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(54) **Title:** ABIN-MEDIATED PROTECTION AGAINST LUNG INFLAMMATORY DISEASE

(57) **Abstract:** The present invention relates to the treatment of airway inflammation. Specifically, the airway inflammations which form the subject of the present invention are mediated by the transcription factor NF-κB. The treatment comprises the use of A20-binding inhibitors of NF-B activation (ABINs). The A20-binding inhibitors of NF-κB activation (ABINs) of the present invention are used in the preparation of a medicament for treating airway inflammation. The airway inflammations of the present invention include asthma and chronic obstructive pulmonary disease (COPD).

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ABIN-MEDIATED PROTECTION AGAINST LUNG INFLAMMATORY DISEASE

Field of the Invention

The present invention relates to the treatment of airway inflammation. Specifically, the airway inflammations which form the subject of the present invention are mediated by the transcription factor NF-κB. The treatment comprises the use of inhibitors of NF-κB activation (ABINs). Furthermore, the inhibitors of NF-κB activation (ABINs) of the present invention are used in the preparation of a medicament for treating airway inflammation. The ABINs are used to treat airway inflammations of different origin, especially asthma, and chronic obstructive pulmonary disease (COPD).

Background of the Invention

The transcription factor NF-κB plays a pivotal role in immune and inflammatory responses through the regulation of genes encoding pro-inflammatory cytokines, chemokines, and adhesion molecules. Activation of the NF-κB pathway is involved in the pathogenesis of chronic inflammatory diseases, such as asthma, rheumatoid arthritis and inflammatory bowel disease (Barnes and Karin, 1997).

Airway inflammation is a characteristic of many lung disorders including asthma and chronic obstructive pulmonary disease (COPD). Several lines of evidence suggest that NF-κB activation of cytokine genes is an important contributor to the pathogenesis of inflammatory lung diseases, which are characterized by the infiltration of inflammatory cells and the deregulation of many cytokines in the lung (Bochner et al., 1994; Christman et al., 2000; Yamamoto and Gaynor, 2001). The ability of NF-κB to activate transcription of genes encoding adhesion molecules and chemo-attractant proteins would lead to the recruitment of inflammatory cells to the lung, a hallmark of asthma and COPD (Barnes and Karin, 1997; Baldwin 2001; Barnes 2003).

The onset, development and clinical manifestations of asthma are driven by a T helper 2 lymphocyte (Th2)-based immune response to an antigen. Inhaled antigen provokes the activation of $CD4^+$ Th2 cytokine cells and the production of specific cytokines (IL-4, -5, -9 and -13) (Chung and Barnes, 1999; Barnes 2001). Cytokine expression results in the recruitment of eosinophils, leading to chronic airway inflammation. Binding and subsequent antigen dependent cross-linking of IgE bound to its receptor results in eosinophil and mast cell activation/degranulation, leading to the local release of mediators, mucus hyperproduction, airflow obstruction and airway hyperresponsiveness (AHR). Moreover, bronchial epithelial cells are able to release various mediators, including chemokines, to initiate inflammatory immune responses (Van der Velden et al., 1998). Recent studies have demonstrated the in vivo expression of eotaxin in bronchial epithelium as well as its increase in asthmatic patients

(Komiya et al., 2003). This chemokine is a specific chemoattractant for eosinophils and it has been shown to cause selective infiltration of these cells into the lung (Mattoli et al., 1997).

Chronic obstructive pulmonary disease (COPD) is a progressive syndrome of expiratory airflow limitation caused by chronic inflammation of the airways and lung parenchyma (Sutherland and Martin, 2003). The airway inflammatory response in COPD is initiated by smoking in the overwhelming majority of cases, and chronic exposure to cigarette smoke initiates a series of events that causes damage to central airways, peripheral airways, and terminal airspaces, leading to physiologic and clinical abnormalities. Although COPD shares some clinical features with asthma, another prevalent airway inflammatory disease, there are distinct differences in the phenotypic characteristics of airway inflammation between COPD and asthma. The eosinophil is the most prominent inflammatory cell in asthma, with mast cells, lymphocytes, and macrophages playing important but less prominent roles. In COPD the cellular composition of the airway inflammatory infiltrate differs, with neutrophils, macrophages, and lymphocytes assuming prominence and the eosinophil playing a minor role, except in the setting of exacerbations. Chemokines, such as MIP-2, and proinflammatory cytokines (IL-1β, TNFα) are involved in the recruitment of neutrophils into the airway space (Mizgerd 2002). This also results in the development of mucus hypersecretion and morphological changes, leading to airflow limitation and respiratory failure (Reid and Sallenave, 2003).

Because of the direct implication of NF-κB activation in inflammation, NF-κB and its regulators have drawn much interest as targets of anti-inflammatory compounds (Barnes 2000; Yamamoto and Gaynor, 2001). In unstimulated cells, NF-κB is usually kept inactive in the cytoplasm through association with IκB (Inhibitor of NF-κB) proteins. Once phosphorylated, upon cell stimulation by cytokines such as TNF and IL-1, IκB is ubiquitinated and degraded by the proteasome, allowing the nuclear translocation of NF-κB. Besides IκB, some other cellular compounds that inhibit NF-κB activation induced by several stimuli have been characterised. Among these factors, the NF-κB-dependent zinc finger protein A20 is involved in the negative feedback regulation of NF-κB activation in response to TNF. Three novel intracellular protein inhibitors of NF-κB (ABIN-1, ABIN-2 and ABIN-3) each belonging to a family of ABIN proteins were identified, which all interact with the C-terminal zinc finger domain of A20, and which have the potential to inhibit TNF-, IL1- and LPS-induced NF-κB activation upon overexpression (Beyaert et al., 2000; Heyninck et al., 1999; Van Huffel et al., 2001; Genbank AJ320534). ABIN-1 and ABIN-2 are widely expressed in several tissues, but ABIN-3 shows a slightly more restricted expression. Moreover, ABIN-3 expression is inducible in monocytes by TNF, LPS and by Listeria infection. ABIN-3 has therefore also been described as LIND (Listeria INDuced; Staege et al., 2001).

Recently, it was shown that in vivo adenoviral gene transfer of murine ABIN-1 prevented completely TNF-mediated lethal hepatitis in mice (WO03000280). However, although the role of ABIN in inhibition of NF-κB induction is clearly established, a protective role of ABIN proteins in lung inflammatory diseases has never been demonstrated nor suggested. Surprisingly we found that modulation of the expression level of ABIN gives a protective effect in lung inflammatory diseases that is similar to, if not better than, direct interaction at the IκB level, by locking the NF-κB in the cytosol by a non-degradable IκB mutation.

Summary of the Invention

The present invention relates to the use of ABIN, or a functional fragment thereof, or a variant thereof, for the manufacture of a medicament for the treatment of airway inflammation. To date three ABINs are known.

Although the complete protein can be used it is also envisaged to use a part of the ABIN protein provided that this part has the same or at least a similar physiological activity. As an example of such a partial protein the present invention relates to molecules comprising the consensus amino acid sequence depicted in SEQ ID N° 4, SEQ ID N° 5 and/or SEQ ID N°6.

Other examples of such partial ABINs are functional fragments that comprise the amino acid sequences as depicted in SEQ ID N°3 and that interact with protein A20. A selected sequence comprises amino acids 420-647 of SEQ ID N° 2, interacts with protein A20 and inhibits NF-κB activation. Said ABINs, functional fragments or variants may be fused to sequences that allow the delivery of the fusion protein in eukaryotic cells

All ABIN sequences mentioned herein and the variants thereof are also used in the treatment of airway inflammation. As a non-limiting example of this type of airway inflammation, tests have been performed on asthma and chronic obstructive pulmonary disease. The asthma and chronic obstructive pulmonary disease used to illustrate the present invention are allergen-induced. However, the invention is not limited thereto, and can be used in other forms of airway inflammation, such as acute respiratory distress syndrome or systemic inflammatory response syndrome. Preferably said airway inflammation is a chronic airway inflammation, such as cystic fibrosis, sarcoidosis or mineral dust disease (asbestosis/silicosis).

The present invention also illustrates the use of a nucleotide sequence encoding ABIN, or a functional fragment or variant thereof for the manufacture of a medicament for the treatment of airway inflammation. Functional fragments or variants of nucleic acids, as used here, are nucleic acids that encode the functional fragments or variants of the ABIN proteins, as described above. In such a case instead of using the protein for therapeutic or prophylactic purposes the DNA encoding the protein or fragments thereof are used. The desired DNA sequence is cloned in a suitable expression vector. Preferably, the nucleic acids are administered for *in vivo* or *ex vivo* gene therapy uses. Non-viral vector delivery systems

include DNA plasmids, naked nucleic acid, and nucleic acid complexed with a delivery vehicle such as a liposome. Viral vector delivery systems include DNA and RNA viruses, which have either episomal or integrated genomes after delivery to the cell.

The present invention also relates to specific pharmaceutical compositions comprising the peptides or nucleic acid sequences together with suitable means for the delivery. The specific composition used for delivery of the molecules depends on the typical delivery route, which is chosen. A preferred delivery route in the present context is by way of inhalation or intratracheal instillation.

The present invention is illustrated by way of example the protective effect of ABIN against allergen-induced asthma, more specifically the protective effect of ABIN-1 and ABIN–2. The overexpression ABIN is shown to result in a considerable reduction of the allergen-induced eosinophil infiltration into the lungs. In addition the overexpression of ABIN further resulted in the reduction of specific allergen induced IgE-levels in serum.

The present invention further discloses other parameters which, are influenced in a positive way by overexpression of ABIN. These include but are not limited to the reduction of eotaxin, IL-4 and IL-1 β levels in bronchoalveolar lavage (BAL) fluid of allergen-induced lung inflammation.

The present invention further discloses the protective effect of ABIN-1 on LPS-induced airway inflammation. The effect of the ABIN-1 being a considerable reduction of LPS-induced neutrophil infiltration into the lung.

The present invention further relates to screening assays for the identification and/or validation of pharmaceutical compounds.

Description of the Figures

Figure 1: Lung expression of ABIN-1 upon AdABIN-1 treatment.

Mice were treated at day 0 intratracheally with AdABIN-1, and lungs were removed daily from day 0 till day 6. Lung homogenates were subjected to SDS-PAGE and E-tagged ABIN-1 expression was revealed by Western blotting using an anti-E-Tag antibody coupled to HRP. N.I.: Non-treated mice used as negative control. Ct: Positive control = lysate from HEK293T cells transfected with an ABIN-1 expression vector.

Figure 2: Lung immunohistochemistry for E-tagged ABIN-1 following AdABIN-1 treatment.

Two days after intratracheal virus administration, lungs were removed and fixed in 4% paraformaldehyde and then embedded in paraffin. Sections were immunostained using Anti-E-

Tag-HRP antibody. Brown precipitates reveal the presence of ABIN-1 proteins. **A**, non-injected mice (x100). **B** and **C**, two days after virus administration (x100 and x200).

Figure 3: Assessment of total cell number and differential cell counts in bronchoalveolar lavage of allergen-sensitised mice.

Bronchoalveolar lavage fluid was collected 24h after the last Ova challenge. Total cell number, as well as differential cell counts for macrophages (MF), neutrophils (N), eosinophils (Eo), lymphocytes (L) and monocytes (Mo) counts are plotted for each group (n=5; mean +/-SEM). Neg: negative control = mice sensitised with PBS and challenged with OVA (PBS/OVA); Pos: positive control = mice sensitised and challenged with Ova (OVA/OVA); AdRR5, AdIκB^s and AdABIN-1: mice sensitised and challenged with Ova, but treated with recombinant adenoviruses AdRR5, AdIκB^s and AdABIN-1 respectively.

Figure 4: Histological evaluation of lung inflammation following Ova sensitisation and treatment with different recombinant adenoviruses.

Paraffin embedded lung sections were prepared 24h after the last Ova challenge, and stained with haematoxylin and eosin (H&E). **A,B,** negative control = PBS/OVA (x200 and x400); **C,D,** positive control = OVA/OVA (x200 and x400); **E,F**, OVA/OVA mice treated with AdRR5 (x200 and x400); **G,H**, OVA/OVA mice treated with AdIκB^s (x200 and x400); **I,J,K**, OVA/OVA mice treated with AdABIN-1 (x100, x200 and x400). Arrows indicate eosinophil (pink cytoplasm) infiltration.

Figure 5: Assessment of OVA-specific IgE levels in serum, and cytokine levels in BAL fluid of allergen-sensitised mice treated with different adenoviruses.

A. Detection of OVA-specific IgE in serum 24h after the last challenge; **B.** Expression of eotaxin in BAL fluid 24h after the last challenge; **C.** expression of IL-4 in BAL fluid 24 h after the last challenge; **D.** expression of IL-1β in BAL fluid 24 h after the last challenge. Results are the average of 5 mice/group +/- SEM.

Negative control = PBS sensitised and Ova challenged; Positive control = Ova sensitised/challenged; AdRR5, AdIκB^s and AdABIN-1 = Ova sensitised/challenged, followed by intratracheal administration of recombinant adenovirus AdRR5, AdIκB^s or AdABIN-1, respectively, 24h before the first challenge.

Figure 6: Assessment of total cell number and differential cell counts in BAL fluid of LPS-treated mice.

Bronchoalveolar lavage fluid was collected 24h after LPS treatment. Total cell number, as well as differential cell counts for macrophages (MF), neutrophils (N), eosinophils (Eo), lymphocytes (L) and monocytes (Mo) are plotted for each group (n=5; mean+/-SEM). PBS/PBS (negative control) = mice treated intratracheally (IT) and intranasally (IN) with PBS; PBS/LPS = mice treated IT with PBS IT and IN with LPS; AdRR/LPS and AdABIN/LPS = mice

treated IT with recombinant adenoviruses AdRR5 and AdABIN-1, respectively, followed by IN treatment with LPS.

Figure 7: Assessment of total cell number and differential cell counts in bronchoalveolar lavage of allergen-sensitised mice, for ABIN-2 treatment.

Bronchoalveolar lavage fluid was collected 24h after the last Ova challenge. Total cell number, as well as differential cell counts for macrophages (MΦ), neutrophils, eosinophils and lymphocytes counts are plotted for each group (n=5; mean +/-SEM). Neg: negative control = mice sensitised with PBS and challenged with OVA (PBS/OVA); Pos: positive control = mice sensitised and challenged with Ova (OVA/OVA); AdRR, AdmABIN-1 and AdhABIN-2: mice sensitised and challenged with Ova, but treated with recombinant adenoviruses AdRR5, AdABIN-1 and AdhABIN-2 respectively.

Figure 8: Assessment of IL-13 levels in BAL fluid of allergen-sensitised/challenged mice treated with AdRR5, AdIκBα^{S} or AdABIN-1.

Expression of IL-13 in BAL fluid 24 h after the last OVA challenge. Results are the average of 5 mice/group +/- SEM.

Figure 9: Histological evaluation of mucus production following Ova sensitisation/challenge and treatment with AdRR5, AdIκBα^{S} or AdABIN-1.

Paraffin embedded lung sections were prepared 24h after the last Ova challenge, and stained with Periodic Acid-Schiff (PAS) reagents (Sigma) to visualise mucus-producing (reddish-purple) goblet cells in the airway epithelium. **A,B,** negative control = PBS/OVA (x100 and x400); **C,D,** positive control = OVA/OVA (x100 and x400); **E,F**, OVA/OVA mice treated with AdRR5 (x100 and x400); **G,H**, OVA/OVA mice treated with AdIκBα^{sr} (x100 and x400); **I,J.** OVA/OVA mice treated with AdABIN-1 (x100 and x400).

Definitions

Nucleotide sequence as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, this term includes double- and single-stranded DNA, and RNA. It also includes known types of modifications, for example, methylation, "caps" substitution of one or more of the naturally occurring nucleotides with an analog.

Overexpression of a protein as used here means that cells or organs treated with the nucleic acid, protein, medicament or therapeutic formulation do produce more of said protein than the untreated control, when kept under the same condition. Preferably, this can be obtained by placing the coding sequence for said protein downstream a constitutive promoter. Alternatively, overexpression can be obtained by stabilisation of the messenger of said protein, or by stabilisation of said protein itself. *Coding sequence* is a nucleotide sequence, which is transcribed into mRNA and/or translated into a polypeptide when placed under the control of

appropriate regulatory sequences. The boundaries of the coding sequence are determined by a translation start codon at the 5'-terminus and a translation stop codon at the 3'-terminus. A coding sequence can include, but is not limited to mRNA, cDNA, recombinant nucleotide sequences or genomic DNA, while introns may be present as well under certain circumstances.

Protein A20 ("A20") means the TNF induced zinc finger protein, described by Dixit *et al.*, 1990; Opipari *et al.*, 1990 and Tewari *et al.,* 1995, or an active fragment thereof, such as the zinc finger containing part (amino acids 387-790 of human A20, amino acids 369-775 of murine A20).

The terms *protein* and *polypeptide* as used in this application are interchangeable. *Polypeptide* refers to a polymer of amino acids and does not refer to a specific length of the molecule. This term also includes post-translational modifications of the polypeptide, such as glycosylation, phosphorylation, ubiquitination and acetylation.

IκB superrepressor ($I\kappa B^s$) means a nondegradable mutant form of IκB-α, with S32A and S36A mutations, that locks NF-κB in a cytosolic protein complex, preventing its nuclear action.

Protein transduction means any system whereby a protein can be delivered into a cell or into a subcellular compartiment of said cell. Preferably, said cell is a eukaryotic cell. Said delivery may be combined with a specific targeting to a certain cell type and/or to a certain subcellular compartiment. *Facilitating* protein transduction, as used here, means that, by fusion or chemical coupling of the protein to the transducing sequence at least a part of the fusion protein or chemical coupled protein is delivered into the cell.

Detailed Description of the Invention

A first aspect of the invention is the use of ABIN, as represented in SEQ ID N°2, or a functional fragment or variant thereof for the preparation of a medicament for the treatment of airway inflammation comprising lung inflammatory diseases. The term 'ABIN' relates to ABIN, ABIN-2 and ABIN-3 as disclosed in Beyaert *et al.*, 2000; Heyninck *et al.*, 1999; Van Huffel *et al.*, 2001, genbank AJ320534 and WO 99/57133. More specifically, the term ABIN relates to any polypeptide that comprises the consensus amino acid sequence(s) as depicted in SEQ ID N° 4 and/or SEQ ID N° 5 which are also disclosed in WO 99/57133 that is hereby incorporated by reference, or to a polypeptide comprising the SEQ ID N°6, possibly in combination with SEQ ID N° 4 and/or SEQ ID N° 5. Said sequences may be fused or chemically coupled to a sequence facilitating transduction of the fusion or chemical coupled proteins into eukaryotic cells. Sequences, facilitating protein transduction are known to the person skilled in the art and include, but are not limited to Protein Transduction Domains. As a non-limiting example, such sequences has been described in EP1512696. Preferably, said sequence is is selected from the group consisting of the HIV TAT protein and a polyarginine sequence. A second aspect of

the invention is the use of a nucleotide sequence encoding ABIN, as represented in SEQ ID N°1, or a functional fragment or a variant thereof, for the manufacture of a medicament for the treatment of airway inflammation. A functional fragment of ABIN is a polypeptide that is still able to interact with protein A20 and/or capable of modulating NF-κB activation. Preferably, said modulation is an inhibition of NF-κB activation. Functional fragments are, as a non limiting example, fragments that comprise at least amino acids 420-647 of SEQ ID N°2, preferably at least amino acids 390-647, more preferably at least 54-647 (SEQ ID N° 3). Preferentially said fragment is essentially consisting of at least amino acids 420-647 of SEQ ID N°2, preferably at least amino acids 390-647, more preferably at least 54-647 (SEQ ID N° 3). Variants are polypeptides with at least 65% identity on amino acid level, preferably 70% identity, as measured by BLAST (Altschul *et al.*, 1997). Variants have one or more common characteristics, such as but not limited to biological activity, immunological reactivity, conformation etc. As a non-limiting example, Naf1 alpha protein (AJ011895), Naf1 beta protein (AJ011896), virion-associated nuclear shuttling protein (AY012155), and Listeria induced LIND protein (AJ320534) are considered as variants. A functional fragment or variant of the ABIN protein or of the nucleotide sequence encoding ABIN, as used here, is a protein sequence, having some of the common characteristics of the ABIN protein or a nucleic acid sequence that encodes a functional fragment or variant of ABIN, as described above.

Said nucleic acid sequence may be cloned in a suitable expression vector, as will be detailed below.

In case a nucleic acid is used, said medicament is preferably intended for delivery of said nucleic acid into the cell, in a gene therapy treatment. A large number of delivery methods are well known to those of skill in the art. Preferably, the nucleic acids are administered for *in vivo* or *ex vivo* gene therapy uses. Non-viral vector delivery systems include DNA plasmids, naked nucleic acid, and nucleic acid complexed with a delivery vehicle such as a liposome. Viral vector delivery systems include DNA and RNA viruses, which have either episomal or integrated genomes after delivery to the cell. Methods of non-viral delivery of nucleic acids include lipofection, microinjection, biolistics, virosomes, liposomes, immunoliposomes, polycation or lipid: nucleic acid conjugates, naked DNA, artificial virions, and agent-enhanced uptake of DNA. Lipofection is described in, e.g., US Pat. No. 5,049,386, US Pat No. 4,946,787; and US Pat. No. 4,897,355 and lipofection reagents are sold commercially (e.g., Transfectam™ and Lipofectin™). Cationic and neutral lipids that are suitable for efficient receptor-recognition lipofection of polynucleotides include those of Flegner, WO 91/17424, WO 91/16024. Delivery can be to cells (*ex vivo* administration) or target tissues (*in vivo* administration). The preparation of lipid: nucleic acid complexes, including targeted liposomes such as immunolipid complexes, is well known to one of skill in the art (*see, e.g.,* Crystal, 1995; Blaese *et al.,* 1995; Behr, 1994; Remy *et al.,* 1994; Gao and Huang, 1995; U.S. Pat.

Nos. 4,186,183, 4,217,344, 4,235,871, 4,261,975, 4,485,054, 4,501,728, 4,774,085, 4,837,028, and 4,946,787). The use of RNA or DNA viral based systems for the delivery of nucleic acids take advantage of highly evolved processes for targeting a virus to specific cells in the body and trafficking the viral payload to the nucleus. Viral vectors can be administered directly to patients (*in vivo*) or they can be used to treat cells *in vitro* and the modified cells are administered to patients (*ex vivo*). Conventional viral based systems for the delivery of nucleic acids include amongst others retroviral, lentivirus, adenoviral, adeno-associated and herpes simplex virus vectors for gene transfer. Viral vectors are currently the most efficient and versatile method of gene transfer in target cells and tissues. Integration in the host genome is possible with the retrovirus, lentivirus, and adeno-associated virus gene transfer methods, often resulting in long-term expression of the inserted transgene. Additionally, high transduction efficiencies have been observed in many different cell types and target tissues.

In cases where transient expression of the nucleic acid is preferred, adenoviral based systems, including replication deficient adenoviral vectors may be used. Adenoviral based vectors are capable of very high transduction efficiency in many cell types and do not require cell division. With such vectors, high titer and levels of expression have been obtained. This vector can be produced in large quantities in a relatively simple system. Adeno-associated virus ("AAV") vectors, including recombinant adeno-associated virus vectors are also used to transduce cells with target nucleic acids, e.g., in the *in vitro* production of nucleic acids and peptides, and for *in vivo* and *ex vivo* gene therapy procedures (*see, e.g.,* U.S. Patent No. 4,797,368; WO 93/24641; Kotin, 1994; The construction of recombinant AAV vectors is described in a number of publications, including U.S. Pat. No. 5,173,414; Hermonat & Muzyczka, 1984; Samulski *et al.,* 1989).

Gene therapy vectors can be delivered *in vivo* by administration to an individual patient, typically by systemic administration (e.g., intravenous, intraperitoneal, intramuscular, intratracheal, subdermal, or intracranial infusion) or topical application. Alternatively, vectors can be delivered to cells *ex vivo*, such as cells explanted from an individual patient (e.g., lymphocytes, bone marrow aspirates, tissue biopsy) or universal donor hematopoietic stem cells, followed by reimplantation of the cells into a patient, usually after selection for cells which have incorporated the vector. *Ex vivo* cell transfection for diagnostics, research, or for gene therapy (e.g., via re-infusion of the transfected cells into the host organism) is well known to those of skill in the art. In a preferred embodiment, cells are isolated from the subject organism, transfected with a nucleic acid (gene or cDNA), and re-infused back into the subject organism (e.g., patient). Various cell types suitable for *ex vivo* transfection are well known to those of skill in the art (*see, e.g.,* Freshney *et al.,* 1994 and the references cited therein for a discussion of how to isolate and culture cells from patients).

A further aspect of the invention is the use of an ABIN inducing and/or activating and/or stabilizing compound for the preparation of a medicament for the treatment of airway inflammation. As a non-limiting example, phytohemagglutinin (PHA) is an ABIN inducing compound (Gupta *et al.*, 2000). In the case of ABIN-3, LPS induces expression of this protein in THP1 monocytes. Stabilization of ABIN-2 mRNA can be induced by 17β-estradiol, which results in the binding of AUF1 to AU-rich elements in the 3'-untranslated region of ABIN-2 mRNA (Arao et al., 2004).

In a further embodiment the invention provides a method for the production or manufacture of a medicament or a pharmaceutical composition comprising ABIN or a functional fragment or variant thereof and further more mixing said polypeptide with a pharmaceutically acceptable carrier. Alternatively, the pharmaceutical composition may comprise an ABIN inducing compound in stead of ABIN itself.

The administration of said pharmaceutical composition may be by way of oral, inhaled or parenteral administration. The active compound may be administered alone or preferably formulated as a pharmaceutical composition. A unit dose will normally contain 0.01 to 50 mg for example 0.01 to 10 mg, or 0.05 to 2 mg of compound or a pharmaceutically acceptable salt thereof. Unit doses will normally be administered once or more than once a day, for example 2, 3, or 4 times a day, more usually 1 to 3 times a day, such that the total daily dose is normally in the range of 0.0001 to 1 mg/kg; thus a suitable total daily dose for a 70 kg adult is 0.01 to 50 mg, for example 0.01 to 10 mg or more usually 0.05 to 10 mg. It is greatly preferred that the compound or a pharmaceutically acceptable salt thereof is administered in the form of a unit-dose composition, such as a unit dose oral, parenteral, or inhaled composition. Such compositions are prepared by admixture and are suitably adapted for oral, inhaled or parenteral administration, and as such may be in the form of tablets, capsules, oral liquid preparations, powders, granules, lozenges, reconstitutable powders, injectable and infusable solutions or suspensions or suppositories or aerosols. Tablets and capsules for oral administration are usually presented in a unit dose, and contain conventional excipients such as binding agents, fillers, diluents, tabletting agents, lubricants, disintegrants, colourants, flavourings, and wetting agents. The tablets may be coated according to well-known methods in the art. Suitable fillers for use include cellulose, mannitol, lactose and other similar agents. Suitable disintegrants include starch, polyvinylpyrrolidone and starch derivatives such as sodium starch glycollate. Suitable lubricants include, for example, magnesium stearate. Suitable pharmaceutically acceptable wetting agents include sodium lauryl sulphate. These solid oral compositions may be prepared by conventional methods of blending, filling, tabletting or the like. Repeated blending operations may be used to distribute the active agent throughout those compositions employing large quantities of fillers. Such operations are, of course, conventional in the art. Oral liquid preparations may be in the form of, for example,

aqueous or oily suspensions, solutions, emulsions, syrups, or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, gelatin, hydroxyethylcellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats, emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example, almond oil, fractionated coconut oil, oily esters such as esters of glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and if desired conventional flavouring or colouring agents. Oral formulations also include conventional sustained release formulations, such as tablets or granules having an enteric coating. Preferably, compositions for inhalation are presented for administration to the respiratory tract as a snuff or an aerosol or solution for a nebulizer, or as a microfine powder for insufflation, alone or in combination with an inert carrier such as lactose. In such a case the particles of active compound suitably have diameters of less than 50 microns, preferably less than 10 microns, for example between 1 and 5 microns, such as between 2 and 5 microns. Alternatively, coated nanoparticles can be used, with a particle size between 30 and 500 nm. A favored inhaled dose will be in the range of 0.05 to 2 mg, for example 0.05 to 0.5 mg, 0.1 to 1 mg or 0.5 to 2 mg. For parenteral administration, fluid unit dose forms are prepared containing a compound of the present invention and a sterile vehicle. The active compound, depending on the vehicle and the concentration, can be either suspended or dissolved. Parenteral solutions are normally prepared by dissolving the compound in a vehicle and filter sterilising before filling into a suitable vial or ampoule and sealing. Advantageously, adjuvants such as a local anaesthetic, preservatives and buffering agents are also dissolved in the vehicle. To enhance the stability, the composition can be frozen after filling into the vial and the water removed under vacuum. Parenteral suspensions are prepared in substantially the same manner except that the compound is suspended in the vehicle instead of being dissolved and sterilised by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the active compound. Where appropriate, small amounts of bronchodilators for example sympathomimetic amines such as isoprenaline, isoetharine, salbutamol, phenylephrine and ephedrine; xanthine derivatives such as theophylline and aminophylline and corticosteroids such as prednisolone and adrenal stimulants such as ACTH may be included. As is common practice, the compositions will usually be accompanied by written or printed directions for use in the medical treatment concerned.

With regard to the protein transduction with ABIN or ABIN-fragments into target cells, it has been shown that a series of small protein domains, termed protein transduction domains (PTDs), cross biological membranes efficiently and independently of transporters or specific

receptors, and promote the delivery of peptides and proteins into cells. For example, the TAT protein from human immunodeficiency virus (HIV-1) is able to deliver biologically active proteins in vivo. Similarly, the third alpha-helix of Antennapedia homeodomain, and VP22 protein from herpes simplex virus promote the delivery of covalently linked peptides or proteins into cells (reviewed in Ford et al., 2001). Protein delivery based on a short amphipathic peptide carrier, Pep-1, is efficient for delivery of a variety of peptides and proteins into several cell lines in a fully biologically active form, without the need for prior chemical covalent coupling (Morris et al., 2001). The capacity of VP22 chimeric proteins to spread from the primary transduced cell to surrounding cells can improve gene therapy approaches (Zender et al., 2002).

The present invention is further illustrated by way of examples, which are not considered to be limiting. The examples specifically show that it is possible to (over)express the ABIN protein in lungs of test animals.

The protective effect of ABIN-1 and ABIN-2 on ovalbumin induced asthma was tested. The overexpression of ABIN resulted in a considerable reduction of the allergen-induced eosinophil infiltration into the lungs. In addition the overexpression of ABIN further resulted in the reduction of specific allergen induced IgE-levels in serum.

Further tests on BAL fluid of allergen-induced lung inflammation showed a reduction of eotaxin, IL-4 and IL-1 β levels.

In a further group of tests the protective effect of ABIN-1 on LPS-induced airway inflammation, as a model for COPD was tested. The results showed a considerable reduction of LPS-induced neutrophil infiltration into the lung.

Examples

Example 1: Generation of the ABIN-1 and ABIN-2 adenovirus

The murine ABIN-1 cDNA (SEQ ID No 1), N-terminally fused to an E-tag, was amplified via PCR with forward (5'cgggatccgccatgggtgcgccggtgcc3') and reverse (5'ccccaagcttaaatgacccactgcagcc3') primers that contained restriction sites for BamHI and HindIII, respectively. The resulting fragment was cloned into a BamHI and HindIII opened pLpA.CMV shuttle vector (Gomez-Foix et al., 1992), and cotransfected with pJM17 (McGrory et al., 1988) by DNA/calcium phosphate coprecipitation in HEK293 cells. In vivo recombination of the shuttle vector expressing the ABIN transgene with the pJM17 backbone resulted in the production of a replication-deficient E1-deleted adenovirus type 5 (AdABIN-1). A control virus (AdRR5), which does not express a transgene, was generated in a similar way. Following recombination, recombinant plaques were isolated, extracted DNA was verified via PCR, and expression of the correct transgene was confirmed by means of Western Blotting. High titer virus stocks were prepared in HEK293 cells and purified via single CsCl banding. The

infectious unit titer was determined in a plaque assay that was performed on confluent HEK293 cells with different virus dilutions. The plaques of lysed cells were counted and calculated as plaque forming units (pfu) per ml virus stock.

AdABIN-2 was constructed in a similar way on the base of the human ABIN-2 sequence (genbank accession number AJ304866), using the same vector

Ad$I\kappa B^s$ is an adenovirus that expresses an $I\kappa B$ superrepressor ($I\kappa B^s$), which means a nondegradable mutant form of $I\kappa B$-α with S32A and S36A mutations (Roff et al., 1996). The latter locks NF-κB in a cytosolic protein complex, preventing its nuclear action. AdRR5 is a control adenovirus expressing no transgene.

Example 2: Expression of ABIN-1 upon adenoviral delivery in lung

A replication deficient adenovirus encoding E-tagged murine ABIN-1 under the control of a CMV promoter (AdABIN-1) was used to demonstrate the expression of ABIN-1 in lung tissue. Balb/c mice were intratracheally (IT) administered with $2x10^9$ plaque forming units (pfu) of virus. Lungs were removed daily, cut in small pieces, and homogenized by douncing in lysis buffer (1% NP-40, 200 mM NaCl, 10mM Tris-HCl pH 7.5, 5 mM EDTA, 10% glycerol) supplemented with 0.1 mM of aprotinine and leupeptine. After 20 minutes incubation on ice, homogenates were centrifuged for 15 minutes at high speed at 4°C. Protein concentration was determined by Bradford assay (Biorad). 50 μg of proteins were subjected to SDS-PAGE and immunoblotted with anti-E-Tag coupled to horseradish peroxidase (HRP). Signals were revealed by ECL (Amersham). The highest expression of ABIN-1 was detected 24h after IT administration and then slowly declined over the next days (figure 1).

For immunohistochemistry, lungs were removed and fixed in 4 % paraformaldehyde, and embedded in paraffin. 5μm sections were subjected to immunostaining using the anti-E-Tag–HRP antibody. Lungs removed two days after adenovirus administration were used. The expression of ABIN-1 was clearly detectable in alveolar and bronchiolar epithelia (figure 2).

Example 3: Evaluation of the protective effect of ABIN-1 on ovalbumin (OVA)-induced asthma

Example 3A: Allergen-induced asthma model

5- to 6-week old female BALB/c mice were sensitised by three intra-peritoneal injections of 10 μg ovalbumin (OVA) adsorbed to 1 mg $Al(OH)_3$ (alum). Negative control mice were injected with PBS. At day 20, mice were treated via intratracheal instillation with recombinant adenoviruses. 24h later, mice were challenged with two intratracheal injections of 80 μl OVA solution (20 μg per mouse) at days 21 and 22. Bronchoalveolar lavage (BAL), lung removal and serum collection were performed 24h after the last challenge.

BAL was performed under anaesthesia with an intraperitoneal injection of avertin (2.5% in PBS-low endotoxin). A 23-gauge cannula was installed into the trachea, and cells were collected by washing the airway lumen with 2 x 0.5 ml PBS. After centrifugation, supernatants

were stored at -20°C for cytokine measurement by ELISA. 200 cells were counted and differential cell counts were determined by morphology criteria on a cytospin preparation stained with May-Grunwald-Giemsa (Sigma).

Lungs were fixed with 4% paraformaldehyde and embedded in paraffin. Eosin and Haematoxylin (E&H) staining of 5μm sections was done for histological analysis.

Example 3B: AdABIN-1 and AdIκB^s reduce allergen-induced eosinophil infiltration into lungs

Mice that were challenged and sensitised with OVA showed a marked influx of cells into the airways, evidenced by increases in cells recovered from the BAL. Typical of antigen-induced airway responses, the number of cells recovered from BAL was accounted for in large part by the influx of eosinophils. Also the number of infiltrating macrophages was increased. OVA-sensitised/challenged mice treated with AdABIN-1 and AdIκB^s showed a strong reduction in the number of infiltrating eosinophils and macrophages, whereas OVA-sensitised/challenged mice treated with AdRR5 or with PBS did not (figure 3). Also histological analysis of 5μm sections with eosin and haematoxylin (E&H) staining showed a reduction of eosinophil lung infiltration in OVA-sensitised/challenged mice treated with AdABIN-1 and AdIκB^s (figure 4).

Example 3C: AdABIN-1 reduces OVA-specific IgE levels in serum of allergen-treated mice

Immunoglobulin E (IgE) and mast cells are believed to play important roles in allergic inflammation. The IgE can capture the antigen presented to the airways and the immune complexes so formed can augment allergic airway response in a high-affinity IgE receptor (FcepsilonRI)-dependent manner.

Levels of OVA-specific IgE in sera, collected 24h after the last challenge, were measured by ELISA. ELISA plates (Nunc 96-well Immunoplates) were prepared by coating the surface overnight at 4°C with 50μl of Ova (100 μg/ml) in carbonate buffer and blocking non-specific binding activity with 10% foetal calf serum (FCS) in PBS. Diluted samples were incubated in each well for 2 hours. Bound IgE was detected with sheep anti-mouse IgE antibody. Secondary anti-sheep IgG antibody coupled to HRP was added, the bound HRP enzyme was detected with tetramethylbenzidine (TMB), and the absorbance was read at 450 nm. Values of IgE levels were determined using a standard curve established on the basis of a serial dilution of supernatant of an OVA-specific IgE hybridoma. Arbitrary units were used according to the OD_{50} of the standard curve.

OVA sensitisation and challenge induced a 1000-fold increase in mouse serum IgE levels. However, a 10-20 fold decrease in IgE levels could be seen in sera from mice treated with AdABIN-1 compared to mice treated with empty virus or even mice treated with AdIκB^s (figure 5A).

Example 3D: AdABIN-1 and AdIκB^s reduce allergen-induced eotaxin levels in BAL fluid

The NF-κB regulated chemokine eotaxin is thought to be important in the development and maintenance of the asthma phenotype (Williams and Jose, 2000). Eotaxin is critical to the recruitment and activation of eosinophils into the airways and potentially also in the development of pulmonary fibrosis as a result of sensitisation and challenge with allergen. Human eotaxin is a NF-κB-regulated gene that is inducible expressed by airway epithelial cells in response to inflammatory cytokines and allergen challenge. The murine eotaxin gene also has a NF-κB binding site in its promoter. NF-κB in cooperation with STAT-6, which is activated in response to Th2 cytokines IL-4, IL-5, and IL-13, is believed to be responsible for enhanced eotaxin expression in asthma.

Eotaxin levels in BAL fluid were determined with a cytokine-specific ELISA according to the manufacturer's instructions (Quantin, R&D Systems Europe), and were found to be increased 4-fold in asthmatic mice. However, eotaxin levels were reduced 30% and 55% after treatment of mice with AdABIN-1 and AdIκB^s, respectively, as compared to the OVA-sensitised/challenged mice that were treated with PBS or the AdRR5 virus (figure 5B).

Example 3E: AdABIN-1 and AdIκB^s reduce allergen-induced IL-4 levels in BAL fluid

The IL-4 signalling pathway controls the most important cellular developmental events that underlie asthma. These include T helper (Th) type 2 cell activation, B cell activation and immunoglobulin E secretion, mast cell development, and effector events related exclusively to immune effects on the lung such as goblet cell metaplasia and airway hyperresponsiveness (Corry and Kheradmand, 2000).

Expression of IL-4 in the BAL fluids of Ova sensitised/challenged mice was assayed by ELISA. Plates were coated with anti-IL-4 monoclonal antibody (18191D Pharmingen) in carbonate buffer overnight at 4°C. Plates were blocked with 1% BSA in PBS. Diluted samples were incubated in each well overnight at 4°C. A standard curve was made using recombinant IL-4. After washing the plates, biotinylated anti-IL-4 monoclonal antibody was added and incubated for 1h at room temperature. Plates were treated with HRP-conjugated streptavidine at room temperature for 1h and then incubated with peroxidase substrate (TMB). The absorbance was measured at 450 nm.

Mice treated with AdABIN-1 showed an almost complete suppression of IL-4 levels in BAL fluid. Mice treated with AdIκB^s also showed a 7-fold decrease in IL-4 levels (figure 5C). These results are consistent with a reduction of eosinophilia and eotaxin production in AdABIN-1 and AdIκB^s treated mice.

Example 3F: AdABIN-1 and AdIκB^s reduce allergen-induced IL-1β levels in BAL fluid

Allergen challenge and clinical asthma are associated with synthesis and release of pro-inflammatory cytokines such as IL-1 and TNF. IL-1 is required for allergen-specific Th2 cell

activation and the development of airway hypersensitivity response (Nakae et al., 2003).

IL-1β levels in BAL fluid were determined with a cytokine-specific ELISA according to the manufacturer's instructions (Quantin, R&D Systems Europe). Ova sensitised/challenged mice showed high levels of IL-1β in the BAL. However, mice treated with AdABIN-1 showed an almost complete inhibition of IL-1β levels in BAL fluid. A similar effect could be seen upon treatment with AdIκB^s, although the effect was less pronounced (figure 5D).

Example 4: Evaluation of the protective effect of ABIN-1 on LPS-induced airway inflammation

Example 4A: LPS-induced airway inflammation as a model for COPD

LPS (endotoxin) is one of the agents ubiquitously present as contaminant on airborne particles (Vernooy et al., 2002). LPS-induced airway inflammation is used as a model for COPD.

5- to 6-week old female BALB/c mice were treated via intratracheal instillation with $2x10^9$ pfu of AdABIN-1, AdRR5 or only buffer (PBS). 24 later, mice received intranasally 5 μg LPS in 25 μl sterile PBS or PBS alone (negative control). Another 24h later, BAL and lungs were isolated and further processed as described above.

Example 4B: AdABIN-1 reduces LPS-induced neutrophil infiltration into the lung

Mice that received an LPS inhalation showed a marked influx of cells into the airways, evidenced by increases in cells recovered from the BAL. Typical of LPS-induced airway responses, the number of cells recovered from BAL was accounted for in large part by the influx of neutrophils. Also the number of infiltrating macrophages was increased. LPS-treated mice that were pretreated with AdABIN-1 showed a strong reduction in the number of infiltrating neutrophils, whereas LPS-treated mice that received AdRR5 or only PBS did not (figure 6).

Example 5: Evaluation of the protective effect of ABIN-2 on ovalbumin (OVA)-induced asthma

5- to 6-week old female BALB/c mice were sensitised and treated as described in example 3A. Mice that were challenged and sensitised with OVA showed a marked influx of cells into the airways, evidenced by increases in cells recovered from the BAL. Typical of antigen-induced airway responses, the number of cells recovered from BAL was accounted for in large part by the influx of eosinophils. Also the number of infiltrating macrophages was increased. OVA-sensitised/challenged mice treated with AdABIN-2 showed a strong reduction in the number of infiltrating eosinophils and macrophages, comparable with the reduction that is obtained with AdABIN-1, whereas OVA-sensitised/challenged mice treated with AdRR5 or with PBS did not (Figure 7).

Example 6: AdABIN-1 reduces allergen-induced IL-13 levels in BAL fluid

Growing evidence shows that interleukin 13 (IL-13) may play an essential role in the development of airway inflammation and bronchial hyper-responsiveness, two defining

features of asthma. Although the underlying mechanisms remain unknown, a number of reports have shown that IL-13 may exert its deleterious effects in asthma by directly acting on airway resident cells, including epithelial cells and airway smooth muscle cells (Wills-Karp, 2004). IL-13 levels in BAL fluid were determined with a cytokine-specific ELISA according to the manufacturer's instructions (Quantin, R&D Systems Europe), and were found to be increased 56-fold in asthmatic mice. However, IL-13 levels were reduced 50% after treatment of mice with AdABIN-1, as compared to the OVA-sensitised/challenged mice that were treated with PBS or the AdRR5 virus (Figure 8).

Example 7: AdABIN-1 reduces allergen-induced mucin production in the lungs of asthmatic mice

Airways of healthy mice have few, if any, mucus-producing goblet cells. However, airway inflammation is known to increase the number of goblet cells (hyperplasia) and the production of mucus. Mucus hypersecretion is a hallmark of asthma that contributes to airway obstruction. While the etiology is not well understood, hypersecretion has been linked to the presence of cytokines such as IL-4 and IL-13 in the inflamed airway. Many of these have been shown to induce mucin gene expression, a major component of mucus (Cohn et al., 2002). Moreover, NF-κB has been implicated in the regulation of mucin gene expression under some conditions (Li et al., 1998).

Mice that were sensitised with OVA showed a marked goblet hyperplasia in the airway epithelium after allergen challenge, which was associated with high mucus production, as revealed by Periodic Acid-Schiff (PAS) staining. However, OVA-sensitised/challenged mice treated with AdABIN-1 or AdIκBα^{S} showed a strong reduction in mucus production, whereas OVA-sensitised/challenged mice treated with AdRR5 or with PBS did not. These results are consistent with the observed reduction of IL-4 and IL-13 production in AdABIN-1 treated mice (Figure 8).

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Claims

1. The use of ABIN, or a functional fragment thereof, or a variant thereof, for the manufacture of a medicament for the treatment of airway inflammation.

2. The use according to claim 1 wherein said ABIN comprises the consensus amino acid sequence depicted in SEQ ID N° 4, SEQ ID N° 5 and/or SEQ ID N°6.

3. The use according to claim 1 wherein said functional fragment is a fragment that comprises the amino acid sequences as depicted in SEQ ID N°3 and that interacts with protein A20.

4. The use according to claim 1 wherein said functional fragment is a fragment that comprises amino acids 420-647 of SEQ ID N° 2, that interacts with protein A20 and/or inhibits NF-κB activation.

5. The use according to any of the preceding claims, whereby said ABIN, functional fragment or variant is fused or chemically coupled to a sequence facilitating protein transduction.

6. The use according to claim 5 whereby said fusion sequence is selected from the group consisting of the HIV TAT protein and a polyarginine sequence

7. The use of a nucleotide sequence encoding ABIN, or a functional fragment or variant thereof for the manufacture of a medicament for the treatment of airway inflammation.

8. The use according to claim 7 wherein the nucleotide sequence is cloned in a vector.

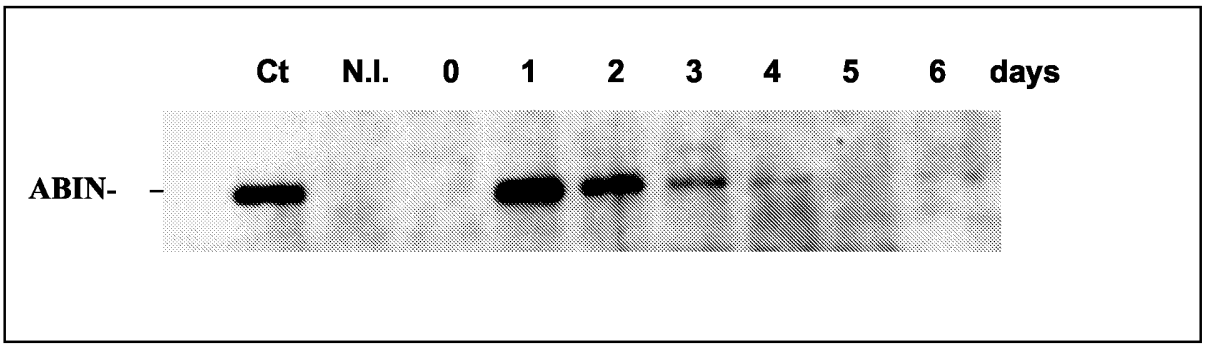
9. The use according to claim 8, whereby said vector is a gene therapy vector.

10. The use of an ABIN inducing and/or stabilizing compound for the manufacture of a medicament for the treatment of airway inflammation.

11. The use according to any of the preceding claims wherein the airway inflammation is asthma or chronic obstructive pulmonary.

Figures

Figure 1:



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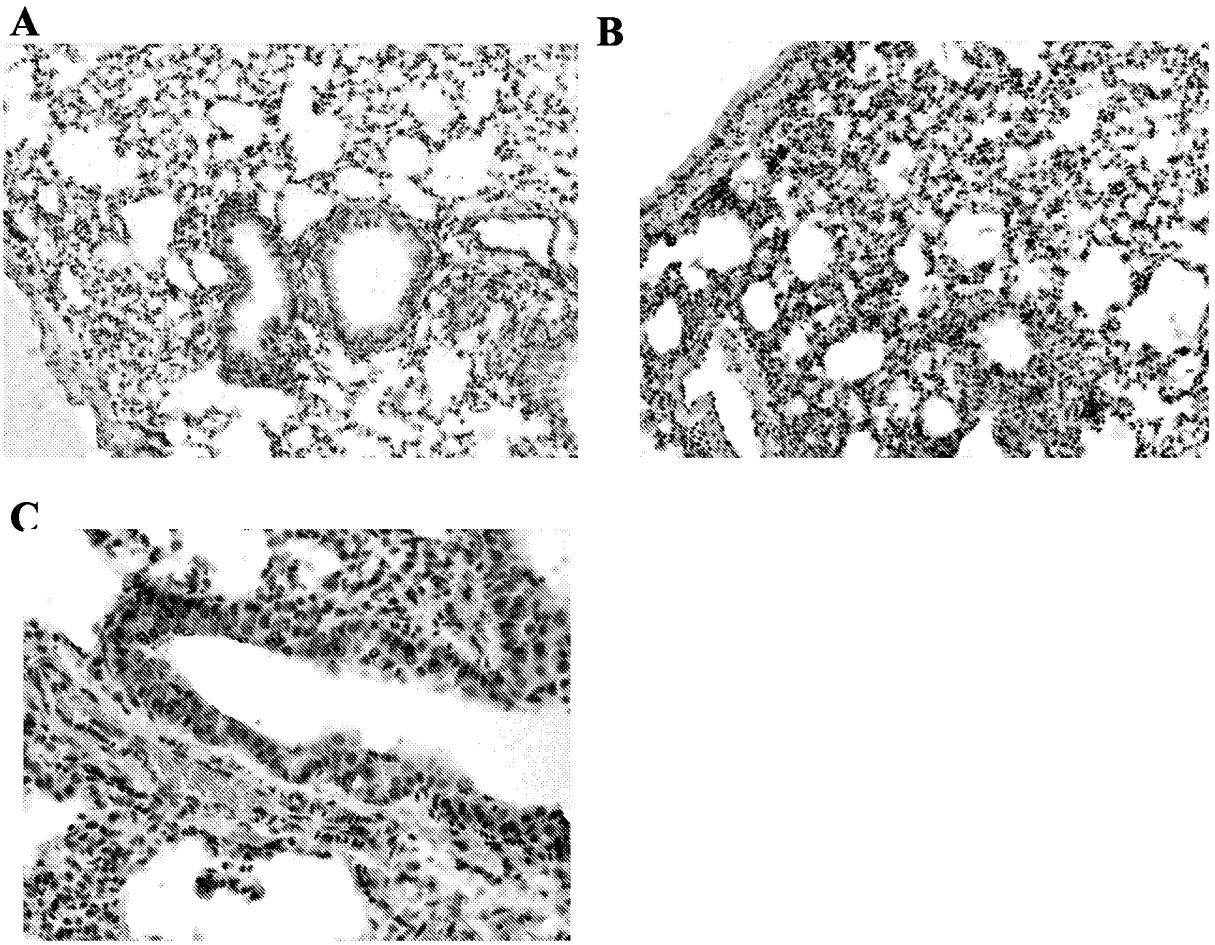
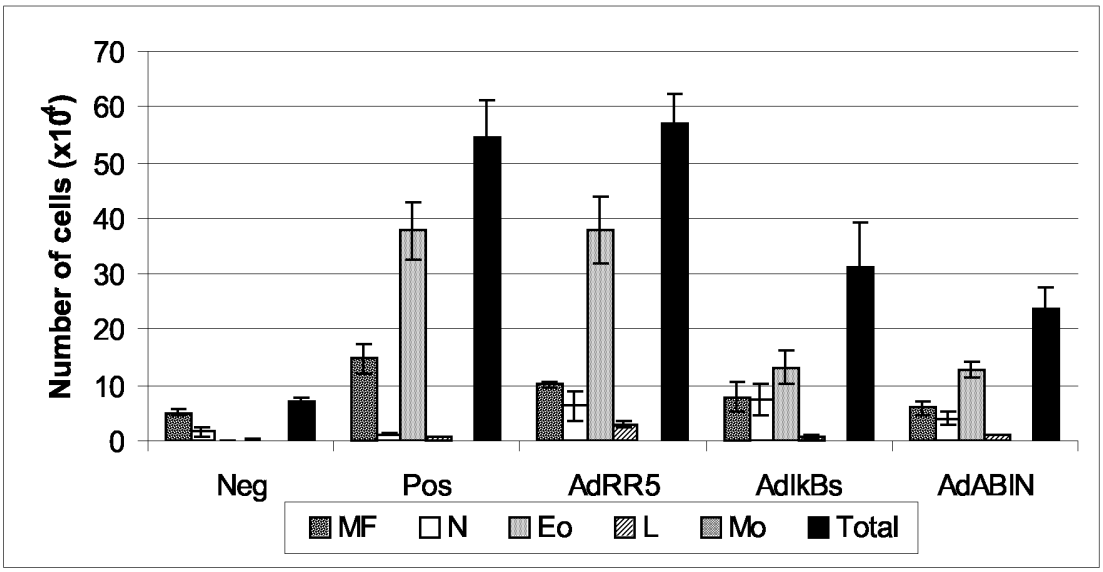


Figure 3:



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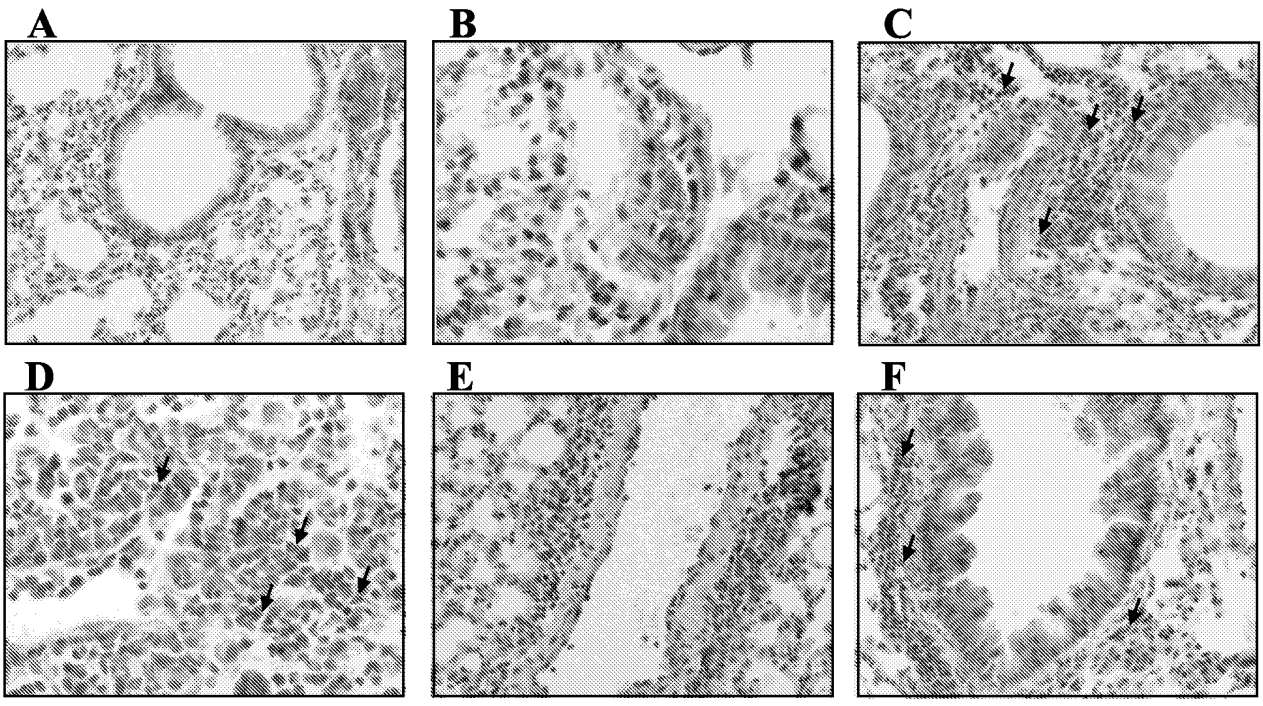

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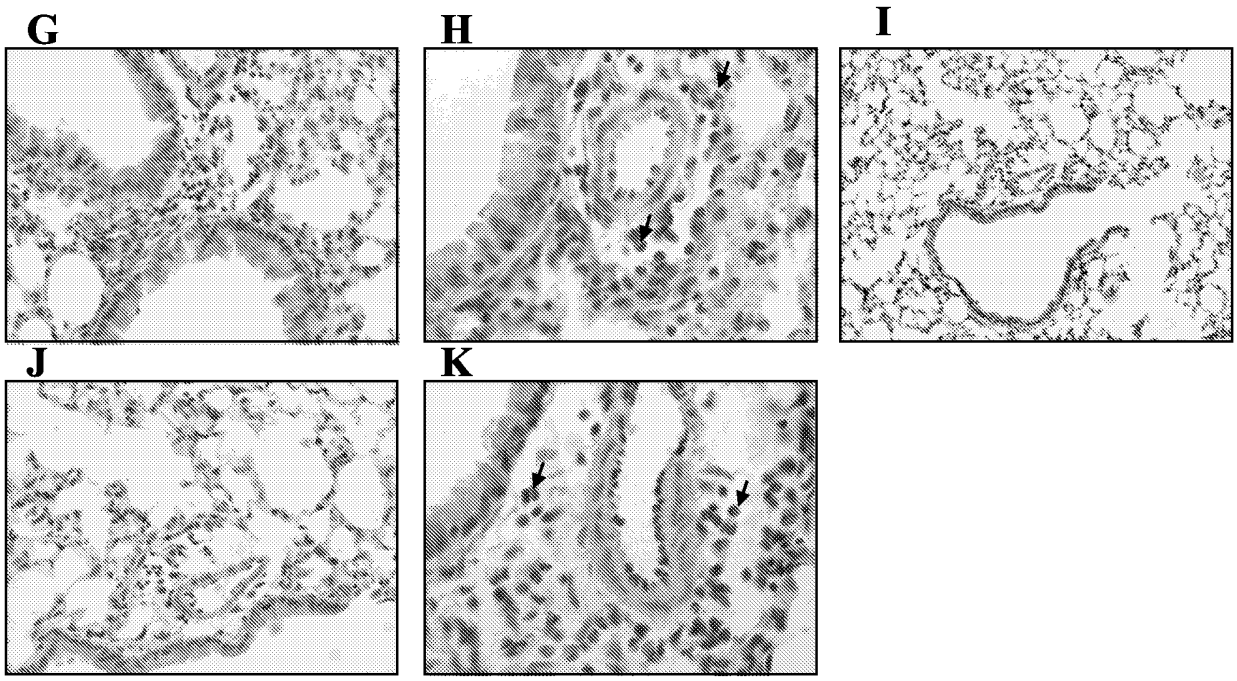


Figure 5 :

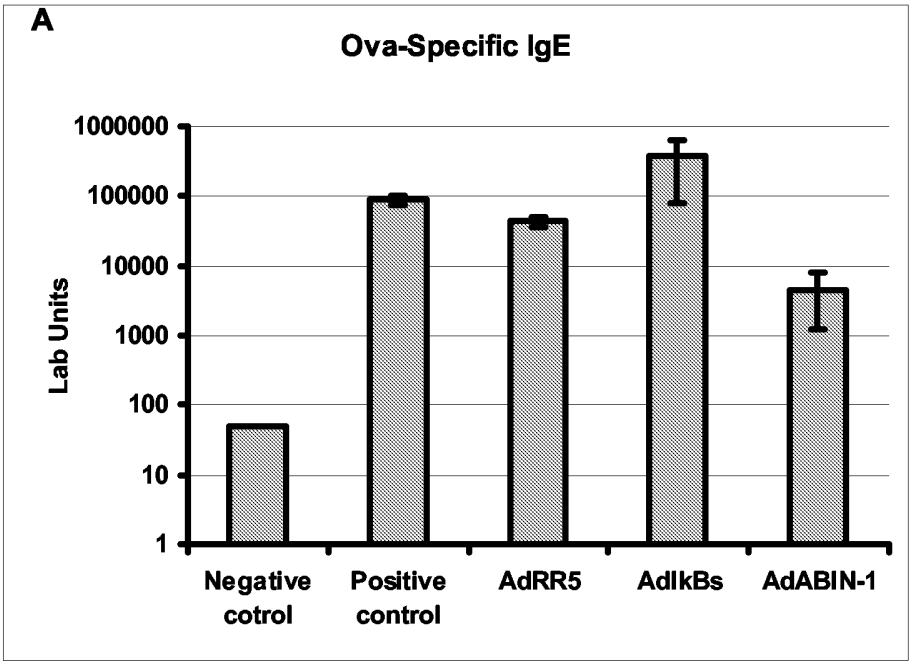
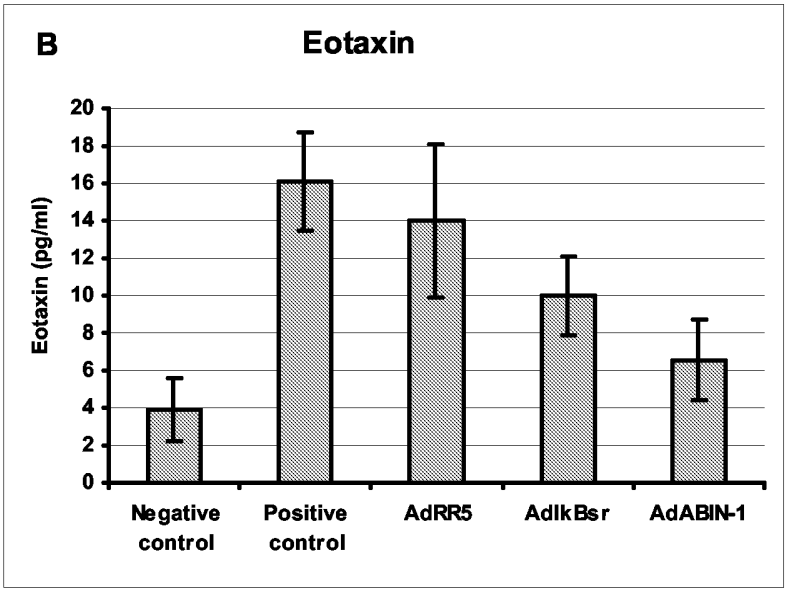
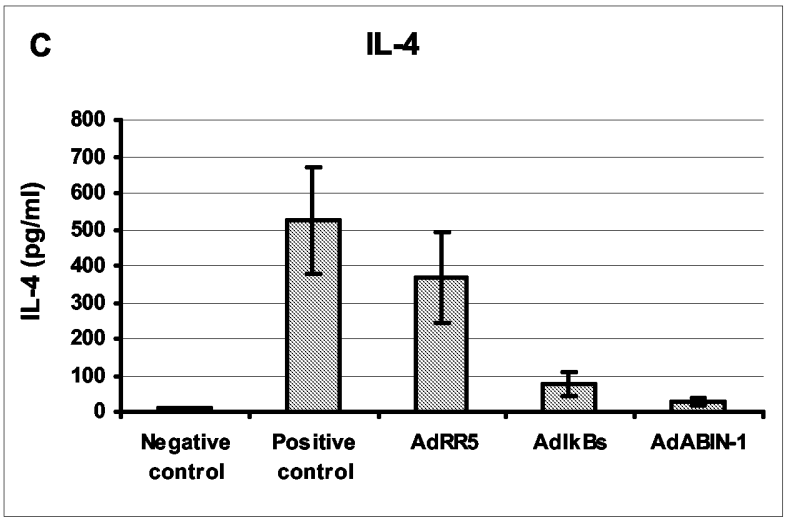


Figure 5 (cont.):





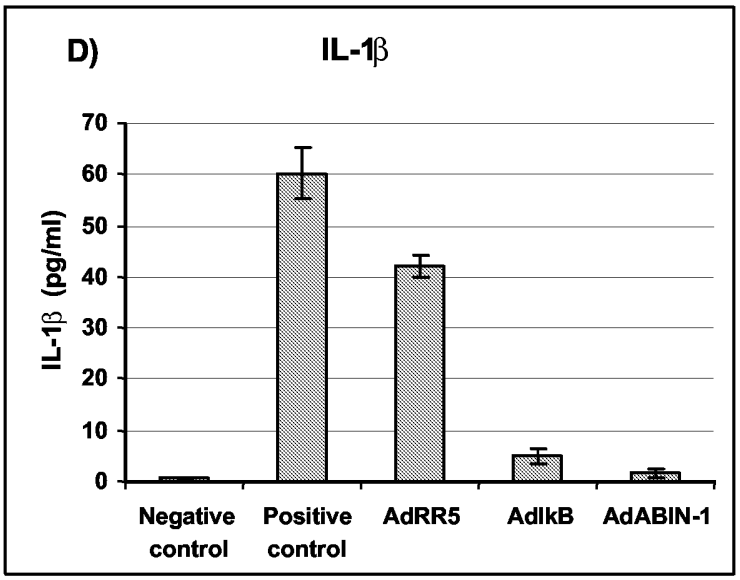


Figure 6 :

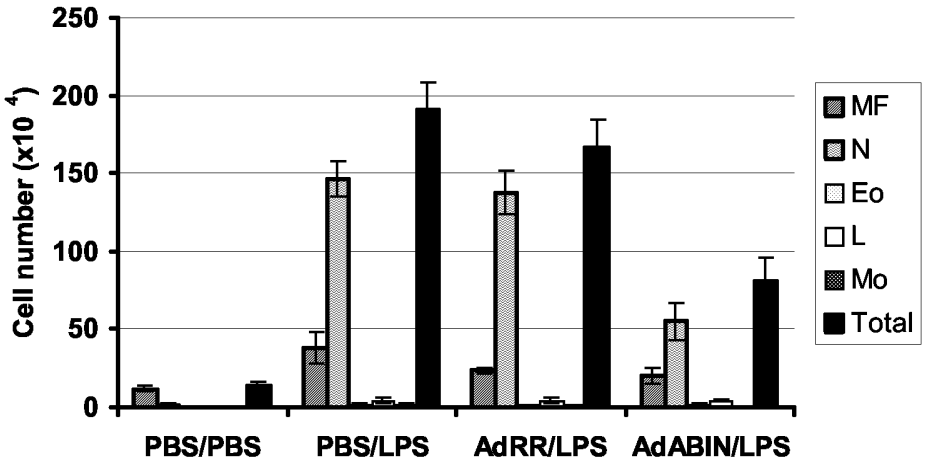


Figure 7 :

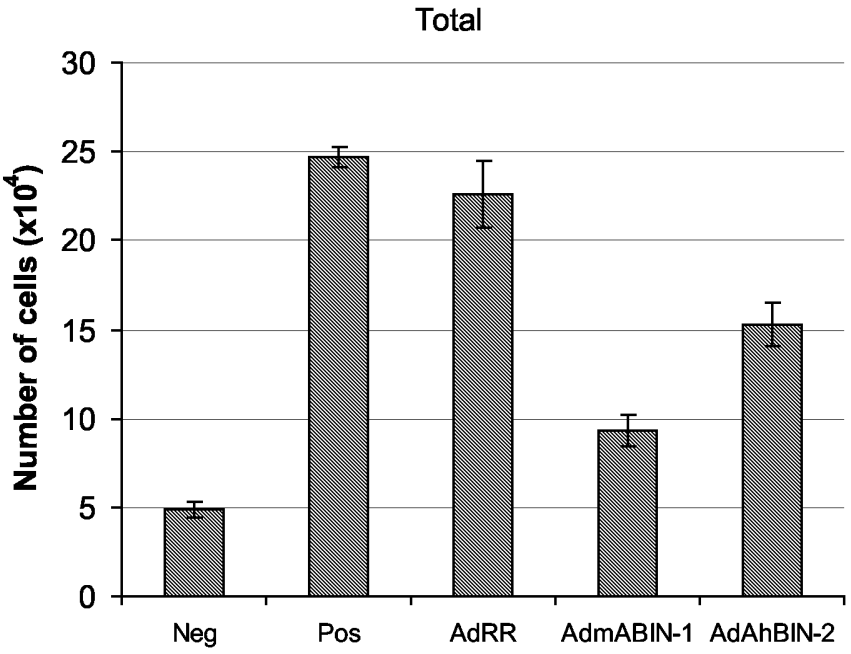


Figure 7 (cont.):

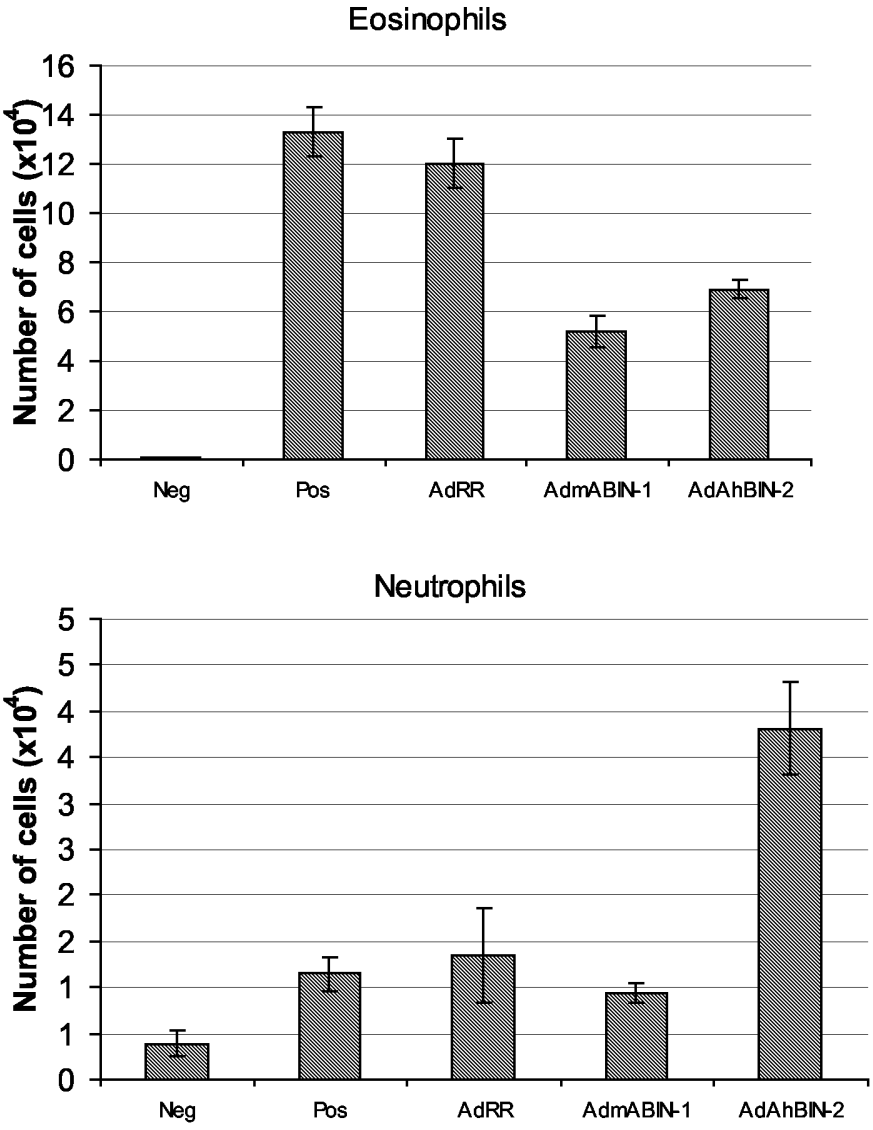


Figure 7 (cont.2) :

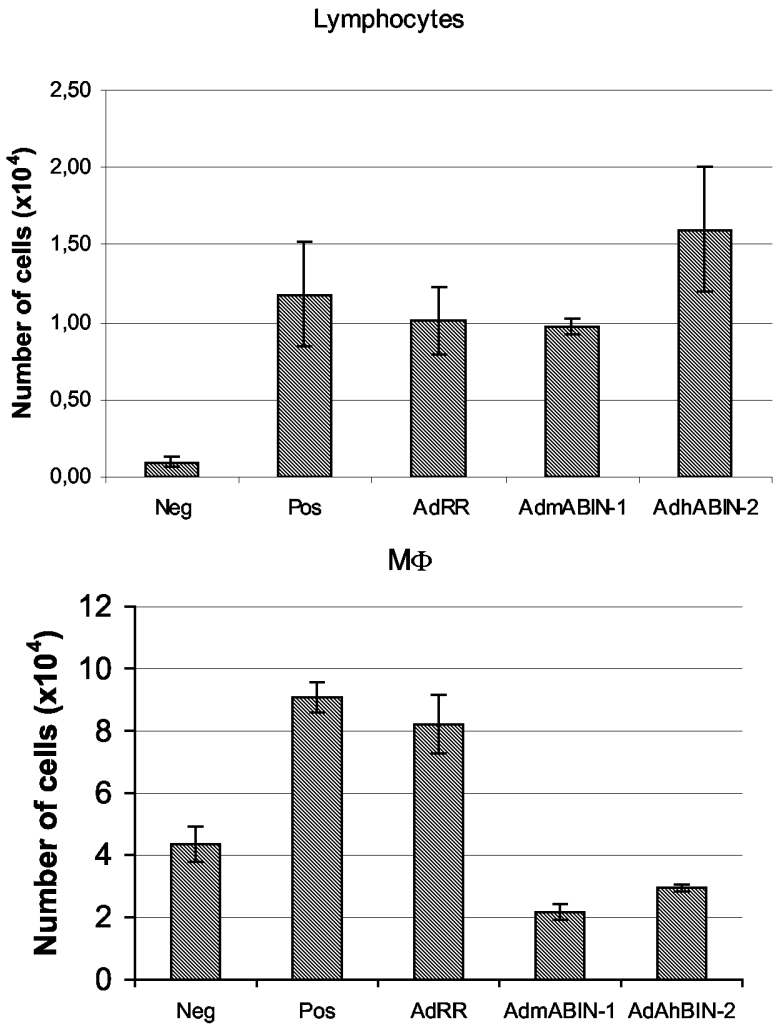


Fig. 8:

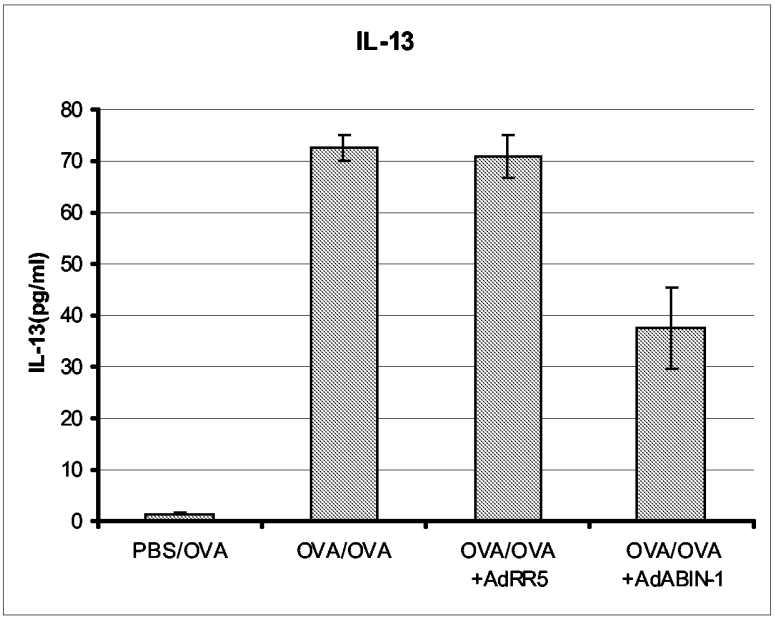
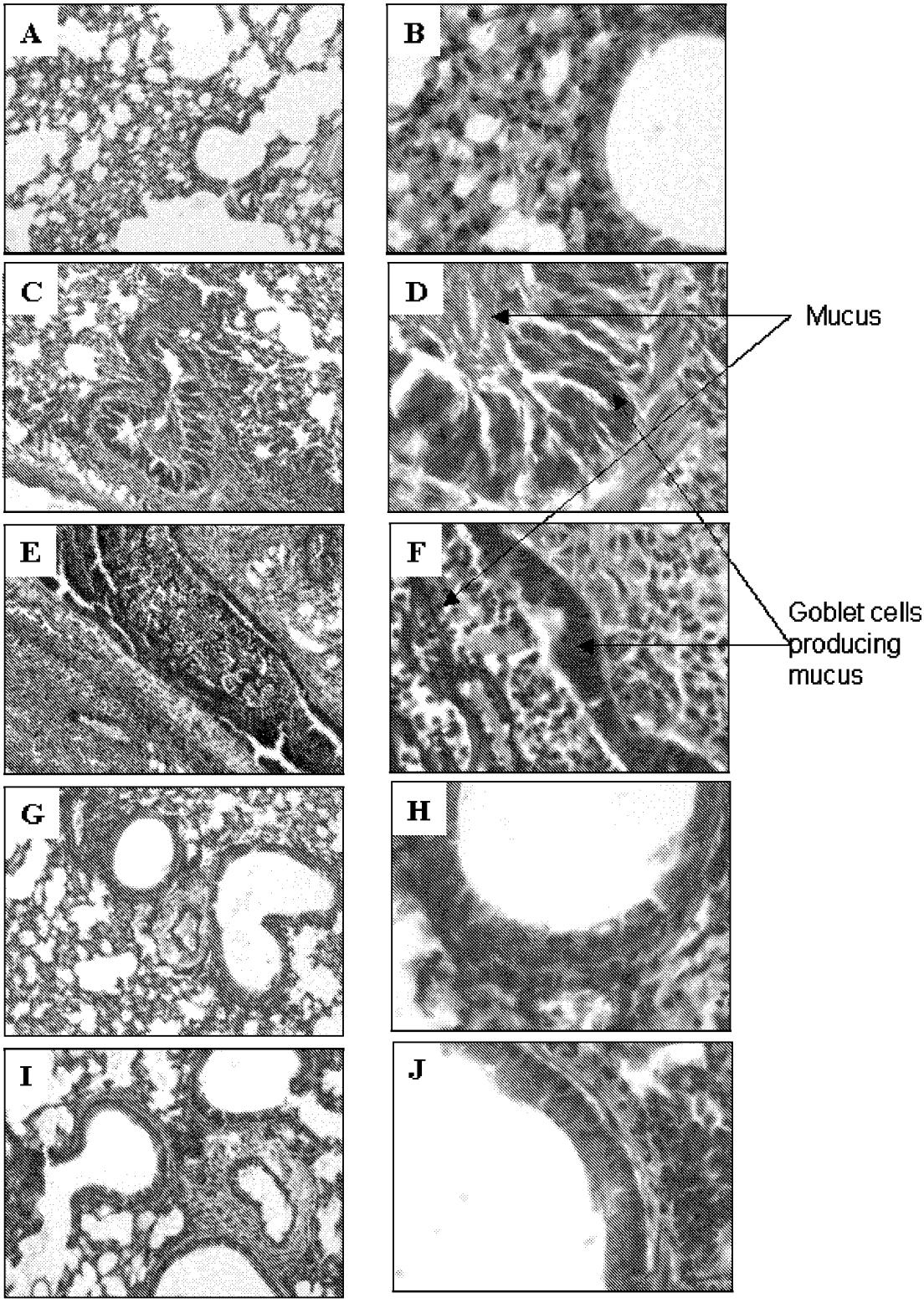


Fig. 9:



INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2005/054440

A. CLASSIFICATION OF SUBJECT MATTER
A61K38/17 A61P11/06

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)
A61P 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 practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data, PAJ, PASCAL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99/57133 A (VLAAMS INTERUNIV INST BIOTECH ; BEYAERT RUDI (BE); FIERS WALTER (BE);) 11 November 1999 (1999-11-11) cited in the application	1-4,7-11
Y	claims 1-21 page 14, line 30 - page 15, line 11	5,6
X	US 2004/092430 A1 (BEYAERT RUDI ET AL) 13 May 2004 (2004-05-13) paragraphs '0058!, '0059!	1-4,7-11
Y		5,6

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

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
5 January 2006	16/01/2006

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, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Fayos, C

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INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GARIEPY J ET AL: "Vectorial delivery of macromolecules into cells using peptide-based vehicles" TRENDS IN BIOTECHNOLOGY, ELSEVIER, AMSTERDAM,, GB, vol. 19, no. 1, 1 January 2001 (2001-01-01), pages 21-28, XP004227647 ISSN: 0167-7799 abstract page 22; table 1	5,6
A	WO 03/000280 A (VLAAMS INTERUNIV INST BIOTECH ; DELAEI FLIP (BE); LIBERT CLAUDE (BE);) 3 January 2003 (2003-01-03) cited in the application claims 1-10 page 3, lines 13-19	1-11
P,X	EL BAKKOURI KARIM ET AL: "Adenoviral gene transfer of the NF-kappa B inhibitory protein ABIN-1 decreases allergic airway inflammation in a murine asthma model." THE JOURNAL OF BIOLOGICAL CHEMISTRY. 6 MAY 2005, vol. 280, no. 18, 6 May 2005 (2005-05-06), pages 17938-17944, XP002361265 ISSN: 0021-9258 the whole document	1-11

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