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(54) Titre : METHODE PERMETTANT D'IDENTIFIER DES SUBSTANCES QUI INFLUENCENT POSITIVEMENT DES AFFECTIONS INFLAMMATOIRES DE MALADIES CHRONIQUES INFLAMMATOIRES DES VOIES RESPIRATOIRES

(54) Title: METHOD FOR IDENTIFYING SUBSTANCES WHICH POSITIVELY INFLUENCE INFLAMMATORY CONDITIONS OF CHRONIC INFLAMMATORY AIRWAY DISEASES

(57) Abrégé/Abstract:
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Method For Identifying Substances Which Positively Influence Inflammatory Conditions Of Chronic Inflammatory Airway Diseases

Introduction

The present invention belongs to the field of modulation of inflammatory processes, in particular of chronic inflammatory airway diseases, in which macrophages play an important role. The inflammatory processes can be modulated according to the invention by influencing the biological activity of a protein which is identified to be involved in the inflammatory process.

Examples for chronic inflammatory airway diseases, in which macrophages play an important role is chronic bronchitis (CB). CB may occur with or without airflow limitation and includes chronic obstructive pulmonary disease (COPD). CB is a complex disease encompassing symptoms of several disorders: chronic bronchitis which is characterized by cough and mucus hypersecretion, small airway disease, including inflammation and peribronchial fibrosis, emphysema, and airflow limitation. CB is characterized by an accelerated and irreversible decline of lung function. The major risk factor for developing CB is continuous cigarette smoking. Since only about 20% of all smokers are inflicted with CB, a genetic predisposition is also likely to contribute to the disease.

The initial events in the early onset of CB are inflammatory, affecting small and large airways. An irritation caused by cigarette smoking attracts macrophages and neutrophils the number of which is increased in the sputum of smokers. Perpetual smoking leads to an ongoing inflammatory response in the lung by releasing mediators from macrophages, neutrophils and epithelial cells that recruit inflammatory cells to sites of the injury. So far there is no therapy available to reverse the course of CB. Smoking cessation may reduce the decline of lung function.

Only a few drugs are known to date to provide some relief for patients. Long-lasting β2-agonists and anticholinergics are applied to achieve a transient bronchodilation. A

variety of antagonists for inflammatory events are under investigation like, LTB_4-inhibitors.

There is a continuous need to provide drugs for treating chronic inflammatory airway diseases. Chronic inflammatory airway diseases can be attributed to activated inflammatory immune cells, e.g. macrophages. There is therefore a need for drugs modulating the function of macrophages in order to eliminate a source of inflammatory processes.

Description Of The Invention

In the present invention it was found that macrophages involved in an inflammatory process, particularly in a chronic inflammatory airway disease, more particularly in chronic bronchitis or COPD, show a pattern of differentially expressed nucleic acid sequence and protein expression which differs from the pattern of gene expression of macrophages from healthy donors or donors in an irritated state, which latter do contain macrophages in an activated state. Therefore, macrophages show different activation levels under different inflammatory conditions. For example, it is shown in the present invention that macrophages involved in an inflammatory process in COPD smokers show different gene expression pattern than macrophages from healthy smokers, indicating that in COPD smokers macrophages are in a different, hereinafter named “hyperactivated” state. The present invention provides for the possibility to inhibit the hyperactivation or to reduce the hyperactive state of a macrophage by allowing the identification of substances which modulate a protein selected from the group consisting of MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase, all depicted in the Sequence Listing hereinafter, involved in the hyperactivation or maintaining the hyperactive state of a macrophage.

The term “chronic inflammatory airway disease” as used hereinafter includes, for example, Chronic Bronchitis (CB) and Chronic Obstructive Pulmonary Disease (COPD). The preferred meaning of the term “chronic inflammatory airway disease” is CB and COPD, the more preferred meaning is CB or COPD.

The invention is based on the identification of a nucleic acid sequence differentially expressed in a hyperactivated macrophage compared to a macrophage which is not hyperactivated. Such a nucleic acid sequence encodes a protein selected from the group consisting of MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase, which protein is involved in the hyperactivation or maintaining the hyperactive state of a macrophage involved in an inflammatory process, preferably in a chronic inflammatory airway disease. Such differentially expressed nucleic acid sequence or protein encoded by such nucleic acid sequence is in the following also named differentially expressed nucleic acid sequence or protein of the invention, respectively. In particular, the present invention teaches a link between phenotypic changes in macrophages due to differentially expressed nucleic acid sequence and protein expression pattern and involvement of macrophages in inflammatory processes and, thus, provides a basis for a variety of applications. For example, the present invention provides a method and a test system for determining the expression level of a macrophage protein of the invention or differentially expressed nucleic acid sequence of the invention and thereby provides e.g. for methods for diagnosis or monitoring of inflammatory processes with involvement of hyperactivated macrophages in mammalian, preferably human beings, especially such beings suffering from an inflammatory process, preferably in a chronic inflammatory airway disease. The invention also relates to a method for identifying a substance by means of a differentially expressed nucleic acid sequence or protein of the invention, which substance modulates, i.e. acts as an inhibitor or activator on the said differentially expressed nucleic acid sequence or protein of the invention and thereby positively influences chronic inflammatory processes by inhibition of the hyperactivation or reduction of the hyperactive state of macrophages, and thereby allows treatment of mammals, preferably human beings, suffering from a said disease. The invention also relates to a method for selectively modulating such a differentially expressed nucleic acid sequence or protein of the invention in a macrophage comprising administering a substance determined to be a modulator of said protein or differentially expressed nucleic acid sequence. The present invention includes the use of said substances for

treating beings in need of a treatment for an inflammatory process, preferably a chronic inflammatory airway disease.

In the present invention in a first step a differentially expressed nucleic acid sequence of the invention is identified which has a different expression pattern in a hyperactivated macrophage compared to a macrophage which is not hyperactivated. For the sake of conciseness this description deals particularly with investigation of macrophages involved in COPD, however, equivalent results may be obtained with samples from subjects suffering from other chronic inflammatory airway diseases, e.g. other chronic bronchitis symptoms. The investigation of the different expression pattern leads to the identification of a series of differentially expressed nucleic acid sequences expressed in dependency on the activation state of a macrophage involved in an inflammatory process, as exemplified in the Examples hereinbelow.

Briefly, such a differentially expressed nucleic acid sequence of the invention is identified by comparative expression profiling experiments using a cell or cellular extract from a hyperactivated macrophage, i.e. for example from the site of inflammation in COPD and from the corresponding site of control being not suffering from said disease, however, suffering under the same irritating condition like cigarette smoke exposure.

In a second step the proteins are identified which are encoded by the differentially expressed nucleic acid sequence, i.e. proteins playing a role in mediating the hyperactivation or in maintaining the hyperactivated state. A group of differentially expressed nucleic acid sequences of the invention can be identified to encode a protein which is selected from the group consisting of MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase. A said protein is involved in the hyperactivation or maintaining the hyperactive state which is characterized in that it is expressed in a macrophage that is hyperactivated according the invention at a lower or higher level than the control level in a macrophage which is not hyperactivated.

Accordingly, the invention concerns a protein selected from the group consisting of MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase. A protein selected from the said group is hereinafter also named protein of the invention. The said proteins of the invention are depicted hereinafter in the Sequence Listing.

The biological activity of MIF (SEQ ID NO. 1, 2) according to the present invention, i.e. mediating the involvement of a macrophage in an inflammatory process according to the invention, e.g. by inhibition of macrophage migration, is dependent, for example, on counteracting suppressive effects of glucocorticoids and/or on another MIF function like inducing inflammatory response to invasion of bacteria or any other function of MIF relevant for its biological activity according to the invention.

The invention also concerns a functional equivalent, derivative, variant, mutant or fragment of MIF. Functional in this context means having a function of the MIF that is involved in its biological activity according to the invention.

The biological activity of DAD1 (SEQ ID NO. 3, 4) according to the present invention, i.e. mediating the involvement of a macrophage in an inflammatory process according to the invention, is dependent, for example, on binding to an oligosaccaryltransferase complex and/or on any other DAD1 function relevant for its biological activity according to the invention.

The invention also concerns a functional equivalent, derivative, variant, mutant or fragment of DAD1. Functional in this context means having a function of DAD1 that is involved in its biological activity according to the invention.

The biological activity of ARL4 (SEQ ID NO. 5, 6) according to the present invention, i.e. mediating the involvement of a macrophage in an inflammatory process according to the invention, is dependent, for example, on interaction with proteins involved in vesicular and membrane trafficking and/or on any other ARL4 function relevant for its biological activity according to the invention.

The invention also concerns a functional equivalent, derivative, variant, mutant or fragment of ARL4. Functional in this context means having a function of ARL4 that is involved in its biological activity according to the invention.

The biological activity of GNS (SEQ ID NO. 7, 8) according to the present invention, i.e. mediating the involvement of a macrophage in an inflammatory process according to the invention, is dependent, for example, on binding and/or recognizing a substrate, e.g. heparan and/or on its hydrolytic activity and/or on any other GNS function relevant for its biological activity according to the invention.

The invention also concerns a functional equivalent, derivative, variant, mutant or fragment of GNS. Functional in this context means having a function of GNS that is involved in its biological activity according to the invention.

The biological activity of Transglutaminase 2 (SEQ ID NO. 9, 10) according to the present invention, i.e. mediating the involvement of a macrophage in an inflammatory process according to the invention, is dependent, for example, on formation of (γ-glutamyl)lysine isopeptide bonds and/or on any other Transglutaminase 2 function, e.g. substrate recognition, relevant for its biological activity according to the invention.

The invention also concerns a functional equivalent, derivative, variant, mutant or fragment of Transglutaminase 2. Functional in this context means having a function of Transglutaminase 2 that is involved in its biological activity according to the invention.

The biological activity of Stearyl-CoA-Desaturase (SEQ ID NO. 11, 12) according to the present invention, i.e. mediating the involvement of a macrophage in an inflammatory process according to the invention, is dependent, for example, on binding to a substrate, e.g. palmitoyl-CoA and/or stearyl-CoA and/ or on its oxidative activity and/or on any other Stearyl-CoA-Desaturase function, e.g. substrate recognition, relevant for its biological activity according to the invention.

The invention also concerns a functional equivalent, derivative, variant, mutant or fragment of Stearyl-CoA-Desaturase. Functional in this context means having a function of Stearyl-CoA-Desaturase that is involved in its biological activity according to the invention.

The biological activity of UDP-Glucose Ceramide Glycosyltransferase (SEQ ID NO. 13, 14) according to the present invention, i.e. mediating the involvement of a macrophage in an inflammatory process according to the invention, is dependent, for example, on binding to a substrate, e.g. UDP-glucose and/or ceramide and/ or on its transferring activity and/or on any other UDP-Glucose Ceramide Glycosyltransferase function, e.g. substrate recognition, relevant for its biological activity according to the invention.

The invention also concerns a functional equivalent, derivative, variant, mutant or fragment of UDP-Glucose Ceramide Glycosyltransferase. Functional in this context means having a function of UDP-Glucose Ceramide Glycosyltransferase that is involved in its biological activity according to the invention.

According to the present invention, the biological activity of a protein selected from the group consisting of MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase, if expressed at a lower level than the control level, is preferably activated in order to inhibit hyperactivation or reduce a hyperactivated state of a macrophage, and if expressed at a higher level than the control level, is preferably inhibited in order to inhibit hyperactivation or reduce a hyperactivated state of a macrophage.

In one embodiment the present invention concerns a test method for determining a substance to be an activator or inhibitor of protein selected from the group consisting of MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase. Since such a protein is involved in a chronic inflammatory airway disease and plays a role in mediating inflammation, a substance modulating the biological activity of such a protein can be used for treating a chronic

inflammatory airway diseases or can be used as lead compound for optimization of the function of the substance in a way that the optimized substance is suitable for treating chronic inflammatory airway diseases. For performing a method of the invention, a test system according to the invention can be used.

The present invention also concerns a test system for determining whether a substance is an activator or an inhibitor of a protein selected from the group consisting of MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase. A test system useful for performing a method of the invention comprises a cellular or a cell-free system. For example, one embodiment of to the invention concerns a test system that is designed in a way to allow the testing of substances acting on the expression level of the differentially expressed nucleic acid sequence e.g. using expression of a reporter-gene, e.g. luciferase gene or the like, as a measurable readout. Another embodiment of the invention concerns a test system that is designed in a way to allow the testing of substances directly interacting with a respective function of a protein of the invention or interfering with the respective activation of a function a protein of the invention by a natural or an artificial but appropriate activator of the respective protein selected from the group consisting of: MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase, e.g. an appropriate kinase or the like.

A test system according to the invention comprises a protein selected from the group consisting of: MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase, or a functional equivalent, derivative, variant, mutant or fragment of a said protein of the invention, a nucleic acid encoding a said protein or encoding a functional equivalent, derivative, variant, mutant or fragment of a said protein of the invention and/or regulatory elements, wherein a functional equivalent, derivative, variant, mutant or fragment of a said protein of the invention or a nucleic acid encoding a said protein or a functional equivalent, derivative, variant, mutant or fragment of a said protein of the invention is capable to interact with a substance which should be tested in a way that direct interaction leads to a measurable read-out indicative for the change of a respective

biological activity of a said protein according to the invention and /or for the change of expression of a said protein of the invention.

A test system of the invention comprises, for example, elements well known in the art. For example, cell-free systems may include, for example, a said protein or a functional equivalent, derivative, variant, mutant or fragment of a said protein of the invention, a nucleic acid encoding a said protein or encoding a functional equivalent, derivative, variant, mutant or fragment of a said protein of the invention in soluble or bound form or in cellular compartments or vesicles. Suitable cellular systems include, for example, a suitable prokaryotic cell or eukaryotic cell, e.g. such comprising a said protein of the invention or a functional equivalent, derivative, variant, mutant or fragment of a said protein of the invention, a nucleic acid encoding a said protein or encoding a functional equivalent, derivative, variant, mutant or fragment of a said protein of the invention. A cell suitable for use in a said test system of the invention may be obtained by recombinant techniques, e.g. after transformation or transfection with a recombinant vector suitable for expression of a desired protein of the invention or functional equivalent, derivative, variant, mutant or fragment of a said protein of the invention, or may e.g. be a cell line or a cell isolated from a natural source expressing a desired protein of the invention or functional equivalent, derivative, variant, mutant or fragment of a said protein. A test system of the invention may include a natural or artificial ligand of the protein selected from the group consisting of MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase if desirable or necessary for testing whether a substance of interest is an inhibitor or activator of a said protein of the invention.

A test method according to the invention comprises measuring a read-out, e.g. a phenotypic change in the test system, for example, if a cellular system is used a phenotypic change of the cell. Such change may be a change in a naturally occurring or artificial response, e.g. a reporter gene expression of the cell to a protein selected from the group consisting of: MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase activation or inhibition, e.g. as detailed in the Examples hereinbelow.

A test method according to the invention can on the one hand be useful for high throughput testing suitable for determining whether a substance is an inhibitor or activator of the invention, but also e.g. for secondary testing or validation of a hit or lead substance identified in high throughput testing.

The present invention also concerns a substance identified in a method according to the invention to be an inhibitor or activator of a protein selected from the group consisting of: MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase. A substance of the present invention is any compound which is capable of activating or preferably inhibiting a function of a protein selected from the group consisting of: MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase. An example of a way to activate or inhibit a function of a protein selected from the group consisting of: MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase is by influencing the expression level of a said protein selected from the group consisting of: MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase. Another example of a way to activate or inhibit a function of a protein selected from the group consisting of: MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase is to apply a substance directly binding a protein selected from the group consisting of: MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase and thereby activating or blocking functional domains of a said protein of the invention, which can be done reversibly or irreversibly, depending on the nature of the substance applied.

Accordingly, a substance useful for activating or inhibiting biological activity of a protein selected from the group consisting of MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase includes a substance acting on the expression of differentially expressed nucleic acid sequence, for example a nucleic acid fragment hybridizing with the corresponding gene or regulatory sequence and thereby influencing gene

expression, or a substance acting on a protein selected from the group consisting of: MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase itself or on its activation or inhibition by other naturally occurring cellular components, e.g. an other protein acting enzymatically on a said protein of the invention, e.g. a protein kinase.

Therefore, the invention concerns, for example, a substance which is a nucleic acid sequence coding for the gene of a protein of the invention, or a fragment, derivative, mutant or variant of such a nucleic acid sequence, which nucleic acid sequence or a fragment, derivative, mutant or variant thereof is capable of influencing the gene expression level, e.g. a nucleic acid molecule suitable as antisense nucleic acid, ribozyme, or for triple helix formation.

The invention also concerns a substance which is e.g. an antibody or an organic or inorganic compound directly binding to or interfering with the activation of a protein selected from the group consisting of: MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase and thereby affecting its biological activity.

In a further aspect, the present invention relates to a method for determining an expression level of a nucleic acid coding for a protein of the invention, preferably messenger RNA, or protein of the invention itself, in cell, preferably in a macrophage, more preferably in a macrophage isolated form a site of inflammation, even more preferably from a site of inflammation in a subject suffering from a chronic inflammatory airway disease. Such a method can be used, for example, for testing whether a substance is capable of influencing differentially expressed nucleic acid sequence expression levels in a method outlined above for determining whether a substance is an activator or inhibitor according to the present invention. A method for determining an expression level according to the invention can, however, also be used for testing the activation state of a macrophage, e.g. for diagnostic purposes or for investigation of the success of treatment for a disease which is caused by the hyperactivated macrophage. Said macrophage is preferably a mammalian, more preferably a human cell. Accordingly, macrophages of the present invention are

preferably obtainable from the site of inflammation in a mammal and more preferably from a site of inflammation in a human being. Accordingly, the invention also relates to a method for diagnosis of a chronic inflammatory disease, or monitoring of such disease, e.g. monitoring success in treating beings in need of treatment for such disease, comprising determining an expression level of a nucleic acid coding for a protein of the invention, preferably messenger RNA, or protein of the invention itself in a macrophage.

A method for determining expression levels of a nucleic acid coding for a protein of the invention, preferably messenger RNA, or protein of the invention itself can, depending on the purpose of determining the expression level, be performed by known procedures such as measuring the concentration of respective RNA transcripts via hybridization techniques or via reporter gene driven assays such as luciferase assays or by measuring the protein concentration of said protein of the invention using respective antibodies.

The present invention also relates to the use of a substance according to the invention for the treatment for a chronic inflammatory airway disease. Another embodiment of the present invention relates to a pharmaceutical composition comprising at least one of the substances according to the invention determined to be an activator or an inhibitor. The composition may be manufactured in a manner that is itself known, e.g. by means of conventional mixing, dissolving, granulating, dragee-making, levigating, powdering, emulsifying, encapsulating, entrapping or lyophilizing processes.

In order to use substances activating or inhibiting according to the invention as drugs for treatment for chronic inflammatory airway diseases, the substances can be tested in animal models for example an animal suffering from an inflammatory airway disorder or a transgenic animal expressing protein of the invention.

Toxicity and therapeutic efficacy of a substance according to the invention can be determined by standard pharmaceutical procedures, which include conducting cell culture and animal experiments to determine the IC_{50}, LD_{50} and ED_{50}. The data

obtained are used for estimating the animal or more preferred the human dose range, which will also depend on the dosage form (tablets, capsules, aerosol sprays ampules, etc.) and the administration route (for example transdermal, oral, buccal, nasal, enteral, parenteral, inhalative, intratracheal, or rectal).

A pharmaceutical composition containing at least one substance according to the invention as an active ingredient can be formulated in conventional manner. Methods for making such formulations can be found in manuals, e.g. “Remington Pharmaceutical Science”. Examples for ingredients that are useful for formulating at least one substance according to the present invention are also found in WO 99/18193, which is hereby incorporated by reference.

In a further aspect the invention concerns a method for treating a chronic inflammatory airway disease. Such method comprises administering to a being, preferably to a human being, in need of such treatment a suitable amount of a pharmaceutical composition comprising at least one substance determined to be an activator or inhibitor by a method according to the invention.

In an other embodiment the invention relates to a method for selectively modulating the concentration of a protein of the invention in a macrophage, comprising administering a substance determined to be an activator or inhibitor of protein of the invention.

The following examples are meant to illustrate the present invention, however, shall not be construed as limitation. However, the Examples describe most preferred embodiments of the invention.

Examples

Example 1: Comparative Expression Profiling

The following is an illustration of how comparative expression profiling can be performed in order to identify protein of the invention.

1.1. Selection of Subjects

Three groups of subjects are studied: healthy non-smokers, healthy smokers and patients with COPD.

In order to assess lung function subjects have to perform spirometry. A simple calculation based on age and height is used to characterize the results. COPD subjects are included if their FEV_1 % predicted is <70%. Healthy smokers are age and smoking history matched with the COPD subjects but have normal lung function. Healthy non-smokers have normal lung function and have never smoked. The latter group has a methacholine challenge to exclude asthma. This technique requires increasing doses of methacholine to be given to the subject, with spirometry between each dose. When the FEV_1 falls 20% the test is stopped and the PC_{20} is calculated. This is the dose of methacholine causing a 20% fall in FEV_1 and we will require a value of >32 as evidence of absence of asthma. All subjects have skin prick tests to common allergens and are required to have negative results. This excludes atopic individuals. The clinical history of the subjects is monitored and examined in order to exclude concomitant disease.

1.2. BAL (bronchoalveolar lavage) Procedure

Subjects are sedated with midazolam prior to the BAL. Local anaesthetic spray is used to anaesthetize the back of the throat. A 7mm Olympus bronchoscope is used. The lavaged area is the right middle lobe. 250 ml of sterile saline is instilled and immediately aspirated. The resulting aspirate contains macrophages.

1.3. BAL Processing

BAL is filtered through sterile gauze to remove debris. The cells are washed twice in HBSS, resuspended in 1ml HBSS (Hank's Balanced Salt Solution) and counted. The macrophages are spun to a pellet using 15 ml Falcon blue-cap polypropylen, resuspended in Trizol reagent (Gibco BRL Life Technologies) at a concentration of 1 ml Trizol reagent per 10 million cells and then frozen at -70°C.

1.4. Differential Gene Expression Analysis

Total RNA is extracted from macrophage samples obtained according to Example 1.3. Cell suspensions in Trizol are homogenized through pipetting and incubated at room temperature for 5 minutes. 200 µl chloroform per ml Trizol is added, the mixture carefully mixed for 15 seconds and incubated for 3 more minutes at room temperature. The samples are spun at 10000g for 15 minutes at 4°C. The upper phase is transferred into a new reaction tube and the RNA is precipitated by adding 0.5 ml isopropanol per ml Trizol for 10 minutes at room temperature. Then, the precipitate is pelleted by using a microcentifuge for 10 minutes at 4°C with 10000g, the pellet is washed twice with 75% ethanol, air dried and resuspended in DEPC-H_2O.

An RNA cleanup with Qiagen RNeasy Total RNA isolation kit (Qiagen) is performed in order to improve the purity of the RNA. The purity of the RNA is determined by agarose gelelectrophoresis and the concentration is measured by UV absorption at 260 nm.

5 µg of each RNA is used for cDNA synthesis. First and second strand synthesis are performed with the SuperScript Choice system (Gibco BRL Life Technologies). In a total volume of 11 µl RNA and 1 µl of 100 µM T7-$(dt)_{24}$ primer, sequence shown in SEQ ID NO. 15, are heated up to 70°C for 10 minutes and then cooled down on ice for 2 minutes. First strand buffer to a final concentration of 1x, DTT to a concentration of 10 mM and a dNTP mix to a final concentration of 0.5 mM are added to a total volume of 18 µl. The reaction mix is incubated at 42°C for 2 minutes and 2 µl of Superscript II reverse transcriptase (200 U/µl) are added. For second strand synthesis 130 µl of a mix containing 1.15x second strand buffer, 230 µM dNTPs, 10 U E.coli DNA ligase (10U/µl), E.coli DNA polymerase (10 U/µl), RNase H (2U/µl) is added to the reaction of the first strand synthesis and carefully mixed with a pipette. Second strand synthesis is performed at 16°C for 2 hours, then 2 µl of T4 DNA polymerase (5 U/µl) are added, incubated for 5 minutes at 16°C and the reaction is stopped by adding 10 µl 0.5 M EDTA.

Prior to cRNA synthesis the double stranded cDNA is purified. The cDNA is mixed with an equal volume of phenol:chloroform:isoamylalcohol (25:24:1) and spun through the gel matrix of phase lock gels (Eppendorf) in a microcentrifuge in order to separate the cDNA from unbound nucleotides. The aqueous phase is precipitated with ammoniumacetate and ethanol. Subsequently, the cDNA is used for in vitro transcription. cRNA synthesis is performed with the ENZO BioArray High Yield RNA Transcript Labeling Kit according to manufacturer's protocol (ENZO Diagnostics). Briefly, the cDNA is incubated with 1x HY reaction buffer, 1x biotin labeled ribonucleotides, 1x DTT, 1x RNase Inhibitor Mix and 1x T7 RNA Polymerase in a total volume of 40 µl for 5 hours at 37°C. Then, the reaction mix is purified via RNeasy columns (Qiagen), the cRNA precipitated with ammonium acetate and ethanol and finally resuspended in DEPC-treated water. The concentration is determined via UV spectrometry at 260 nm. The remaining cRNA is incubated with 1x fragmentation buffer (5x fragmentation buffer: 200 mM Tris acetate, pH 8.1, 500 mM KOAc, 150 mM MgOAc) at 94°C for 35 minutes.

For hybridization of the DNA chip 15 µg of cRNA is used, mixed with 50 pM biotin-labeled control B2 oligonucleotide, sequence shown SEQ ID NO. 16, 1x cRNA cocktail, 0.1 mg/ml herring sperm DNA, 0.5 mg/ml acetylated BSA, 1x MES (2-[N-morpholino]-ethanesulfonic acid) hybridization buffer in a total volume of 300 µl. The hybridization mixture is heated up to 99°C for 5 minutes, cooled down to 45°C for 10 minutes and 200 µl of the mix are used to fill the probe array. The hybridization is performed at 45°C at 60 rpm for 16 hours.

After the hybridization the hybridization mix on the chip is replaced by 300 µl non-stringent wash buffer (100 mM MES, 100 mM NaCl, 0.01% Tween 20). The chip is inserted into an Affymetrix Fluidics station and washing and staining is performed according to the EukGE-WS2 protocol. The staining solution per chip consists of 600 µl 1x stain buffer (100 mM MES, 1 M NaCl, 0.05% Tween 20), 2 mg/ml BSA, 10 µg/ml SAPE (streptavidin phycoerythrin) (Dianova), the antibody solution consists of 1x stain buffer, 2 mg/ml BSA, 0.1 mg/ml goat IgG, 3 µg/ml biotinylated antibody. After the washing and staining procedure the chips are scanned on the HP Gene Array Scanner (Hewlett Packard).

Data Analysis is performed by pairwise comparisons between chips hybridized with RNA isolated from COPD smokers and chips hybridized with RNA isolated from healthy smokers.

The following is an illustration of differentially expressed genes and their function as identified according to the approach of the present invention.

Example 2: MIF

A gene identified as consistently upregulated in individuals with COPD codes for MIF. MIF is secreted by pituitary cells, macrophages, and T cells and its synthesis can be induced by proinflammatory stimuli such as LPS, TNFα, and IFN-γ. MIF itself has proinflammatory activity by counteracting suppressive effects of glucocorticoids and by inducing inflammation in response to invasion of bacteria. Neutralizing MIF can prevent septic shock in certain mouse models (Calandra et al. 1994, Bernhagen et al. 1998, Calandra et al. 2000)

MIF is consistently found upregulated (42%) in COPD smokers compared to healthy smokers. This is shown by "fold change" values (Tab. 1). The p value for comparing COPD smokers and healthy smokers is 0.03.

Tab.1: Deregulation of MIF: “fold change” (FC) values for each patient are listed for the comparisons between obstructed and healthy smokers.

comp	FC	comp	FC	comp	FC	comp	FC
1 vs 2	-1.3	5 vs 43	3.9	39 vs 57	-2.0	68 vs 66	2.8
1 vs 37	8.0	5 vs 56	1.9	39 vs 58	1.0	68 vs 69	2.3
1 vs 43	1.8	5 vs 57	1.5	39 vs 62	1.0	68 vs 76	5.0
1 vs 56	-1.3	5 vs 58	2.9	44 vs 2	1.4	68 vs 78	3.2
1 vs 57	-1.6	5 vs 62	2.0	44 vs 37	14.4	70 vs 65	1.1
1 vs 58	1.2	6 vs 2	-1.6	44 vs 43	3.0	70 vs 66	1.4
1 vs 62	-1.2	6 vs 37	6.5	44 vs 56	1.4	70 vs 69	1.1
3 vs 2	-1.6	6 vs 43	1.5	44 vs 57	1.1	70 vs 76	2.6
3 vs 37	6.3	6 vs 56	-1.6	44 vs 58	2.1	70 vs 78	1.6

3 vs 43	1.4	6 vs 57	-2.0	44 vs 62	1.5	71 vs 65	2.1
3 vs 56	-1.6	6 vs 58	1.0	64 vs 65	2.0	71 vs 66	2.7
3 vs 57	-2.1	6 vs 62	-1.5	64 vs 66	2.6	71 vs 69	2.2
3 vs 58	-1.1	39 vs 2	-1.6	64 vs 69	2.1	71 vs 76	4.9
3 vs 62	-1.5	39 vs 37	1.0	64 vs 76	4.7	71 vs 78	3.1
5 vs 2	1.9	39 vs 43	1.0	64 vs 78	3.0		
5 vs 37	18.5	39 vs 56	-1.5	68 vs 65	2.1		

2.1. Cloning of MIF

MIF is cloned from a total RNA extracted from human THP-1 cells. 5 μg RNA is reverse transcribed into cDNA with 5 ng oligo$(dt)_{18}$ primer, 1x first strand buffer, 10 mM DTT, 0.5 mM dNTPs and 2 U Superscript II (Gibco BRL) at 42°C for 50 minutes. Then, the reaction is terminated at 70°C for 15 minutes and the cDNA concentration is determined by UV-spectrophotometry. For amplification of MIF 100 ng of the cDNA and 10 pmoles of sequence-specific primers for MIF (forward primer, SEQ ID NO. 17 and reverse primer, SEQ ID NO. 18) are used for PCR. Reaction conditions are: 2 minutes of 94°C, 35 cycles with 30 seconds at 94°C, 30 seconds at 53°C, 90 seconds at 72°C, followed by 7 minutes at 72°C with Taq DNA-polymerase. The reaction mix is separated on a 2% agarose gel, a band of about 360bp is cut out and purified with the QIAEX II extraction kit (Qiagen). The concentration of the purified band is determined and about 120 ng are incubated with 300 ng of pDONR201, the donor vector of the Gateway system (Life Technologies), 1x BP clonase reaction buffer, BP clonase enzyme mix in a total volume of 20 μl for 60 minutes at 25°C. Then, reactions are incubated with 2 μl of proteinase K and incubated for 10 minutes at 37°C. The reaction mix is then electroporated into competent DB3.1 cells and plated on Kanamycin-containing plates. Clones are verified by sequencing. A clone, designated pDONR-MIF, with identical sequence to the database entry (acc. L19686) is used for further experiments.

2.2. Generation of a transfection vector for MIF

The vector containing MIF described under 1.1. is used to transfer the cDNA for MIF to the expression vector pcDNA3.1(+)/attR that contains the "attR1" and "attR2" recombination sites of the Gateway cloning system (Life Technologies) where MIF is expressed under the control of the CMV promoter. 150 ng of the "entry vector" pDONR-MIF is mixed with 150 ng of the "destination vector" pcDNA3.1(+)/attR, 4 µl of the LR Clonase enzyme mix, 4 µl LR Clonase reaction buffer, added up with TE (Tris/EDTA) to 20 µl and incubated at 25°C for 60 minutes. Then, 2 µl of proteinase K solution is added and incubated for 10 minutes at 37°C. 1 µl of the reaction mix is transformed into 50 µl DH5α by a heat-shock of 30 seconds at 42°C after incubating cells with DNA for 30 minutes on ice. After heat-shock of the cells 450 µl of S.O.C. is added and cells are incubated at 37°C for 60 minutes. Cells (100 µl) are plated on LB plates containing 100 µg/ml ampicillin and incubated over night.
A colony that contains pcDNA3.1(+)/attR with MIF as an insert is designated pcDNA/MIF and used for transfection studies.

2.3. Expression of recombinant MIF

The vector containing MIF described under 1.1. is used to transfer the cDNA for MIF to the expression vectors gpET28abc/attR that contains the "attR1" and "attR2" recombination sites of the Gateway cloning system (Life Technologies). These vectors allow the expression of recombinant hig-tagged MIF in bacteria under the control tog the T7 promoter. 150 ng of the "entry vector" pDONR-MIF is mixed with 150 ng of the "destination vector" gpET28abc/attR, 4 µl of the LR Clonase enzyme mix, 4 µl LR Clonase reaction buffer, added up with TE (Tris/EDTA) to 20 µl and incubated at 25°C for 60 minutes. Then, 2 µl of proteinase K solution is added and incubated for 10 minutes at 37°C. 1 µl of the reaction mix is transformed into 50 µl DH5α by a heat-shock of 30 seconds at 42°C after incubating cells with DNA for 30 minutes on ice. After heat-shock of the cells 450 µl of S.O.C. is added and cells are incubated at 37°C for 60 minutes. Cells (100 µl) are plated on LB plates containing 100 µg/ml ampicillin and incubated over night.
A colony that contains gpET28abc/attR with MIF fused to the his-tag in the correct reading frame is designated pgPET/MIF and used for expression of MIF in bacteria.

2.4. Purification of recombinant MIF

1 l LB broth including 100 μg/ml ampicillin is inoculated with 0.5 ml of an overnight culture of E. coli M15(pREP4) that carries pQE/MIF. The culture is incubated at 37°C with vigorous shaking until OD_{600} of 0.6. Expression is induced by adding 1 mM IPTG and the culture is grown further for 4 hours. Cells are harvested by centrifugation at 4000xg for 20 minutes at 4°C. Pellet is frozen at -20°C.

Cells are thawed on ice and resuspended in 2 ml/g cell pellet of lysis buffer (50 mM NaH_2PO4, pH 8.0, 300 mM NaCl, 10 mM imidazole). Then, lysozyme is added to 1 mg/ml and incubated on ice for 30 minutes. Then, cells are sonicated (six bursts of 10 seconds at 300 W). 10 μg/ml RNase A and 5 μg/ml DNase I is added and incubated on ice for 10 minutes. Then, lysates are cleared by spinning debris at 10000xg for 20 minutes at 4°C. Then, protease inhibitors (40 μg/ml bacitracin, 4 μg/ml leupeptin, 4 μg/ml chymostatin, 10 μg/ml pefabloc, 100 μM PMSF) are added. 3 ml of Ni-NTA resin (Qiagen) are added to the lysate and filled into a column. Binding to the resin is allowed for 60 minutes at 4°C during gentle shaking. Then, column outlet is opened, the resin washed twice with 12 ml wash buffer (50 mM NaH_2PO4, pH 8.0, 300 mM NaCl, 20 mM imidazole) and eluted with four times 3 ml of elution buffer (50 mM NaH_2PO_4, pH 8.0, 300 mM NaCl, 250 mM imidazole). The elution fraction that contains the recombinant protein is determined by SDS-PAGE and protein concentration of the purified protein is determined by the method of Bradford.

2.5. Purification of $CD4^+$ T cells and mononuclear cells from periperal blood

10 ml blood of healthy volunteers is diluted with 25 ml PBS and layered carefully on top of 15 ml ficoll in a 50 ml Falcon tube. The tube is spun at 400x g for 40 minutes at room temperature. Cells are removed with a pasteur pipet and washed in 50 ml PBS at 500x g for 10 minutes at RT.

$CD4^+$ lymphocytes are isolated with the help of magnetic beads. The cell fraction (as described in the previous paragraph) is resuspended 80 μl MACS buffer (PBS, 2 mM EDTA, 0.5% BSA) per $1x10^7$ cells. 20 μl of $CD4^+$ separation beads (Miltenyi Biotech) are added to $1x10^7$ cells, mixed and incubated at 4°C for 15 minutes. Then, 20 volumes of MACS buffer are added and spun at 1000 rpm for 10 minutes. The pellet

is resuspended in 500 µl MACS buffer per $1x10^8$ cells and added to a Miltenyi Separation Column LS^+ that is equilibrated with 3 ml of MACS buffer. Magnetic beads are exposed to a magnetic field for 30 seconds and labeled $CD4^+$ cells are retained. Afterwards, the columns is separated from the magnetic field and $CD4^+$ cells are flushed out with 5 ml of MACS buffer. Cells are spun down and resuspended in RPMI1640, 10% FCS).

Similarly, human mononuclear cells are isolated from whole blood by ficoll density centrifugation. After seeding the cells are washed twice in 24 hours with RPMI 1640, 10% FCS in order to remove non-adherent cells.

2.6. Phenotypic/cellular effects caused by MIF

The following assays are performed with cell lines THP-1 (Tsuchiya et al. 1980), and MonoMac 6 (Ziegler-Heitbrock et al. 1988) that are transiently or stably transfected with MIF and the read-outs are compared to mock-transfected cells. In addition substances according to the invention that stimulate the activity of MIF are added.

Production and Release of Cytokines

Monocytic/macrophage cell lines are stimulated with MIF (1 µg/ml) at cell densities between 2.5 and 5×10^5 cells/ml. Cells are harvested after 0, 1, 3, 6, 12, 24, 48, and 72 hours, the supernatant frozen for further investigation, cells are washed with PBS, and resuspended in 400 µl of RLT buffer (from Qiagen RNeasy Total RNA Isolation Kit) with 143 mM β-mercaptoethanol, the DNA sheared with a 20 g needle for at least 5 times and stored at –70°C.

Stimulation of cells by cigarette smoke is performed by a smoke-enriched media. 100 ml RPMI media without supplements is perfused with the cigarette smoke of 2 cigarettes. The smoke of the cigarettes is pulled into a 50 ml syringe (about 20 volumes of a 50-ml volumes per cigarette) and then perfused into the media. Afterwards, the pH of the media is adjusted to 7.4, and the media is filtersterilized through a 0.2 µm filter. Cells are resuspended in smoke-enriched media and incubated for 10 minutes at 37°C at a density of $1x10^6$ cells/ml. Then, cells are washed twice with RPMI 1640 and seeded in flasks or 24-well plates (MonoMac6) for the times indicated above.

Total RNAs are isolated with the Qiagen RNeasy Total RNA Isolation Kit (Qiagen) according to the manufacturer's protocol. Purified RNA is used for TaqMan analysis. The expression levels of cytokines TNFα, IL-1β, IL-8, and IL-6 are measured.

Detection of secreted cytokines

Proteins in the supernatants of the cultured and stimulated cells are precipitated by adding TCA to a final concentration of 10%. Precipitates are washed twice with 80% ethanol and pellets are resuspended in 50 mM Tris/HCl, pH 7.4, 10 mM $MgCl_2$, 1 mM EDTA. Protein concentration is determined via the Bradford method and 50 □g of each sample are loaded on 12% SDS polyacrylamide gels. Gels are blotted onto PVDF-membranes, blocked for 1 hour in 5% BSA in TBST, and incubated for 1 hour with commercially available antibodies against human TNFα, IL-1β, IL-8, and IL-6. After washing with TBST blots are incubated with anti-human IgG conjugated to horseradish-peroxidase, washed again and developed with ECL chemiluminescence kit (Amersham). Intensity of the bands are visualised with BioMax X-ray films (Kodak) and quantified by densitometry.

Purified $CD4^+$ cells (as described under 2.0) are seeded in 96-well-plates ($5x10^4$ cells/200 µl) in RPMI 1640, 10% FCS and incubated with dexamethasone (10 nM) in the presence or absence of 10 ng/ml MIF. After 24 hours of incubation at 37°C in a humidified atmosphere with 5% CO_2, cytokine release (e.g. IL-2 or IFN-γ (interferon-gamma)) is determined by ELISA. MIF overrides the inhibitory effect of dexamethasone and causes release of cytokines. The counteracting effect of MIF on dexamethasone is modulated by adding substances according to the invention (0.1 - 100 ng/ml) to the reaction mix and calculate the effect as percent inhibition of the MIF-mediated effect.

In order to determine cytokine release (IL-1β, IL-6, IL-8, TNF-α) in monocytes, the cells need to be treated with 1 µg/ml LPS after 1 hour of preincubation with dexamethasone and MIF (according to previous paragraph).

Detection of secreted matrix metalloproteases and other proteases

The procedure is identical to the one used for cytokines. Antibodies used for Western blotting are against human MMP-1, MMP-7, MMP-9, and MMP-12.

Activity of secreted matrix metalloproteases

Protease activity is determined with a fluorescent substrate. Supernatants isolated from stimulated and unstimulated cells (described above) are incubated in a total volume of 50 µl with 1 µM of the substrate (Dabcyl-Gaba-Pro-Gln-Gly-Leu-Glu (EDANS)-Ala-Lys-NH2 (Novabiochem)) for 5 minutes at room temperature. Positive controls are performed with 125 ng purified MMP-12 per reaction. Protease activity is determined by fluorometry with an excitation at 320 nm and an emission at 405 nm.

In an alternative assay to determine proteolytic activity and cell migration a chemotaxis (Boyden) chamber is used. In the wells of the upper part of the chamber cells (10^5 cells per well) are plated on filters coated with an 8 µm layer of Matrigel (Becton Dickinson). In the lower compartment chemoattractants like MIF (1µg/ml), leukotriene B_4 (10 ng/ml), MCP-1 (10 ng/ml) are added to the media. After five days filters are removed, cells on the undersurface that have traversed the Matrigel are fixed with methanol, stained with the Diff-Quik staining kit (Dade Behring) and counted in three high power fields (400x) by light microscopy.

Chemotaxis Assay

In order to determine chemotaxis, a 48 well chemotaxis (Boyden) chamber (Neuroprobe) is used. Cells are starved for 24 hours in RPMI media without FCS. Chemotaxis is stimulated by 100 ng/ml LPS, 10 ng/ml leukotriene B_4,or MCP-1. Additon of MIF (1 µg/ml) is used to block chemotaxis. Substances according to the invention are diluted in RPMI media without FCS and 30 µl is placed in the wells of the lower compartment in order to counteract MIF activity. The upper compartment is separated from the lower compartment by a polycarbonate filter (pore size 8 µm). 50 µl cell suspension (5×10^4) are placed in the well of the upper compartment. The chamber is incubated for 5 hours at 37°C in a humidified atmosphere with 5% CO_2. Then the filter is removed, cells on the upper side are scraped off, cells on the downside are fixed for 5 minutes in methanol and stained with the Diff-Quik staining

set (Dade Behring). Migrated cells are counted in three high-power fields (400x) by light microscopy.

Adherence Assay

Cells are harvested, washed in PBS and resuspended ($4x10^6$/ml) in PBS and 1 μM BCECF ((2'-7'-bis-(carboxyethyl)-5(6')-carboxyfluorescein acetoxymethyl) ester, Calbiochem) and incubated for 20 minutes at 37°C. Cells are washed in PBS and resuspended ($3.3x\ 10^6$/ml) in PBS containing 0.1% BSA. $3x10^5$ cells (90 μl) are added to each well of a 96-well flat bottom plate coated with laminin (Becton Dickinson) and allowed to settle for 10 minutes. Substances according to the invention are added in the presence and absence of MIF (1 μg/ml), and plates are incubated for 20 minutes at 37°C. Cells are washed with PBS containing 0.1% BSA and adherent cells are solubilized with 100 μl of 0.025 M NaOH and 0.1% SDS. Quantification is performed by fluorescence measurement.

Phagocytosis

Cell suspensions ($2.5x10^4$ cells/ml) are seeded in 6-well plates with 5 ml of U937 or THP-1 or in 24-well plates with 2 ml of MonoMac6 and incubated for 1 hour at 37°C in a humidified atmosphere with 5% CO_2. In the presence of MIF, substances according to the invention are added to counteract the actvity of MIF. 40 μl of a dispersed suspension of heat-inactivated Saccharomyces boulardii (20 yeast/cell) are added to each well. Cells are incubated for three more hours, washed twice with PBS and cytocentrifuged. The cytospin preparations are stained with May-Grünwald-Giemsa and phagocytosed particles are counted by light microsopy.

Example 3: DAD1

A gene identified as being downregulated in COPD smokers compared to healthy smokers is DAD1 (defender against apoptotic cell death 1). Originally, DAD1 was discovered as being a negative regulator of apoptosis (Nakashima et al. 1993). By homology to the Ost2 protein in Schizosaccaromyces pombe it was identified as the 16 kDa subunit of the oligosaccaryltransferase complex which catalyzes the transfer

of high mannose oligosaccharides onto asparagine residues in nascent polypeptides. DAD1 is an integral membrane protein and is ubiquitously expressed (Kelleher and Gilmore 1997).

DAD1 is consistently found upregulated (42%) in comparisons between COPD smokers and healthy smokers. This is shown by „fold change“ values (Tab. 2).

Tab. 2: Fold change values (FC) for comparisons between obstructed smokers and healthy smokers. On average DAD1 is upregulated by 1.6fold, the median is 1.5fold.

comp	FC	comp	FC	comp	FC	comp	FC
1 vs 2	-1.1	5 vs 43	2.3	39 vs 57	4.8	68 vs 66	1.4
1 vs 37	2.5	5 vs 56	3.9	39 vs 58	2.5	68 vs 69	1.
1 vs 43	1.5	5 vs 57	4.0	39 vs 62	6.6	68 vs 76	2.2
1 vs 56	2.4	5 vs 58	2.0	44 vs 2	-2.9	68 vs 78	2.1
1 vs 57	2.5	5 vs 62	5.5	44 vs 37	1.1	70 vs 65	-1.3
1 vs 58	1.3	6 vs 2	1.0	44 vs 43	-1.7	70 vs 66	-1.4
1 vs 62	3.4	6 vs 37	2.7	44 vs 56	1.0	70 vs 69	-1.3
3 vs 2	-1.2	6 vs 43	1.6	44 vs 57	1.0	70 vs 76	1.1
3 vs 37	2.3	6 vs 56	2.7	44 vs 58	-1.9	70 vs 78	1.1
3 vs 43	1.4	6 vs 57	2.7	44 vs 62	1.4	71 vs 65	1.1
3 vs 56	2.3	6 vs 58	1.4	64 vs 65	-1.1	71 vs 66	1.0
3 vs 57	2.3	6 vs 62	3.7	64 vs 66	-1.1	71 vs 69	1.2
3 vs 58	1.2	39 vs 2	1.7	64 vs 69	-1.1	71 vs 76	1.6
3 vs 62	3.2	39 vs 37	4.8	64 vs 76	1.3	71 vs 78	1.6
5 vs 2	1.4	39 vs 43	2.8	64 vs 78	1.3		
5 vs 37	3.9	39 vs 56	4.7	68 vs 65	1.4		

The protein is cloned and assays are designed and performed in an analogous manner to the cloning and assays described hereinbefore.

Example 4: ARL4,

A gene identified as being upregulated in COPD smokers compared to healthy smokers is ARL4 (ADP-ribosylation factor-like protein 4). ARLs belong to the family of ADP-ribosylation factors (ARFs). ARFs are involved in vesicular and membrane trafficking. ARL4 is both detected inside and outside of the nucleus and it is speculated that it is involved in cellular differentiation (Jacobs et al. 1999).

ARL4 is consistently found upregulated (45%) in comparisons between COPD smokers and healthy smokers. This is shown by „fold change" values (Tab. 3: The p values for two separate groups comparing COPD smokers and healthy smokers are 0.10 and 0.06.

Tab. 3: Fold change values (FC) for comparisons between obstructed smoker and healthy smokers. On average ARL4 is upregulated by 1.6fold, the median is 1.9fold.

comp	FC	comp	FC	comp	FC	comp	FC
1 vs 2	-1.1	5 vs 43	1.9	39 vs 57	2.5	68 vs 66	2.4
1 vs 37	2.7	5 vs 56	2.2	39 vs 58	1.2	68 vs 69	4.5
1 vs 43	3.2	5 vs 57	1.6	39 vs 62	1.5	68 vs 76	7.8
1 vs 56	4.3	5 vs 58	-1.2	44 vs 2	-3.7	68 vs 78	3.3
1 vs 57	2.0	5 vs 62	1.0	44 vs 37	-1.3	70 vs 65	1.2
1 vs 58	-1.1	6 vs 2	1.2	44 vs 43	-1.1	70 vs 66	1.5
1 vs 62	1.2	6 vs 37	3.4	44 vs 56	1.5	70 vs 69	2.7
3 vs 2	-1.8	6 vs 43	3.6	44 vs 57	-1.7	70 vs 76	4.7
3 vs 37	2.0	6 vs 56	4.1	44 vs 58	-3.5	70 vs 78	1.9
3 vs 43	2.4	6 vs 57	2.7	44 vs 62	-2.7	71 vs 65	1.7
3 vs 56	3.2	6 vs 58	1.3	64 vs 65	-1.1	71 vs 66	2.0
3 vs 57	1.5	6 vs 62	1.6	64 vs 66	1.2	71 vs 69	3.9
3 vs 58	-1.4	39 vs 2	1.1	64 vs 69	2.2	71 vs 76	6.7
3 vs 62	1.0	39 vs 37	3.3	64 vs 76	3.8	71 vs 78	2.8
5 vs 2	-1.3	39 vs 43	4.0	64 vs 78	1.6		
5 vs 37	1.8	39 vs 56	4.7	68 vs 65	1.9		

4.1. Cloning of ARL4

ARL4 is cloned from a total RNA extracted from human 3T3-L1. 5 μg RNA is reverse transcribed into cDNA with 5 ng oligo$(dt)_{18}$ primer, 1x first strand buffer, 10 mM DTT, 0.5 mM dNTPs and 2 U Superscript II (Gibco BRL) at 42°C for 50 minutes. Then, the reaction is terminated at 70°C for 15 minutes and the cDNA concentration is determined by UV-spectrophotometry. For amplification of ARL4100 ng of the cDNA and 10 pmoles of sequence-specific primers for ARL4 (forward primer, SEQ ID NO. 19 and reverse primer, SEQ ID NO. 20) are used for PCR. Reaction conditions are: 2 minutes of 94°C, 35 cycles with 30 seconds at 94°C, 30 seconds at 53°C, 90 seconds at 72°C, followed by 7 minutes at 72°C with Taq DNA-polymerase. The PCR product is separated on a 2% agarose gel, a band of about 600bp is cut out and purified with the QIAEX II extraction kit (Qiagen). This product is digested with BamH1 and HindIII and cloned into pQE-30 (Qiagen) that is digested with BamHI and HindIII. A clone, designated pQE/ARL4 with identical sequence to the database entry (acc.U73960) is used for further experiments.

4.2 Expression of ARL4

1 l LB broth including 100 μg/ml ampicillin is inoculated with 0.5 ml of an overnight culture of E. coli M15(pREP4) that carries pQE/ARL4. The culture is incubated at 37°C with vigorous shaking until OD_{600} of 0.6. Expression is induced by adding 1 mM IPTG and the culture is grown further for 4 hours. Cells are harvested by centrifugation at 4000xg for 20 minutes at 4°C. Pellet is frozen at -20°C.
Cells are thawed on ice and resuspended in 2 ml/g cell pellet of lysis buffer (50 mM NaH_2PO4, pH 8.0, 300 mM NaCl, 10 mM imidazole). Then, lysozyme is added to 1 mg/ml and incubated on ice for 30 minutes. Then, cells are sonicated (six bursts of 10 seconds at 300 W). 10 μg/ml RNase A and 5 μg/ml DNase I is added and incubated on ice for 10 minutes. Then, lysates are cleared by spinning debris at 10000xg for 20 minutes at 4°C. Then, protease inhibitors (40 μg/ml bacitracin, 4 μg/ml leupeptin, 4 μg/ml chymostatin, 10 μg/ml pefabloc, 100 μM PMSF) are added. 3 ml of Ni-NTA resin (Qiagen) are added to the lysate and filled into a column. Binding to the resin is allowed for 60 minutes at 4°C during gentle shaking. Then,

column outlet is opened, the resin washed twice with 12 ml wash buffer (50 mM NaH_2PO4, pH 8.0, 300 mM NaCl, 20 mM imidazole) and eluted with four times 3 ml of elution buffer (50 mM NaH_2PO4, pH 8.0, 300 mM NaCl, 250 mM imidazole). The elution fraction that contains the recombinant protein is determined by SDS-PAGE and protein concentration of the purified protein is determined by the method of Bradford.

4.3 GTPγS binding assay

Recombinant ARL4 (1 µM) is incubated at 37 °C with [^{35}S]GTPS or [^{3}H]GDP (10 µM, ~1000 cpm/pmol) in 50 mM Hepes (pH7.5), 1 mM dithiothreitol, 1 mM MgCl2 with or without (as indicated in the figure legends) 2 mM EDTA (1 µM or 1 mM free Mg2+), 100 mM KCl. Substances according to the invention are preincubated with ARL4 for 5 minutes at 4°C in a concentration range from 0.5 to 300 nM before starting the GTPγS binding reaction. At various time points (10 seconds to 30 minutes) samples of 25 µl (25 pmoles of ARF) are removed, diluted into 2 ml of ice-cold 20 mM Hepes (pH 7.5), 100 mM NaCl, and 10 mM $MgCl_2$, and filtered on 25-mm BA 85 nitrocellulose filters (Schleicher & Schüll). Filters are washed twice with 2 ml of the same buffer, dried, and quantified by scintillation counting.

Example 5: GNS,

A gene identified as being downregulated in COPD smokers compared to healthy smokers is Glucosamine-6-sulphatase (GNS). GNS hydrolysis the 6-sulfate group of the N-acetyl-d-glucosamine 6-sulfate units of heparan (Kresse et al. 1980). GNS is consistently found downregulated (44%) in comparisons between COPD smokers and healthy smokers. This is shown by „fold change“ values (Tab. 4).The p values for two separate groups comparing COPD smokers and healthy smokers are 0.05 and 0.006.

Tab. 4: Fold change values (FC) for comparisons between obstructed smoker and healthy smokers. On average is downregulated by -2.0fold, the median is -1.8fold

comp	FC	comp	FC	comp	FC	comp	FC
1 vs 2	1.0	5 vs 43	-4.6	39 vs 57	-2.4	68 vs 66	-3.6
1 vs 37	1.0	5 vs 56	-1.7	39 vs 58	-3.3	68 vs 69	-2.3
1 vs 43	-3.7	5 vs 57	-3.1	39 vs 62	-1.1	68 vs 76	-2.6
1 vs 56	-1.1	5 vs 58	-4.0	44 vs 2	-1.2	68 vs 78	-2.6
1 vs 57	-2.3	5 vs 62	1.0	44 vs 37	-1.2	70 vs 65	-1.4
1 vs 58	-3.0	6 vs 2	1.0	44 vs 43	-4.3	70 vs 66	-1.6
1 vs 62	1.0	6 vs 37	1.1	44 vs 56	-1.3	70 vs 69	1.0
3 vs 2	-1.5	6 vs 43	-3.5	44 vs 57	-2.6	70 vs 76	-1.1
3 vs 37	-1.4	6 vs 56	1.0	44 vs 58	-3.7	70 vs 78	-1.1
3 vs 43	-5.0	6 vs 57	-2.2	44 vs 62	-1.2	71 vs 65	-2.1
3 vs 56	-1.8	6 vs 58	-3.0	64 vs 65	-2.3	71 vs 66	-2.5
3 vs 57	-3.1	6 vs 62	1.1	64 vs 66	-2.6	71 vs 69	-1.7
3 vs 58	-3.9	39 vs 2	1.0	64 vs 69	-1.7	71 vs 76	-1.8
3 vs 62	-1.3	39 vs 37	-1.1	64 vs 76	-1.9	71 vs 78	-1.8
5 vs 2	-1.7	39 vs 43	-3.8	64 vs 78	-1.9		
5 vs 37	-1.7	39 vs 56	1.0	68 vs 65	-3.1		

The protein is cloned and assays are designed and performed in an analogous manner to the cloning and assays described hereinbefore.

Example 6: Transglutaminase 2

A gene identified as being downregulated in COPD smokers compared to healthy smokers is transglutaminase 2. This enzyme belongs to a family of calcium-dependent transglutaminases that catalyze the covalent cross-linking of specific proteins by the formulation of (γ-glutamyl)lysine bonds and the conjugation of polyamines to proteins (Folk 1980). Transglutaminases can also be secreted. The physiological functions are not well understood, it may be involved in the specialized processing of the matrix that occurs during bone formation, wound healing, and other remodeling processes (Lu et al. 1995).

Transglutaminase 2 is consistently found downregulated (55%) in comparisons between COPD smokers and healthy smokers. This is shown by „fold change" values (Tab. 5). The p values for two separate groups comparing COPD smokers and healthy smokers are 0.04 and 0.16.

Tab.5: Fold change values (FC) for comparisons between obstructed smoker and healthy smokers. On average is downregulated by 2.3fold, the median is -2.35fold

comp	FC	comp	FC	comp	FC	comp	FC
1 vs 2	1.0	5 vs 43	-5.6	39 vs 57	-2.3	68 vs 66	-2.8
1 vs 37	-3.6	5 vs 56	-1.4	39 vs 58	-3.9	68 vs 69	-7.4
1 vs 43	-6.9	5 vs 57	-3.7	39 vs 62	1.0	68 vs 76	-4-4
1 vs 56	-1.5	5 vs 58	-7.5	44 vs 2	1.0	68 vs 78	-3.4
1 vs 57	-3.6	5 vs 62	1.0	44 vs 37	-3.2	70 vs 65	1.5
1 vs 58	-8.9	6 vs 2	2.2	44 vs 43	-7.7	70 vs 66	1.2
1 vs 62	1.0	6 vs 37	-2.2	44 vs 56	-1.9	70 vs 69	-2.5
3 vs 2	1.0	6 vs 43	-3.6	44 vs 57	-3.8	70 vs 76	-1.4
3 vs 37	-2.5	6 vs 56	1.0	44 vs 58	-11.3	70 vs 78	1.0
3 vs 43	-4.5	6 vs 57	-2.5	44 vs 62	1.0	71 vs 65	-1.8
3 vs 56	-1.2	6 vs 58	-4.7	64 vs 65	1.4	71 vs 66	-2.4
3 vs 57	-2.8	6 vs 62	-1.2	64 vs 66	1.1	71 vs 69	-6.9
3 vs 58	-5.9	39 vs 2	1.0	64 vs 69	-2.7	71 vs 76	-3.9
3 vs 62	1.0	39 vs 37	-1.8	64 vs 76	-1.5	71 vs 78	-2.8
5 vs 2	1.0	39 vs 43	-2.9	64 vs 78	-1.1		
5 vs 37	-3.3	39 vs 56	1.2	68 vs 65	-2.1		

The protein is cloned and assays are designed and performed in an analogous manner to the cloning and assays described hereinbefore.

Example 7: Stearyl-CoA-Desaturase

A gene identified as being downregulated in COPD smokers compared to healthy smokers is Stearoyl-CoA-Desaturase. Stearoyl-CoA-Desaturase catalyzes the

oxidation of palmitoyl-CoA and stearoyl-CoA at the Δ^9 position to form the mono-unsaturated fatty acyl-CoA esters, palmitoleoyl-CoA and aoleoyl-CoA, respectively (Enoch et al. 1976).

Stearoyl-CoA-desaturase is consistently found downregulated (48%) in comparisons between COPD smokers and healthy smokers. This is shown by „fold change“ values (Tab. 6). The p values for two separate groups comparing COPD smokers and healthy smokers are 0.03 and 0.15.

Tab. 6: Fold change values (FC) for comparisons between obstructed smoker and healthy smokers. On average is downregulated by 2.3fold, the median is -1.9fold

comp	FC	comp	FC	comp	FC	comp	FC
1 vs 2	-1.7	5 vs 43	-5.8	39 vs 57	-3.9	68 vs 66	-2.5
1 vs 37	1.0	5 vs 56	-2.1	39 vs 58	-7.3	68 vs 69	-1.2
1 vs 43	-4.0	5 vs 57	-3.7	39 vs 62	-1.8	68 vs 76	-1.2
1 vs 56	1.0	5 vs 58	-6.5	44 vs 2	-1.1	68 vs 78	-1.5
1 vs 57	-2.4	5 vs 62	-2.3	44 vs 37	1.3	70 vs 65	-1.5
1 vs 58	-4.6	6 vs 2	-3.0	44 vs 43	-2.4	70 vs 66	-1.2
1 vs 62	-1.1	6 vs 37	-1.8	44 vs 56	1.4	70 vs 69	1.5
3 vs 2	-1.8	6 vs 43	-7.1	44 vs 57	-1.5	70 vs 76	1.5
3 vs 37	-1.1	6 vs 56	-2.2	44 vs 58	-2.9	70 vs 78	1.3
3 vs 43	-4.4	6 vs 57	-4.3	44 vs 62	1.3	71 vs 65	-2.5
3 vs 56	-1.2	6 vs 58	-8.2	64 vs 65	-4.2	71 vs 66	-1.9
3 vs 57	-2.7	6 vs 62	-2.4	64 vs 66	-3.3	71 vs 69	1.0
3 vs 58	-5.0	39 vs 2	-2.7	64 vs 69	-1.7	71 vs 76	-1.1
3 vs 62	-1.2	39 vs 37	-1.6	64 vs 76	-1.7	71 vs 78	-1.3
5 vs 2	-2.9	39 vs 43	-6.4	64 vs 78	-2.2		
5 vs 37	-1.9	39 vs 56	-1.7	68 vs 65	-3.3		

The protein is cloned and assays are designed and performed in an analogous manner to the cloning and assays described hereinbefore.

Example 8: UDP-Glucose Ceramide Glycosyltransferase

A gene identified as being downregulated in COPD smokers compared to healthy smokers is UDP-glucose Ceramide Glucosyltransferase. This enzyme catalyzes the transfer of glucose from UDP-glucose to ceramide. The product glucosyl-ceramid serces as the core structure of more than 300 glycoshingolipids that are involved in multiple cellular processes as differentiation, adhesion, proliferation, and cell-cell recognition (Basu et al. 1968, Ichikawa et al. 1996).

Ceramide Glucosyltransferase is consistently found downregulated (48%) in comparisons between COPD smokers and healthy smokers. This is shown by „fold change" values (Tab. 7).

Tab. 7: Fold change values (FC) for comparisons between obstructed smoker and healthy smokers. On average is downregulated by 1.2fold, the median is -1.9fold

comp	FC	comp	FC	comp	FC	comp	FC
1 vs 2	1.3	5 vs 43	-2.4	39 vs 57	-1.6	68 vs 66	-4.0
1 vs 37	-2.4	5 vs 56	-2.0	39 vs 58	-2.6	68 vs 69	-1.1
1 vs 43	-1.9	5 vs 57	-1.6	39 vs 62	-2.3	68 vs 76	-2.9
1 vs 56	-1.5	5 vs 58	-2.6	44 vs 2	7.2	68 vs 78	-3.4
1 vs 57	-1.3	5 vs 62	-2.0	44 vs 37	1.9	70 vs 65	1.0
1 vs 58	-2.1	6 vs 2	1.0	44 vs 43	2.7	70 vs 66	-2.0
1 vs 62	-1.5	6 vs 37	-4.2	44 vs 56	3.5	70 vs 69	1.5
3 vs 2	1.3	6 vs 43	-2.8	44 vs 57	4.6	70 vs 76	-1.4
3 vs 37	-2.6	6 vs 56	-2.3	44 vs 58	2.7	70 vs 78	-1.8
3 vs 43	-1.9	6 vs 57	-1.8	44 vs 62	3.4	71 vs 65	-2.0
3 vs 56	-1.6	6 vs 58	-3.0	64 vs 65	-1.7	71 vs 66	-4.3
3 vs 57	-1.3	6 vs 62	-2.4	64 vs 66	-3.2	71 vs 69	1.0
3 vs 58	-2.1	39 vs 2	1.0	64 vs 69	-1.1	71 vs 76	-2.5
3 vs 62	-1.7	39 vs 37	-3.5	64 vs 76	-2.5	71 vs 78	-3.7
5 vs 2	1.0	39 vs 43	-2.4	64 vs 78	-2.9		
5 vs 37	-3.1	39 vs 56	-2.2	68 vs 65	-1.9		

The protein is cloned and assays are designed and performed in an analogous manner to the cloning and assays described hereinbefore.

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<210> 8
<211> 552
<212> PRT
<213> Homo sapiens

<400> 8

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Arg His Leu Pro Ser Cys Ser Pro Ala Leu Leu Leu Leu Val Leu Gly
20 25 30

Gly Cys Leu Gly Val Phe Gly Val Ala Ala Gly Thr Arg Arg Pro Asn
35 40 45

Val Val Leu Leu Leu Thr Asp Asp Gln Asp Glu Val Leu Gly Gly Met
50 55 60

Thr Pro Leu Lys Lys Thr Lys Ala Leu Ile Gly Glu Met Gly Met Thr
65 70 75 80

Phe Ser Ser Ala Tyr Val Pro Ser Ala Leu Cys Cys Pro Ser Arg Ala
85 90 95

Ser Ile Leu Thr Gly Lys Tyr Pro His Asn His His Val Val Asn Asn
100 105 110

Thr Leu Glu Gly Asn Cys Ser Ser Lys Ser Trp Gln Lys Ile Gln Glu
115 120 125

Pro Asn Thr Phe Pro Ala Ile Leu Arg Ser Met Cys Gly Tyr Gln Thr
130 135 140

Phe Phe Ala Gly Lys Tyr Leu Asn Glu Tyr Gly Ala Pro Asp Ala Gly
145 150 155 160

Gly Leu Glu His Val Pro Leu Gly Trp Ser Tyr Trp Tyr Ala Leu Glu
165 170 175

Lys Asn Ser Lys Tyr Tyr Asn Tyr Thr Leu Ser Ile Asn Gly Lys Ala
180 185 190

Arg Lys His Gly Glu Asn Tyr Ser Val Asp Tyr Leu Thr Asp Val Leu
195 200 205

Ala Asn Val Ser Leu Asp Phe Leu Asp Tyr Lys Ser Asn Phe Glu Pro
210 215 220

Phe Phe Met Met Ile Ala Thr Pro Ala Pro His Ser Pro Trp Thr Ala
225 230 235 240

Ala Pro Gln Tyr Gln Lys Ala Phe Gln Asn Val Phe Ala Pro Arg Asn
245 250 255

Lys Asn Phe Asn Ile His Gly Thr Asn Lys His Trp Leu Ile Arg Gln
260 265 270

Ala Lys Thr Pro Met Thr Asn Ser Ser Ile Gln Phe Leu Asp Asn Ala
275 280 285

Phe Arg Lys Arg Trp Gln Thr Leu Leu Ser Val Asp Asp Leu Val Glu
290 295 300

Lys Leu Val Lys Arg Leu Glu Phe Thr Gly Glu Leu Asn Asn Thr Tyr
305 310 315 320

Ile Phe Tyr Thr Ser Asp Asn Gly Tyr His Thr Gly Gln Phe Ser Leu
325 330 335

Pro Ile Asp Lys Arg Gln Leu Tyr Glu Phe Asp Ile Lys Val Pro Leu
340 345 350

Leu Val Arg Gly Pro Gly Ile Lys Pro Asn Gln Thr Ser Lys Met Leu
355 360 365

Val Ala Asn Ile Asp Leu Gly Pro Thr Ile Leu Asp Ile Ala Gly Tyr
370 375 380

Asp Leu Asn Lys Thr Gln Met Asp Gly Met Ser Leu Leu Pro Ile Leu
385 390 395 400

Arg Gly Ala Ser Asn Leu Thr Trp Arg Ser Asp Val Leu Val Glu Tyr
405 410 415

Gln Gly Glu Gly Arg Asn Val Thr Asp Pro Thr Cys Pro Ser Leu Ser
420 425 430

Pro Gly Val Ser Gln Cys Phe Pro Asp Cys Val Cys Glu Asp Ala Tyr
435 440 445

Asn Asn Thr Tyr Ala Cys Val Arg Thr Met Ser Ala Leu Trp Asn Leu
450 455 460

Gln Tyr Cys Glu Phe Asp Asp Gln Glu Val Phe Val Glu Val Tyr Asn
465 470 475 480

Leu Thr Ala Asp Pro Asp Gln Ile Thr Asn Ile Ala Lys Thr Ile Asp
485 490 495

Pro Glu Leu Leu Gly Lys Met Asn Tyr Arg Leu Met Met Leu Gln Ser
500 505 510

Cys Ser Gly Pro Thr Cys Arg Thr Pro Gly Val Phe Asp Pro Gly Tyr
515 520 525

Arg Phe Asp Pro Arg Leu Met Phe Ser Asn Arg Gly Ser Val Arg Thr
530 535 540

Arg Arg Phe Ser Lys His Leu Leu
545 550

<210> 9
<211> 3257

<212> DNA

<213> Homo sapiens

<400> 9

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<210> 10
<211> 687
<212> PRT
<213> Homo sapiens

<400> 10

Met Ala Glu Glu Leu Val Leu Glu Arg Cys Asp Leu Glu Leu Glu Thr
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Asn Gly Arg Asp His His Thr Ala Asp Leu Cys Arg Glu Lys Leu Val
20 25 30

Val Arg Arg Gly Gln Pro Phe Trp Leu Thr Leu His Phe Glu Gly Arg
35 40 45

Asn Tyr Gln Ala Ser Val Asp Ser Leu Thr Phe Ser Val Val Thr Gly
50 55 60

Pro Ala Pro Ser Gln Glu Ala Gly Thr Lys Ala Arg Phe Pro Leu Arg
65 70 75 80

Asp Ala Val Glu Glu Gly Asp Trp Thr Ala Thr Val Val Asp Gln Gln
85 90 95

Asp Cys Thr Leu Ser Leu Gln Leu Thr Thr Pro Ala Asn Ala Pro Ile
100 105 110

Gly Leu Tyr Arg Leu Ser Leu Glu Ala Ser Thr Gly Tyr Gln Gly Ser
115 120 125

Ser Phe Val Leu Gly His Phe Ile Leu Leu Phe Asn Ala Trp Cys Pro
130 135 140

Ala Asp Ala Val Tyr Leu Asp Ser Glu Glu Glu Arg Gln Glu Tyr Val
145 150 155 160

Leu Thr Gln Gln Gly Phe Ile Tyr Gln Gly Ser Ala Lys Phe Ile Lys
165 170 175

Asn Ile Pro Trp Asn Phe Gly Gln Phe Gln Asp Gly Ile Leu Asp Ile
180 185 190

Cys Leu Ile Leu Leu Asp Val Asn Pro Lys Phe Leu Lys Asn Ala Gly
195 200 205

Arg Asp Cys Ser Arg Arg Ser Ser Pro Val Tyr Val Gly Arg Val Gly
210 215 220

Ser Gly Met Val Asn Cys Asn Asp Asp Gln Gly Val Leu Leu Gly Arg
225 230 235 240

Trp Asp Asn Asn Tyr Gly Asp Gly Val Ser Pro Met Ser Trp Ile Gly
245 250 255

Ser Val Asp Ile Leu Arg Arg Trp Lys Asn His Gly Cys Gln Arg Val
260 265 270

Lys Tyr Gly Gln Cys Trp Val Phe Ala Ala Val Ala Cys Thr Val Leu
275 280 285

Arg Cys Leu Gly Ile Pro Thr Arg Val Val Thr Asn Tyr Asn Ser Ala
290 295 300

His Asp Gln Asn Ser Asn Leu Leu Ile Glu Tyr Phe Arg Asn Glu Phe
305 310 315 320

Gly Glu Ile Gln Gly Asp Lys Ser Glu Met Ile Trp Asn Phe His Cys
325 330 335

Trp Val Glu Ser Trp Met Thr Arg Pro Asp Leu Gln Pro Gly Tyr Glu
340 345 350

Gly Trp Gln Ala Leu Asp Pro Thr Pro Gln Glu Lys Ser Glu Gly Thr
355 360 365

Tyr Cys Cys Gly Pro Val Pro Val Arg Ala Ile Lys Glu Gly Asp Leu
370 375 380

Ser Thr Lys Tyr Asp Ala Pro Phe Val Phe Ala Glu Val Asn Ala Asp
385 390 395 400

Val Val Asp Trp Ile Gln Gln Asp Asp Gly Ser Val His Lys Ser Ile
405 410 415

Asn Arg Ser Leu Ile Val Gly Leu Lys Ile Ser Thr Lys Ser Val Gly
420 425 430

Arg Asp Glu Arg Glu Asp Ile Thr His Thr Tyr Lys Tyr Pro Glu Gly
435 440 445

Ser Ser Glu Glu Arg Glu Ala Phe Thr Arg Ala Asn His Leu Asn Lys
450 455 460

Leu Ala Glu Lys Glu Glu Thr Gly Met Ala Met Arg Ile Arg Val Gly
465 470 475 480

Gln Ser Met Asn Met Gly Ser Asp Phe Asp Val Phe Ala His Ile Thr
485 490 495

Asn Asn Thr Ala Glu Glu Tyr Val Cys Arg Leu Leu Leu Cys Ala Arg
500 505 510

Thr Val Ser Tyr Asn Gly Ile Leu Gly Pro Glu Cys Gly Thr Lys Tyr
515 520 525

Leu Leu Asn Leu Thr Leu Glu Pro Phe Ser Glu Lys Ser Val Pro Leu
530 535 540

Cys Ile Leu Tyr Glu Lys Tyr Arg Asp Cys Leu Thr Glu Ser Asn Leu
545 550 555 560

Ile Lys Val Arg Ala Leu Leu Val Glu Pro Val Ile Asn Ser Tyr Leu
565 570 575

Leu Ala Glu Arg Asp Leu Tyr Leu Glu Asn Pro Glu Ile Lys Ile Arg
580 585 590

Ile Leu Gly Glu Pro Lys Gln Lys Arg Lys Leu Val Ala Glu Val Ser
595 600 605

Leu Gln Asn Pro Leu Pro Val Ala Leu Glu Gly Cys Thr Phe Thr Val
610 615 620

Glu Gly Ala Gly Leu Thr Glu Glu Gln Lys Thr Val Glu Ile Pro Asp
625 630 635 640

Pro Val Glu Ala Gly Glu Glu Val Lys Val Arg Met Asp Leu Val Pro
645 650 655

Leu His Met Gly Leu His Lys Leu Val Val Asn Phe Glu Ser Asp Lys
660 665 670

Leu Lys Ala Val Lys Gly Phe Arg Asn Val Ile Ile Gly Pro Ala
675 680 685

<210> 11
<211> 1470
<212> DNA
<213> Homo sapiens

<400> 11
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aagccaaaaa aaaaaaaaaa aaaaaaaaaa 1470

<210> 12

<211> 359

<212> PRT

<213> Homo sapiens

<400> 12

Met Pro Ala His Leu Leu Gln Asp Asp Ile Ser Ser Ser Tyr Thr Thr
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Thr Thr Thr Ile Thr Ala Pro Pro Pro Gly Val Leu Gln Asn Gly Gly
20 25 30

Asp Lys Leu Glu Thr Met Pro Leu Tyr Leu Glu Asp Asp Ile Arg Pro
35 40 45

Asp Ile Lys Asp Asp Ile Tyr Asp Pro Thr Tyr Lys Asp Lys Glu Gly
50 55 60

Pro Ser Pro Lys Val Glu Tyr Val Trp Arg Asn Ile Ile Leu Met Ser
65 70 75 80

Leu Leu His Leu Gly Ala Leu Tyr Gly Ile Thr Leu Ile Pro Thr Cys
85 90 95

Lys Phe Tyr Thr Trp Leu Trp Gly Val Phe Tyr Tyr Phe Val Ser Ala
100 105 110

Leu Gly Ile Thr Ala Gly Ala His Arg Leu Trp Ser His Arg Ser Tyr
115 120 125

Lys Ala Arg Leu Pro Leu Arg Leu Phe Leu Ile Ile Ala Asn Thr Met
130 135 140

Ala Phe Gln Asn Asp Val Tyr Glu Trp Ala Arg Asp His Arg Ala His
145 150 155 160

His Lys Phe Ser Glu Thr His Ala Asp Pro His Asn Ser Arg Arg Gly
165 170 175

Phe Phe Phe Ser His Val Gly Trp Leu Leu Val Arg Lys His Pro Ala
180 185 190

Val Lys Glu Lys Gly Ser Thr Leu Asp Leu Ser Asp Leu Glu Ala Glu
195 200 205

Lys Leu Val Met Phe Gln Arg Arg Tyr Tyr Lys Pro Gly Leu Leu Met
210 215 220

Met Cys Phe Ile Leu Pro Thr Leu Val Pro Trp Tyr Phe Trp Gly Glu
225 230 235 240

Thr Phe Gln Asn Ser Val Phe Val Ala Thr Phe Leu Arg Tyr Ala Val
245 250 255

Val Leu Asn Ala Thr Trp Leu Val Asn Ser Ala Ala His Leu Phe Gly
260 265 270

Tyr Arg Pro Tyr Asp Lys Asn Ile Ser Pro Arg Glu Asn Ile Leu Val
275 280 285

Ser Leu Gly Ala Val Gly Glu Gly Phe His Asn Tyr His His Ser Phe
290 295 300

Pro Tyr Asp Tyr Ser Ala Ser Glu Tyr Arg Trp His Ile Asn Phe Asn
305 310 315 320

Thr Phe Phe Ile Asp Trp Met Ala Ala Leu Gly Leu Thr Tyr Asp Arg
325 330 335

Lys Lys Val Ser Lys Ala Ala Ile Leu Ala Arg Ile Lys Arg Thr Gly
340 345 350

Asp Gly Asn Tyr Lys Ser Gly
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<210> 13
<211> 1637
<212> DNA
<213> Homo sapiens

<400> 13
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<210> 14

<211> 394

<212> PRT

<213> Homo sapiens

<400> 14

Met Ala Leu Leu Asp Leu Ala Leu Glu Gly Met Ala Val Phe Gly Phe
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Val Leu Phe Leu Val Leu Trp Leu Met His Phe Met Ala Ile Ile Tyr
20 25 30

Thr Arg Leu His Leu Asn Lys Lys Ala Thr Asp Lys Gln Pro Tyr Ser
35 40 45

Lys Leu Pro Gly Val Ser Leu Leu Lys Pro Leu Lys Gly Val Asp Pro
50 55 60

Asn Leu Ile Asn Asn Leu Glu Thr Phe Phe Glu Leu Asp Tyr Pro Lys
65 70 75 80

Tyr Glu Val Leu Leu Cys Val Gln Asp His Asp Asp Pro Ala Ile Asp
85 90 95

Val Cys Lys Lys Leu Leu Gly Lys Tyr Pro Asn Val Asp Ala Arg Leu
100 105 110

Phe Ile Gly Gly Lys Lys Val Gly Ile Asn Pro Lys Ile Asn Asn Leu
115 120 125

Met Pro Gly Tyr Glu Val Ala Lys Tyr Asp Leu Ile Trp Ile Cys Asp
130 135 140

Ser Gly Ile Arg Val Ile Pro Asp Thr Leu Thr Asp Met Val Asn Gln
145 150 155 160

Met Thr Glu Lys Val Gly Leu Val His Gly Leu Pro Tyr Val Ala Asp
165 170 175

Arg Gln Gly Phe Ala Ala Thr Leu Glu Gln Val Tyr Phe Gly Thr Ser
180 185 190

His Pro Arg Tyr Tyr Ile Ser Ala Asn Val Thr Gly Phe Lys Cys Val
195 200 205

Thr Gly Met Ser Cys Leu Met Arg Lys Asp Val Leu Asp Gln Ala Gly
210 215 220

Gly Leu Ile Ala Phe Ala Gln Tyr Ile Ala Glu Asp Tyr Phe Met Ala
225 230 235 240

Lys Ala Ile Ala Asp Arg Gly Trp Arg Phe Ala Met Ser Thr Gln Val
245 250 255

Ala Met Gln Asn Ser Gly Ser Tyr Ser Ile Ser Gln Phe Gln Ser Arg
260 265 270

Met Ile Arg Trp Thr Lys Leu Arg Ile Asn Met Leu Pro Ala Thr Ile
275 280 285

Ile Cys Glu Pro Ile Ser Glu Cys Phe Val Ala Ser Leu Ile Ile Gly
290 295 300

Trp Ala Ala His His Val Phe Arg Trp Asp Ile Met Val Phe Phe Met
305 310 315 320

Cys His Cys Leu Ala Trp Phe Ile Phe Asp Tyr Ile Gln Leu Arg Gly
325 330 335

Val Gln Gly Gly Thr Leu Cys Phe Ser Lys Leu Asp Tyr Ala Val Ala
340 345 350

Trp Phe Ile Arg Glu Ser Met Thr Ile Tyr Ile Phe Leu Ser Ala Leu
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ttt 63

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Claims

1) A method for determining whether a substance is an activator or an inhibitor of a function of a protein, characterized in that the protein is selected from the group consisting of MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase, or a functional equivalent, derivative, variant, mutant or fragment of a said protein, and characterized in that the method comprises contacting a said protein or functional equivalent, variant, mutant or fragment thereof with a substance to be tested whether it is an inhibitor or activator of a desired function of a said protein, and measuring whether the desired function is inhibited or activated.

2) A method according to claim 1 in which the inhibition or activation of the desired function is measured directly.

3) A method according to claim 1 in which the inhibition or activation of the desired function is measured indirectly.

4) A method according to claim 1 in which the said protein is a mammalian protein.

5) A method according to claim 4 in which the said protein is a human protein.

6) A method according to claim 1 in which the analysis is performed using a cellular system.

7) A method according to claim 1 in which the analysis is performed using a cell-free system.

8) A method for determining an expression level of a protein which is selected from the group consisting of MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase comprising determining the level of a said protein expressed in a macrophage.

9) A method according to claim 8 in which said macrophage is a mammalian macrophage.

10) A method according to claim 9 in which said macrophage is a human macrophage.

11) A method according to claim 8 for diagnosis or monitoring of a chronic inflammatory airway disease.

12) A method according to claim 11 in which the chronic inflammatory airway disease is selected from the group consisting of chronic bronchitis and COPD.

13) A test system for determining whether a substance is an activator or an inhibitor of a function of a protein, characterized in that the protein is selected from the group consisting of MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase or a functional equivalent, variant, mutant or fragment of a said protein.

14) A test system according to claim 13 comprising a cell expressing a protein selected from the group consisting of MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase or a functional equivalent, variant, mutant or fragment of a said protein.

15) A substance determined to be an activator or inhibitor of a protein selected from the group consisting of MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase.

16) A substance which is an activator or inhibitor of a protein selected from the group consisting of MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase for the treatment for a disease.

17) A substance according to claim 16 in which said disease is a chronic inflammatory airway disease.

18) A substance according to claim 17in which said chronic inflammatory airway disease is selected from the group consisting of chronic bronchitis and COPD.

19) A pharmaceutical composition comprising at least one substance determined to be an activator or inhibitor of a protein selected from the group consisting of MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase.

20) Use of a substance determined to be an activator or inhibitor of a protein selected from the group consisting of MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase for preparing a pharmaceutical composition for treating a chronic inflammatory airway disease.

21) Use of a substance according to claim 20 in which the chronic inflammatory airway disease is selected from the group consisting of chronic bronchitis and COPD.

22) A method for treating a chronic inflammatory airway disease which method comprises administering to a being in need of such treatment a suitable amount of a pharmaceutical composition comprising at least one substance determined to be an activator or inhibitor of a protein selected from the group consisting of MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase.

23) A method according to claim 22 for treating a mammal.

24) A method according to claim 22 for treating a human being.

25) A method according to claim 22 for treating a chronic inflammatory airway disease selected from the group consisting of chronic bronchitis and COPD.

26) A method for selectively modulating a protein selected from the group consisting of MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase in a macrophage, comprising administering a substance determined to be an activator or inhibitor of a protein selected from the group consisting of MIF, DAD1, ARL4, GNS, Transglutaminase 2, Stearyl-CoA-Desaturase and UDP-Glucose Ceramide Glycosyltransferase.

27) A method according to claim 26 in which the macrophage is involved in a chronic inflammatory airway disease.

28) A method according to claim 27 in which the chronic inflammatory airway disease is selected from the group consisting of chronic bronchitis and COPD.