneumococcal pneumonia in mice. *Am J Respir Crit Care Med* 177:1322-1330.

21. Gaschler, G. J., M. Skrtic, C. C. Zavitz, M. Lindahl, P. O. Onnervik, T. F. Murphy, S. Sethi, and M. R. Stampfli. 2009. Bacteria challenge in smoke-exposed mice exacerbates inflammation and skews the inflammatory profile. *Am J Respir Crit Care Med* 179:666-675.

22. Mucida, D., and S. Salek-Ardakani. 2009. Regulation of TH17 cells in the mucosal surfaces. *J Allergy Clin Immunol* 123:997-1003.

23. Zelante, T., A. De Luca, P. Bonifazi, C. Montagnoli, S. Bozza, S. Moretti, M. L. Belladonna, C. Vacca, C. Conte, P. Mosci, F. Bistoni, P. Puccetti, R. A. Kastelein, M. Kopf, and L. Romani. 2007. IL-23 and the Th17 pathway promote inflammation and impair antifungal immune resistance. *Eur J Immunol* 37:2695-2706.

24. Colonna, M. 2009. Interleukin-22-producing natural killer cells and lymphoid tissue inducer-like cells in mucosal immunity. *Immunity* 31:15-23.

25. Liang, S. C., X. Y. Tan, D. P. Luxenberg, R. Karim, K. Dunussi-Joannopoulos, M. Collins, and L. A. Fouser. 2006. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med* 203:2271-2279.

26. Sonnenberg, G. F., L. A. Monticelli, T. Alenghat, T. C. Fung, N. A. Hutnick, J. Kunisawa, N. Shibata, S. Grunberg, R. Sinha, A. M. Zahm, M. R. Tardif, T. Sathaliyawala, M. Kubota, D. L. Farber, R. G. Collman, A. Shaked, L. A. Fouser, D. B. Weiner, P. A. Tessier, J. R. Friedman, H. Kiyono, F. D. Bushman, K. M. Chang, and D. Artis. Innate lymphoid cells promote anatomical containment of lymphoid-resident commensal bacteria. *Science* 336:1321-1325.

27. Van Maele, L., C. Carnoy, D. Cayet, P. Songhet, L. Dumoutier, I. Ferrero, L. Janot, F. Erard, J. Bertout, H. Leger, F. Sebbane, A. Benecke, J. C. Renauld, W. D. Hardt, B. Ryffel, and J. C. Sirard. TLR5 signaling stimulates the innate production of IL-17 and IL-22 by CD3(neg)CD127+ immune cells in spleen and mucosa. *J Immunol* 185:1177-1185.

28. Kolls, J. K., P. B. McCray, Jr., and Y. R. Chan. 2008. Cytokine-mediated regulation of antimicrobial proteins. *Nat Rev Immunol* 8:829-835.

29. Sonnenberg, G. F., L. A. Fouser, and D. Artis. Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. *Nat Immunol* 12:383-390.

30. Witte, E., K. Witte, K. Warszawska, R. Sabat, and K. Wolk. Interleukin-22: a cytokine produced by T, NK and NKT cell subsets, with importance in the innate immune defense and tissue protection. *Cytokine Growth Factor Rev* 21:365-379.

31. Wolk, K., S. Kunz, E. Witte, M. Friedrich, K. Asadullah, and R. Sabat. 2004. IL-22 increases the innate immunity of tissues. *Immunity* 21:241-254.

32. Zheng, Y., P. A. Valdez, D. M. Danilenko, Y. Hu, S. M. Sa, Q. Gong, A. R. Abbas, Z. Modrusan, N. Ghilardi, F. J. de Sauvage, and W. Ouyang. 2008. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. *Nat Med* 14:282-289.

33. Cash, H. L., C. V. Whitham, C. L. Behrendt, and L. V. Hooper. 2006. Symbiotic bacteria direct expression of an intestinal bactericidal lectin. *Science* 313:1126-1130.

34. Aujla, S. J., Y. R. Chan, M. Zheng, M. Fei, D. J. Askew, D. A. Pociask, T. A. Reinhart, F. McAllister, J. Edeal, K. Gaus, S. Husain, J. L. Kreindler, P. J. Dubin, J. M. Pilewski, M. M. Myerburg, C. A. Mason, Y. Iwakura, and J. K. Kolls. 2008. IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. *Nat Med* 14:275-281.

35. Graham, A. C., K. D. Carr, A. N. Sieve, M. Indramohan, T. J. Break, and R. E. Berg. IL-22 production is regulated by IL-23 during Listeria monocytogenes infection but is not required for bacterial clearance or tissue protection. *PLoS One* 6:e17171.

36. Kumar, P., M. S. Thakar, W. Ouyang, and S. Malarkannan. IL-22 from conventional NK cells is epithelial regenerative and inflammation protective during influenza infection. *Mucosal Immunol* 6:69-82.

37. Lu, Y. J., J. Gross, D. Bogaert, A. Finn, L. Bagrade, Q. Zhang, J. K. Kolls, A. Srivastava, A. Lundgren, S. Forte, C. M. Thompson, K. F. Harney, P. W. Anderson, M. Lipsitch, and R. Malley. 2008. Interleukin-17A mediates acquired immunity to pneumococcal colonization. *PLoS Pathog* 4:e1000159.

38. Liu, J. Z., M. Pezeshki, and M. Raffatellu. 2009. Th17 cytokines and host-pathogen interactions at the mucosa: dichotomies of help and harm. *Cytokine* 48:156-160.

CLAIMS:

1. A method for the treatment of acute exacerbation of chronic obstructive pulmonary disease in a subject in need thereof comprising administering the subject with a therapeutically effective amount of an agonist of IL-23.

2. The method of claim 1 wherein the acute exacerbation of COPD is caused by a bacterial infection, by a viral infection or by air pollution.

3. The method of claim 2 wherein the bacterial infection is due to *Streptococcus pneumoniae*, *Haemophilus influenzae*, or *Moraxella catarrhalis*.

4. The method of claim 1 wherein the subject experienced an acute exacerbation of COPD or is at risk of experiencing an acute exacerbation of COPD.

5. The method of claim 1 wherein the subject is a frequent exacerbator.

6. The method of claim 1 wherein the treatment is a prophylactic treatment.

7. The method of claim 1 wherein the agonist of IL-23 is delivered to the respiratory tract.

8. The method of claim 1 wherein the agonist of IL-23 is administered to the subject in combination with an antiviral agent or an anti-bacterial agent.

9. The method of claim 8 wherein the antibacterial agent is an antibiotic.

10. The method of claim 1 wherein the IL-23 agonist is administered to the subject in combination with at least one corticosteroid.

11. The method of claim 1 wherein the agonist of IL-23 is interleukin 23.

12. The method of claim 1 wherein the IL-23 is selected from the group consisting of anti-IL-23R antibodies, and anti-IL12Rbeta1, and antibodies which are directed to the complex of IL-23R and IL-12Rbeta1.

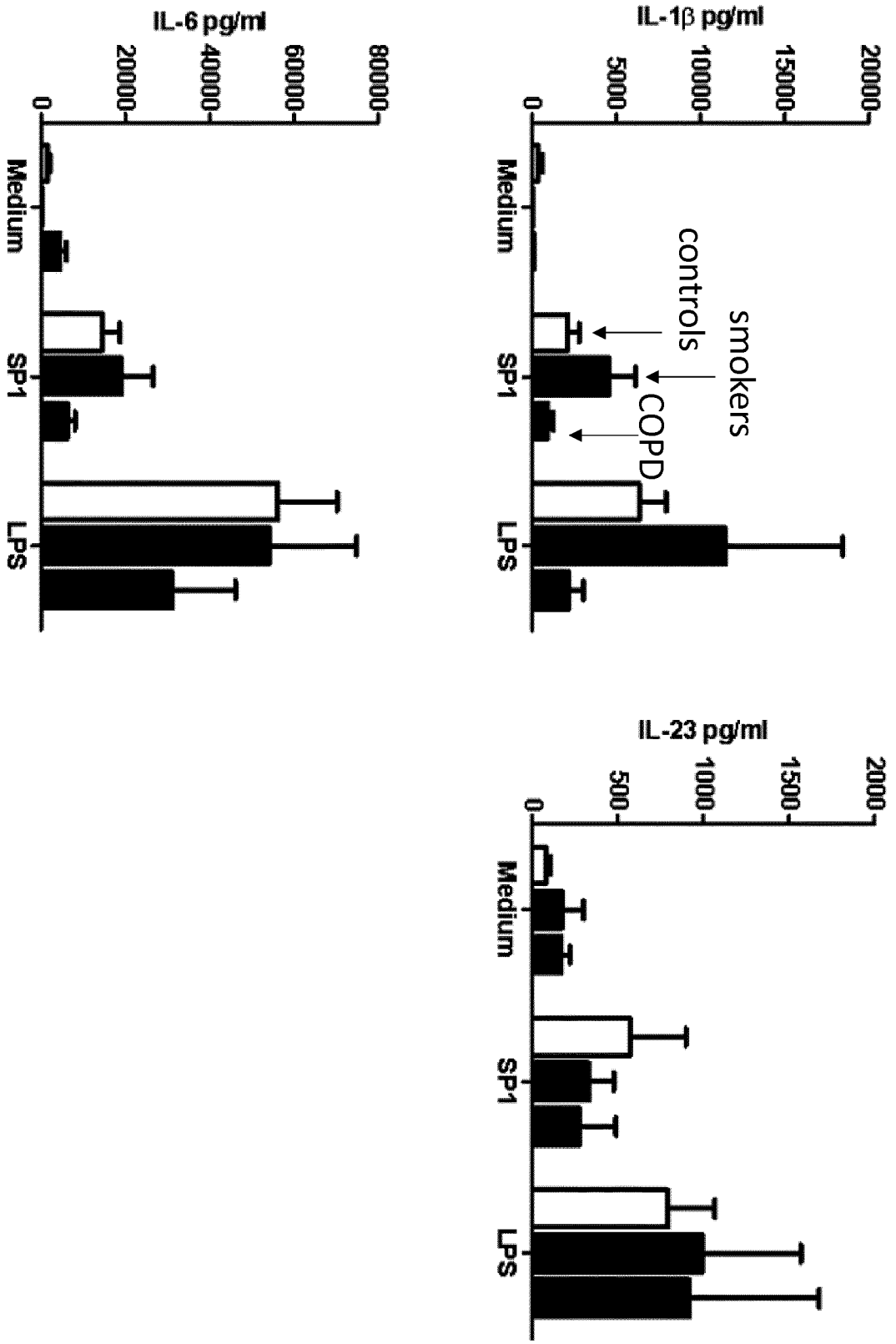
IL-6 pg/ml
0
20000
40000
60000
80000
Medium
SP1
LPS
IL-1β pg/ml
0
5000
10000
15000
20000
controls
smokers
COPD
IL-23 pg/ml
0
500
1000
1500
2000

Figure 1

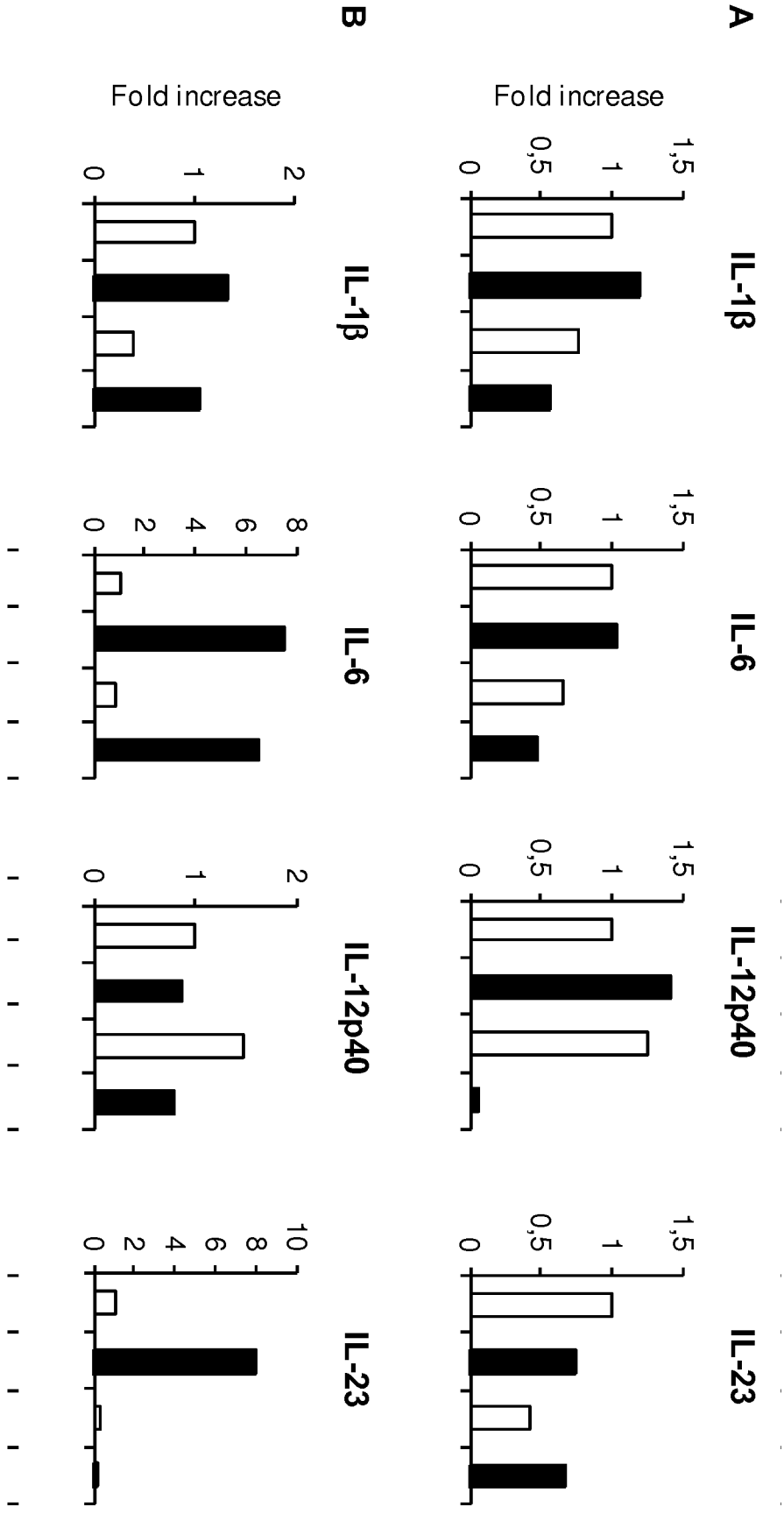
A
B
Fold increase
Fold increase
IL-1β
IL-6
IL-12p40
IL-23
IL-1β
IL-6
IL-12p40
IL-23
0
0,5
1
1,5
0
1
2
0
2
4
6
8
0
1
2
0
2
4
6
8
10

Figure 2 A&B

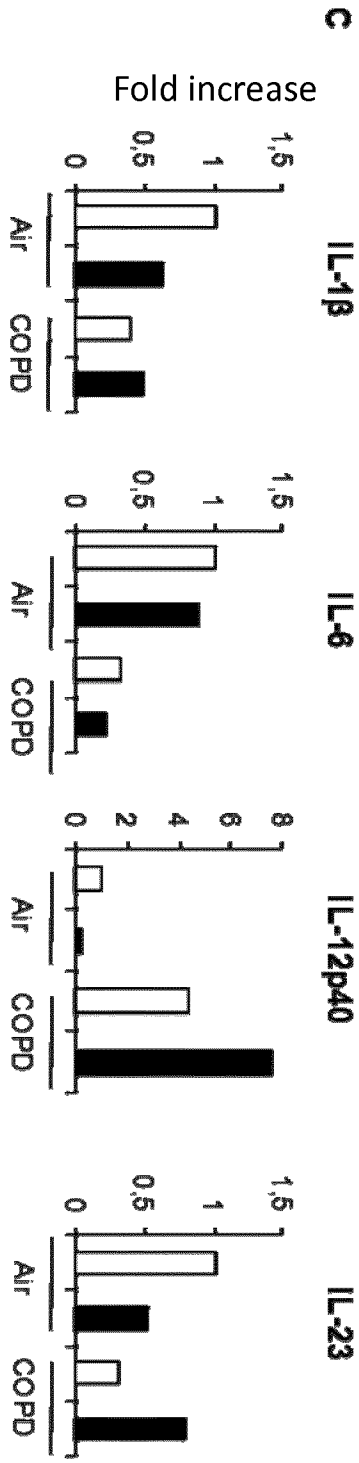

Figure 2 C

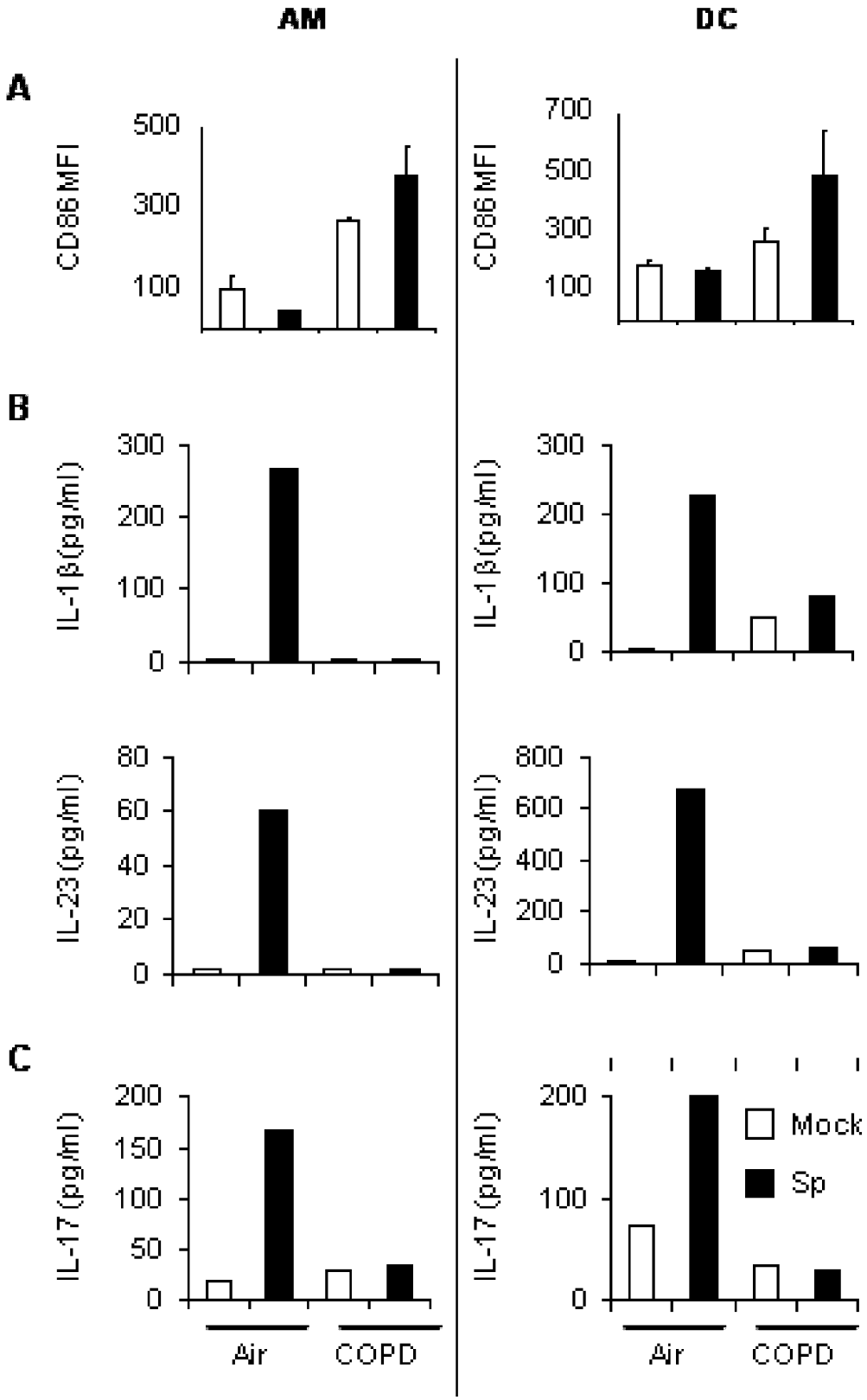
AM
DC
A
CD86 MFI
500
300
100
700
500
300
100
B
IL-1β (pg/ml)
300
200
100
0
300
200
100
0
IL-23 (pg/ml)
80
60
40
20
0
800
600
400
200
0
C
IL-17 (pg/ml)
200
150
100
50
0
200
100
0
Mock
Sp
Air
COPD
Air
COPD

Figure 3

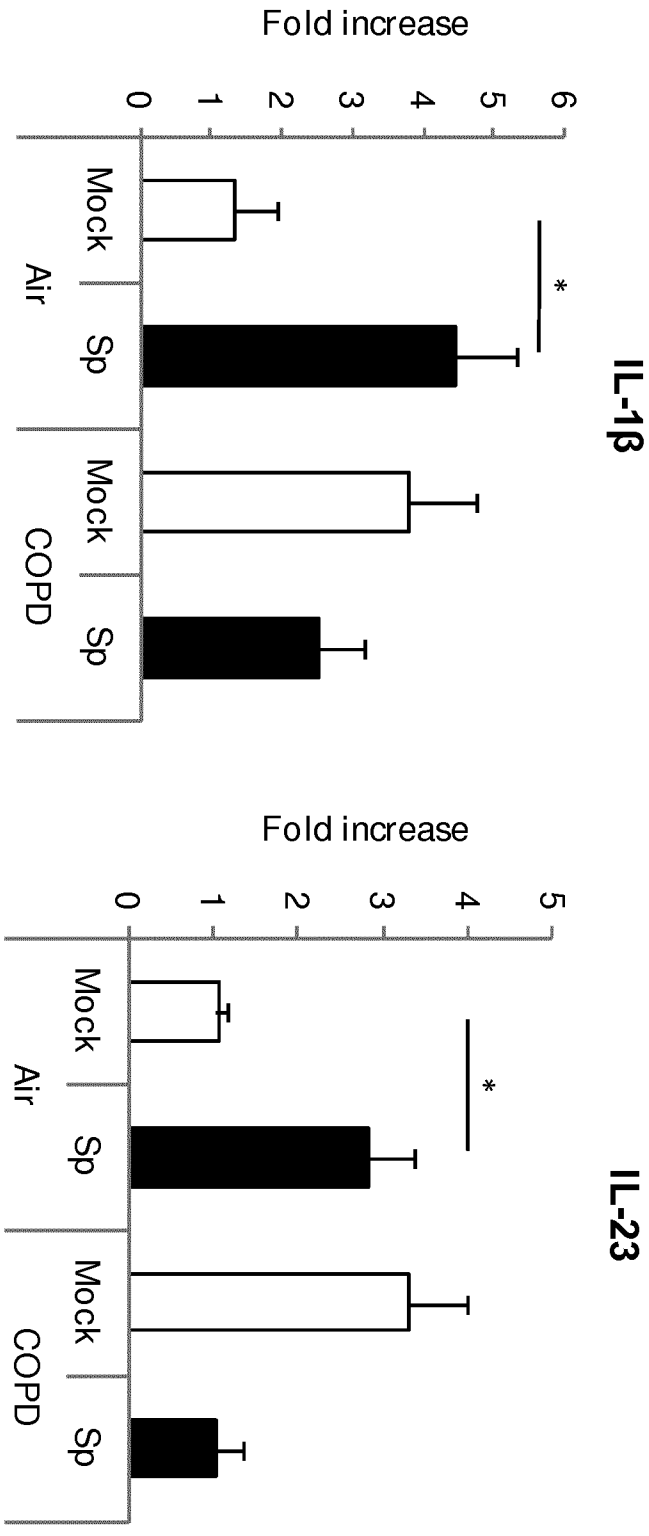
Fold increase
0
1
2
3
4
5
6
Mock
Sp
Air
Mock
Sp
COPD
*
IL-1β
Fold increase
0
1
2
3
4
5
Mock
Sp
Air
Mock
Sp
COPD
*
IL-23

Figure 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/081112

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/20 A61P11/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MICHELLE G. ROY ET AL: "Muc5b is required for airway defence", NATURE, vol. 505, no. 7483, 8 December 2013 (2013-12-08), pages 412-416, XP055181840, United Kingdom ISSN: 0028-0836, DOI: 10.1038/nature12807 *cf. summary part at front page 412, in combination with 2nd para. at the left-sided col. of page 415*	1-12
Y	US 7 422 743 B2 (CHIRICA MADALINE [US] ET AL) 9 September 2008 (2008-09-09) *cf. col. 2, lines 39-44, in combination with col. 5, lines 44-50*	1-12

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 March 2016	16/03/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Stoltner, Anton

3

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	YAMAGATA TOSHIYUKI ET AL: "Agents against cytokine synthesis or receptors", EUROPEAN JOURNAL OF PHARMACOLOGY, vol. 533, no. 1, 2006, pages 289-301, XP028913786, ISSN: 0014-2999, DOI: 10.1016/J.EJPHAR.2005.12.046 *cf. abstract, page 297, "conclusions" at left-sided col.*	1-12
Y	WO 2008/106134 A2 (SCHERING CORP [US]; PRESTA LEONARD G [US]) 4 September 2008 (2008-09-04) *cf. abstract, page 1, para. [0001], para. [0010] at pages 3/4*	1-12
Y	WO 2010/112458 A1 (NOVARTIS AG [CH]; BARDROFF MICHAEL [DE]; CARBALLIDO HERRERA JOSE M [CH) 7 October 2010 (2010-10-07) *cf. abstract, page 1, lines 1-12, 2nd para. at page 4, claim 1*	1-12

3

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2015/081112

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 7422743 B2	09-09-2008	AT 543905 T	15-02-2012
		AU 2004293811 A1	09-06-2005
		BR PI0416211 A	26-12-2006
		CA 2546619 A1	09-06-2005
		CN 1906297 A	31-01-2007
		CN 102784391 A	21-11-2012
		EP 1699925 A1	13-09-2006
		EP 2256203 A1	01-12-2010
		JP 4745980 B2	10-08-2011
		JP 2008501626 A	24-01-2008
		JP 2011093935 A	12-05-2011
		JP 2013253113 A	19-12-2013
		JP 2015155456 A	27-08-2015
		MX PA06005676 A	17-08-2006
		NZ 546962 A	27-11-2009
		US 2004258686 A1	23-12-2004
		US 2008317748 A1	25-12-2008
		US 2010278825 A1	04-11-2010
		US 2011129465 A1	02-06-2011
		US 2012107313 A1	03-05-2012
		US 2013156729 A1	20-06-2013
		WO 2005052157 A1	09-06-2005
		ZA 200604026 A	27-12-2007
WO 2008106134 A2	04-09-2008	AR 065496 A1	10-06-2009
		AU 2008219684 A1	04-09-2008
		BR PI0807991 A2	17-06-2014
		CA 2679381 A1	04-09-2008
		CN 101675076 A	17-03-2010
		EP 2057194 A2	13-05-2009
		EP 2395025 A1	14-12-2011
		JP 5238723 B2	17-07-2013
		JP 2010519902 A	10-06-2010
		JP 2013059351 A	04-04-2013
		JP 2014087374 A	15-05-2014
		KR 20100014568 A	10-02-2010
		NZ 579251 A	29-06-2012
		US 2010166767 A1	01-07-2010
		US 2012148582 A1	14-06-2012
		US 2013064817 A1	14-03-2013
		US 2014170154 A1	19-06-2014
		WO 2008106134 A2	04-09-2008
		ZA 200905964 A	26-05-2010
WO 2010112458 A1	07-10-2010	AU 2010230311 A1	13-10-2011
		CA 2759942 A1	07-10-2010
		CN 102414223 A	11-04-2012
		EA 201101572 A1	30-05-2012
		EP 2424894 A1	07-03-2012
		JP 2012524524 A	18-10-2012
		KR 20120057563 A	05-06-2012
		US 2012034231 A1	09-02-2012
		US 2014248265 A1	04-09-2014
		WO 2010112458 A1	07-10-2010