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<td>Title:</td>
<td>A PAF-ACETYLHYDROLASE AND USE IN THERAPY</td>
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<tr>
<td>57</td>
<td>Abstract</td>
<td>A PAF-acetylhydrolase of SEQ.ID.NO 1, for use in therapy.</td>
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**A PAF-ACETYLMETHOXYDRASE AND USE IN THERAPY**

**New Use**

The present invention relates to the use of a novel lipase in therapy.

Lipoprotein Associated Phospholipase A₂ (Lp-PLA₂) [also known as Platelet Activating Factor Acetyl Hydrolase (PAF acetyl hydrolase)] is responsible, during the conversion of LDL to its oxidised form, for hydrolysing the sn-2 ester of oxidatively modified phosphatidylcholine to give lyso-phosphatidylcholine and an oxidatively modified fatty acid. Both of these products of Lp-PLA₂ action are potent chemoattractants for circulating monocytes. As such, this enzyme is thought to be responsible for the accumulation of cells loaded with cholesterol ester in the arteries, causing the characteristic 'fatty streak' associated with the early stages of atherosclerosis. The amino acid and DNA sequence of the enzyme lipoprotein associated Lp-PLA₂ are disclosed in WO95/00649 (SmithKline Beecham plc).

WO 95/09921 (Icos Corporation) suggests a range of possible therapeutic use for platelet activating factor acetylhydrolase, in regulating pathological inflammatory events such as asthma, anaphylaxis, reperfusion injury and central nervous system ischemia, antigen-induced arthritis, atherogenesis, Crohn’s Disease, ischemic bowel necrosis/necrotising enterocolitis, ulcerative colitis, ischemic stroke, ischemic brain injury, systemic lupus erythematosus, acute pancreatitis, septicemia, acute post streptococcal glomerulonephritis, pulmonary edema resulting from IL-2 therapy, allergic inflammation, ischemic renal failure, preterm labour and adult respiratory distress syndrome. It is being developed by Icos for the treatment of diseases associated with PAF, including acute respiratory distress syndrome, asthma, acute pancreatitis, inflammatory bowel disease, solid organ transplant and necrotizing enterocolitis.

More recently, International application number PCT/GB95/02320 (SmithKline Beecham plc) has disclosed a novel polypeptide of human origin having the amino acid sequence given in SEQ ID NO 1, and fragments, analogs or derivative thereof. In addition, it discloses polynucleotides (DNA or RNA) which encode such polypeptide sequences, in particular a polynucleotide having the DNA sequence given in SEQ ID NO 2. cDNA molecules showing extended identity sections with the cDNA of SEQ ID NO 2 have been identified in cDNA libraries from foetal heart, pineal gland, activated T cells, microvascular endothelial cells and secondary breast tumour tissues. The polynucleotide of SEQ ID NO 2 was discovered in a cDNA library derived from prostate (benign possible hyperplasia). It is structurally related to the lipase family. It contains an open reading frame encoding a protein of about 393 amino acid residues. The protein exhibits the highest degree of homology to Lp-PLA₂ (WO95/00649, WO 95/09912) with 40% identity and 60% similarity over a 390 amino acid stretch. Although the overall identity is only 40%, the residues
identified for the catalytic triad in Lp-PLA2 (WO 95/09912) are conserved between the two polypeptides implying that they are likely to have a similar biochemical function. The positions of the Ser, Asp and His, are underlined below. SEQ ID NO 1 is the lower sequence and Lp-PLA2 the upper sequence. Vertical lines indicate identical residues.

238 DEDHGPKVKNAILKFDMEQLKDSIDREKIAVIGHSFGGATVIQTQLSEDQ 287
... | | | | : | : | : | : | : | : | : | : | : | : | ... 
202 EVTAGQTVFNIFFPGGLDLMLTKGNIDMSRVAVMGH5FGGATAILALAKET 251

288 RFRGIALEAWMFPLGDDEVSYRIPOPLFFINSEYFQYPANIICKKCYSP 337
... | | | | | : | : | : | : | : | : | : | : | : | : | ... 
252 QPRCAVALAWMFPLGDFYPKARGPVFFINTKQMTE5MESVNLKKICAO 301

338 DKEKMITIGSVMENFAKTFATGKIHML..KLGIDDSNAAILDSN 385
302 HESQRITVLGVSVEKQTDFAFVTGNLIGKFSTETRSGEGLDPYEQQEQEVM 351

The aforementioned patent application (International application number PCT/GB95/02320) discloses that inhibitors of the polypeptide may be of use in various therapeutic applications such as atherosclerosis, myocardial infarction, reperfusion injury, acute and chronic inflammation, rheumatoid arthritis, stroke, diabetes and neuropsychiatric illnesses but fails to suggest a possible therapeutic role for the polypeptide per se.

We have now established that this novel polypeptide, already implicated as probable lipase by the sequence comparison shown above, shows PAF-acetylhydrolase activity in vitro.

Accordingly, the present invention provides for the use of a polypeptide having the amino acid sequence given in SEQ ID NO 1, and fragments, analogs or derivative thereof in therapy, in particular for the treatment of diseases associated with PAF.

Within the limits imposed by the application of an active protein therapeutic agent, the clinical indications are already, or will be, indicated by the progression of PAF antagonists in revealing the pathological mechanism of disorders involving PAF. These are detailed in a review by Kotai et al. (Platelet-activating factor antagonists: scientific background and possible clinical applications, Koltai M, Guinot P, Hosford D & Braquet G, Advances in Pharmacology, Vol 28, 1994, Academic Press, NY).

Such diseases include those hereinbefore mentioned for PAF-acetyl hydrolase, in particular, acute respiratory distress syndrome, asthma, acute pancreatitis, inflammatory bowel disease, solid organ transplant and necrotizing enterocolitis, as well as AIDS [Platelet activating factor: a candidate HIV-1 induced neutrotoxin, Gelbard et al. J.Virol, 68, 4828-4635 (1994)].
As used herein, the term 'polypeptide(s)' refers to a polypeptide having the amino acid sequence given in SEQ ID NO 1 and fragments, analogs and derivatives thereof.

The polypeptide of the present invention may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide, preferably a recombinant polypeptide.

The fragment, derivative or analog of the polypeptide of SEQ ID NO 1 may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence, (v) one in which amino acid substitutions have been made to induce a glycosylation pattern to increase half-life and/or bioavailability, or (vi) one in which amino acid substitutions, additions or truncations have been made to enhance the capability of the enzyme to utilise PAF as a substrate. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The terms "fragment," "derivative" and "analog" when referring to the polypeptide of SEQ ID NO 1, means a polypeptide which retains essentially the same biological function or activity as such polypeptide. Thus, an analog includes a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide.

The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment. The polypeptide is preferably in purified form. By purified form is meant at least 80%, more preferably 90%, still more preferably 95% and most preferably 99% pure with respect to other protein contaminants.
The polypeptides of the present invention may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise a therapeutically effective amount of the active agent, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

The pharmaceutical compositions may be administered in a convenient manner such as by the oral, topical, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes. The polypeptides or polynucleotides of the present invention is administered in an amount which is effective for treatment and/or prophylaxis of the specific indication. The amounts and dosage regimens of active agent administered to a subject will depend on a number of factors such as the mode of administration, the nature of the condition being treated and the judgment of the prescribing physician.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutic compounds.

Polypeptide for use in the present invention may be obtained from genetically modified host cells using genetic engineering techniques well known to those skilled in the art.

The polynucleotide for use in the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encodes the polypeptide may be identical to the coding sequence shown in SEQ ID NO 1 or may be a different coding sequence which, as a result of the redundancy or degeneracy of the genetic code, encodes the same polypeptide as the DNA of SEQ ID NO 1. Also included are variants of the hereinabove described polynucleotides which encode fragments, analogs and derivatives of the polypeptide having the amino acid sequence of SEQ ID NO 1. The variant of the polynucleotide may be a naturally occurring allelic variant of the polynucleotide or a non-naturally occurring variant of the polynucleotide.

The polynucleotide may have a coding sequence which is a naturally occurring allelic variant of the coding sequence of SEQ ID NO 2. As known in the
art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide.

The polynucleotide which encodes for the polypeptide of SEQ ID NO 1 may include: only the coding sequence for the polypeptide; the coding sequence for the polypeptide and additional coding sequence such as a leader or secretory sequence or a proprotein sequence; the coding sequence for the polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature polypeptide.

Thus, the term "polynucleotide encoding a polypeptide" encompasses a polynucleotide which includes only coding sequence for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequence.

The present invention therefore includes polynucleotides, wherein the coding sequence for the polypeptide may be fused in the same reading frame to a polynucleotide sequence which aids in expression and secretion of a polypeptide from a host cell, for example, a leader sequence which functions as a secretory sequence for controlling transport of a polypeptide from the cell. The polypeptide having a leader sequence is a proprotein and may have the leader sequence cleaved by the host cell to form the mature form of the polypeptide. The polynucleotides may also encode for a proprotein which is the mature protein plus additional 5' amino acid residues. A mature protein having a prosequence is a proprotein and is an inactive form of the protein. Once the prosequence is cleaved an active mature protein remains. Thus, for example, the polynucleotide of the present invention may encode for a mature protein, or for a protein having a prosequence or for a protein having both a prosequence and a prosequence (leader sequence).

The polynucleotides may also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptide of the present invention. The marker sequence may be a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., et al., Cell, 37:767 (1984)).

Suitable polynucleotides also include those which hybridize to the hereinafore-described sequences if there is at least 50% and preferably 70% identity between the sequences, in particular polynucleotides which hybridize under stringent conditions to the hereinafore-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which
hybridize to the hereinabove described polynucleotides in a preferred embodiment
encode polypeptides which retain substantially the same biological function or
activity as the polypeptide of SEQ ID NO 1.

Suitable host cells are genetically engineered (transduced or transformed or
transfected) with the vectors of this invention which may be, for example, a cloning
vector or an expression vector. The vector may be, for example, in the form of a
plasmid, a cosmid, a phage, etc. The engineered host cells can be cultured in
conventional nutrient media modified as appropriate for activating promoters,
selecting transformants or amplifying the genes. The culture conditions, such as
temperature, pH and the like, are those previously used with the host cell selected for
expression, and will be apparent to the ordinarily skilled artisan.

Suitable expression vectors include chromosomal, nonchromosomal and
synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA;
baculovirus; yeast plasmids; vectors derived from combinations of plasmids and
phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and
pseudorabies. However, any other vector may be used as long as it is replicable and
viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of
procedures. In general, the DNA sequence is inserted into an appropriate restriction
endonuclease site(s) by procedures known in the art. Such procedures and others are
deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an
appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As
representative examples of such promoters, there may be mentioned: LTR or SV40
promoter, the E. coli lac or trp, the phage lambda P_L promoter and other promoters
known to control expression of genes in prokaryotic or eukaryotic cells or their
viruses. The expression vector also contains a ribosome binding site for translation
initiation and a transcription terminator. The vector may also include appropriate
sequences for amplifying expression.

In addition, the expression vectors preferably contain one or more selectable
marker genes to provide a phenotypic trait for selection of transformed host cells such
as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such
as tetracycline or ampicillin resistance in E. coli.

The gene can be placed under the control of a promoter, ribosome binding site
(for bacterial expression) and, optionally, an operator (collectively referred to herein
as "control" elements), so that the DNA sequence encoding the desired protein is
transcribed into RNA in the host cell transformed by a vector containing this
expression construction. The coding sequence may or may not contain a signal
peptide or leader sequence. The protein sequences of the present invention can be
expressed using, for example, the *E. coli* tac promoter or the protein A gene (spa) promoter and signal sequence. Leader sequences can be removed by the bacterial host in post-translational processing. Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are PKK232-8 and PCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda PR, PL and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In addition to control sequences, it may be desirable to add regulatory sequences which allow for regulation of the expression of the protein sequences relative to the growth of the host cell. Regulatory sequences are known to those of skill in the art, and examples include those which cause the expression of a gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Other types of regulatory elements may also be present in the vector, for example, enhancer sequences.

An expression vector is constructed so that the particular coding sequence is located in the vector with the appropriate regulatory sequences, the positioning and orientation of the coding sequence with respect to the control sequences being such that the coding sequence is transcribed under the "control" of the control sequences (i.e., RNA polymerase which binds to the DNA molecule at the control sequences transcribes the coding sequence). Modification of the coding sequences may be desirable to achieve this end. For example, in some cases it may be necessary to modify the sequence so that it may be attached to the control sequences with the appropriate orientation; i.e., to maintain the reading frame. The control sequences and other regulatory sequences may be ligated to the coding sequence prior to insertion into a vector, such as the cloning vectors described above. Alternatively, the coding sequence can be cloned directly into an expression vector which already contains the control sequences and an appropriate restriction site. Modification of the coding sequences may also be performed to alter codon usage to suit the chosen host cell, for enhanced expression.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion
protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein.


In some cases, it may be desirable to add sequences which cause the secretion of the polypeptide from the host organism, with subsequent cleavage of the secretory signal.

Yeast expression vectors are also known in the art. See, e.g., U.S. Patent Nos. 4,446,235; 4,443,539; 4,430,428; see also European Patent Applications 103,409; 100,561; 96,491. pSV2neo (as described in J. Mol. Appl. Genet. 1:327-341) which uses the SV40 late promoter to drive expression in mammalian cells or pCDNA1neo, a vector derived from pCDNA1(Mol. Cell Biol. 7:4125-29) which uses the CMV promoter to drive expression. Both these latter two vectors can be employed for transient or stable (using G418 resistance) expression in mammalian cells. Insect cell expression systems, e.g., Drosophila, are also useful. See for example, PCT applications WO 90/06358 and WO 92/06212 as well as EP 290,261-B1.

Polypeptides can be expressed in host cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.


Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter.
enhancer, the polyoma enhancer on the late side of the replication origin, and
adenovirus enhancers.

Suitable hosts include prokaryotes for example bacterial cells, such as *E. coli*,
*Streptomyces, Salmonella typhimurium*; and eukaryotes for example fungal cells, such
as yeast, insect cells such as *Drosophila* and *Spodoptera frugiperda*, mammalian cells
such as CHO, COS or Bowes melanoma, plant cells, etc.

Introduction of the construct into the host cell can be effected by calcium
phosphate transfection, DEAE-Dextran mediated transfection, or electroporation.
(Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

Following transformation of a suitable host strain and growth of the host strain
to an appropriate cell density, the selected promoter is induced by appropriate means
(e.g., temperature shift or chemical induction) and cells are cultured for an additional
period.

Cells are typically harvested by centrifugation, disrupted by physical or
chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any
convenient method, including freeze-thaw cycling, sonication, mechanical disruption,
or use of cell lysing agents, such methods are well know to those skilled in the art.

Various mammalian cell culture systems can also be employed to express
recombinant protein. Examples of mammalian expression systems include the COS-7
lines of monkey kidney fibroblasts, described by Gluzman. Cell, 23:175 (1981), and
other cell lines capable of expressing a compatible vector, for example, the C127,
3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise
an origin of replication, a suitable promoter and enhancer, and also any necessary
ribosome binding sites, polyadenylation site, splice donor and acceptor sites,
transcriptional termination sequences, and 5' flanking nontranscribed sequences.
DNA sequences derived from the SV40 splice, and polyadenylation sites may be used
to provide the required nontranscribed genetic elements.

Depending on the expression system and host selected, the polypeptide of the
present invention may be produced by growing host cells transformed by an
expression vector described above under conditions whereby the polypeptide of
interest is expressed. The polypeptide is then isolated from the host cells and
purified. If the expression system secretes the polypeptide into growth media, the
polypeptide can be purified directly from the media. If the polypeptide is not
secreted, it is isolated from cell lysates or recovered from the cell membrane fraction.
Where the polypeptide is localized to the cell surface, whole cells or isolated
membranes can be used as an assayable source of the desired gene product.
Polypeptide expressed in bacterial hosts such as *E. coli* may require isolation from
inclusion bodies and refolding. The selection of the appropriate growth conditions and recovery methods are within the skill of the art.

The polypeptide can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue.

Cells may be engineered in vivo for expression of a polypeptide in vivo by, for example, procedures known in the art. As known in the art, a producer cell for producing a retroviral particle containing RNA encoding the polypeptide of the present invention may be administered to a patient for engineering cells in vivo and expression of the polypeptide in vivo. These and other methods for administering a polypeptide of the present invention by such method should be apparent to those skilled in the art from the teachings of the present invention. For example, the expression vehicle for engineering cells may be other than a retrovirus, for example, an adenovirus which may be used to engineer cells in vivo after combination with a suitable delivery vehicle.

"Recombinant" polypeptides refer to polypeptides produced by recombinant DNA techniques; i.e., produced from cells transformed by an exogenous DNA construct encoding the desired polypeptide. "Synthetic" polypeptides are those prepared by chemical synthesis.

A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication in vivo; i.e., capable of replication under its own control.

A "vector" is a replicon, such as a plasmid, phage, or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment.

A "double-stranded DNA molecule" refers to the polymeric form of deoxyribonucleotides (bases adenine, guanine, thymine, or cytosine) in a double-stranded helix, both relaxed and supercoiled. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, inter alia, in linear
DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the sense strand of DNA.

A DNA "coding sequence of" or a "nucleotide sequence encoding" a particular protein, is a DNA sequence which is transcribed and translated into a polypeptide when placed under the control of appropriate regulatory sequences.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. Within the promoter sequence will be found a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes.

DNA "control sequences" refers collectively to promoter sequences, ribosome binding sites, polyadenylation signals, transcription termination sequences, upstream regulatory domains, enhancers, and the like, which collectively provide for the expression (i.e., the transcription and translation) of a coding sequence in a host cell.

A control sequence "directs the expression" of a coding sequence in a cell when RNA polymerase will bind the promoter sequence and transcribe the coding sequence into mRNA, which is then translated into the polypeptide encoded by the coding sequence.

A "host cell" is a cell which has been transformed or transfected, or is capable of transformation or transfection by an exogenous DNA sequence.

A cell has been "transformed" by exogenous DNA when such exogenous DNA has been introduced inside the cell membrane. Exogenous DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell. In prokaryotes and yeasts, for example, the exogenous DNA may be maintained on an episomal element, such as a plasmid. With respect to eukaryotic cells, a stably transformed or transfected cell is one in which the exogenous DNA has become integrated into the chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cell containing the exogenous DNA.

A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth in vitro for many generations.

Two DNA or polypeptide sequences are "substantially homologous" or "substantially the same" when at least about 85% (preferably at least about 90%, and
most preferably at least about 95%) of the nucleotides or amino acids match over a
defined length of the molecule and includes allelic variations. As used herein,
substantially homologous also refers to sequences showing identity to the specified
DNA or polypeptide sequence. DNA sequences that are substantially homologous
can be identified in a Southern hybridization experiment under, for example, stringent
conditions, as defined for that particular system. Defining appropriate hybridization
conditions is within the skill of the art. See, e.g., "Current Protocols in Mol. Biol."
substantially the same can be identified by proteolytic digestion, gel electrophoresis
and microsequencing.

The term "functionally equivalent" intends that the amino acid sequence of the
subject protein is one that will exhibit enzymatic activity of the same kind as that of
the lipase.

A "heterologous" region of a DNA construct is an identifiable segment of
DNA within or attached to another DNA molecule that is not found in association
with the other molecule in nature.

The present invention will be further described with reference to the following
examples; however, it is to be understood that the present invention is not limited to
such examples. All parts or amounts, unless otherwise specified, are by weight.

In order to facilitate understanding of the following examples certain
frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case p preceded and/or followed by
capital letters and/or numbers. The starting plasmids herein are either commercially
available, publicly available on an unrestricted basis, or can be constructed from
available plasmids in accord with published procedures. In addition, equivalent
plasmids to those described are known in the art and will be apparent to the ordinarily
skilled artisan.

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or
two complementary polydeoxynucleotide strands which may be chemically
synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not
ligate to another oligonucleotide without adding a phosphate with an ATP in the
presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has
not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two
double stranded nucleic acid fragments (Maniatis. T.. et al., Id., p. 146). Unless
otherwise provided, ligation may be accomplished using known buffers and
conditions with 10 units to T4 DNA ligase ("ligase") per 0.5 µg of approximately
equimolar amounts of the DNA fragments to be ligated.
EXAMPLE
Gene Cloning

cDNA Library construction
Poly A+ (mRNA) was isolated from human prostate (benign possible hyperplasia) using standard methods (ref Maniatis et al). First strand cDNA was primed using an oligo dT primer. The cDNA library was constructed with the Stratagene ZAP-cDNA synthesis kit, packaged with Gigpack II gold packaging extract and amplified in XLI-blue MRF* bacterial cells. The cDNA inserts were cloned unidirectionally into the vector.

DNA Sequencing
The phage clone containing the EST was excised from the λ Unizap XR bacteriophage vector into the Bluescript phagemid (according to the Stratagene manual) for characterisation. The insert of 1823bp was manually sequenced on both strands (using the Amersham -USB Sequenase 2.0 DNA sequencing kit) by primer walking (SEQ ID 2). The cDNA has an open reading frame with the potential to code for a polypeptide of 393 amino acids (SEQ ID 1). The predicted MW for the full reading frame is 44143Da.

Production of an NSDL* recombinant baculovirus and expression of the functional protein.

The NSDL cDNA was isolated from its host plasmid pBluescript II SK+- as an approximate 1800bp EcoRI-Xhol fragment, the enzyme activity inactivated for 10 minutes at 80° C followed by blunt ending with the Klenow fragment of DNA polymerase I, in the presence of excess dNTPs. The blunt end fragment was ligated into the Sma I site of the baculovirus expression vector, pBacPAK9 (Clontech) with the resulting plasmid transformed into E.Coli (high efficiency JM109 -Promega) and plated on LB/1.5% w/v agar plates containing 100μg/ml ampicillin. Recombinant clones were predicted by DNA restriction enzyme analysis and confirmed by DNA sequencing using the USB Sequenase 2.0 DNA sequencing kit.

* NSDL (novel serine dependent lipase) is name given to the novel polypeptide of SEQ ID NO 1.

Co-transfection of plasmid DNA into baculovirus DNA

DNA from selected clones was diluted to 100ng/μl with water and co-transfected with BakPAK6 DNA into BSU 36I digested baculovirus DNA (Clontech), using lipid mediated transfer (Lipofectin-Life Technologies). The viral DNA was used to infect 1.5x10^6 Spodoptera frugiperda cells (sf-9; ATCC CRL 1711) seeded in 2ml of IPL-41 medium plus lipid and yeast supplements (complete medium). 72 hours post-
transfection, the virus particles released into the medium were harvested and used to create a plaque assay (Summers, M.D. and Smith G.E.; Texas Agricultural Experiment Station Bulletin No 1555, 1987). Ten plaques were chosen at random and each used to infect $5 \times 10^5$ fresh sf-9 cells in 2ml complete medium. Four days post-infection 1.8ml medium from each plaque was used to infect a further $5 \times 10^5$ fresh sf-9 cells. Four days post infection the sf-9 cells were lysed in 200µl 50mM HEPES buffer, 10mM CHAPS pH 7.4, plus 10 µg/ml each of the protease inhibitors pepstatin A, antipain. Leupeptin, aprotinin, the debris removed by centrifugation at 3000xg for 4 minutes and 1µl of the supernatant assayed in a colourimetric assay.

Virus titre was improved by further rounds of infection in sf-9 cells using a moi of 0.01 and protein expression optimised at 2 days post-infection. Purification of NSDL was carried out from a 30 litre culture of infected cells.

**Purification of Recombinant NSDL**

Sf9 cells were lysed by freeze thawing 3 times in liquid nitrogen after the addition of 40mL/L of lysis buffer (50mM Tris (pH 8.5), 5mM Chaps, 1mM EDTA, 1mM DTT, 20% glycerol,50 µg/ml Benzamidine, plus 10µg/ml protease inhibitors. The sample was centrifuged at 50,000xg for 20 minutes to pellet the cell debris, following the addition of DNase to reduce the viscosity.

The supernatant was loaded onto a Q sepharose column (Pharmacia) which had been equilibrated with lysis buffer minus protease inhibitors (buffer A). The column was washed with buffer A and the protein was eluted using a linear gradient of NaCl. (0-1M) The enzyme eluted at 300mM NaCl.

Active fractions were concentrated using a YM30 membrane in an Amicon ultrafiltration cell, followed by dialysis against 50mM Mes,( pH 6.0) ,5mM Chaps, 1mM DTT, 1mM EDTA, 20% Glycerol (buffer B). The sample was loaded onto a blue sepharose 6 fast flow column (Pharmacia), equilibrated with buffer B. The column was washed with buffer B, followed by 50mM Mops, (pH 7.0), 5mM Chaps, 1mM DTT, 1mM EDTA, 20% glycerol, 0.3M NaCl (buffer C) and enzyme activity was eluted using 50mM Tris (pH 8.0), 5mM Chaps, 1mM DTT, 1mM EDTA, 20% glycerol, 1M NaCl (buffer D).

Active fractions were concentrated over a YM30 membrane and dialysed against 10mM Tris (pH 7.4), 5mM Chaps, 1mM DTT, 20% Glycerol (buffer E).

The concentrated protein was applied to a hydroxyapatite column (Biorad), equilibrated with buffer E and the enzyme was eluted with a linear gradient from buffer E alone, to buffer E containing 150mM KH₂PO₄. The activity eluted at around 60mM KH₂PO₄.
The protein was concentrated over a YM30 membrane followed by a 10 fold dilution with buffer A and was then applied to a Resource Q column (Pharmacia) pre-equilibrated with buffer A. The enzyme does not bind to the column and is collected by washing with buffer A, contaminants are eluted using buffer A plus 1M NaCl.

Activity of Recombinant NSDL

Activity assays were carried out as described in Tew D G, Southan C, Rice S Q. J., Rice G., Lawrence M P, Li H, Gloger I S, Saul H F, Moores K & MacPhee C H., Purification, properties, sequencing and cloning of a lipoprotein associated, serine dependent phospholipase which is involved in the oxidative modification of low density lipoproteins. Arteriosclerosis, 16, 591-599, 1996. The Km values for PAF as a substrate with purified NSDL was determined to be 42 mM and the turn over rate for PAF determined as 35 mmol/min/mg.
M is defined as either A or C, where the actual base is unclear from either DNA strand.
Claims

1. A polypeptide having the amino acid sequence of SEQ ID NO 1 or a fragment, analog or derivative of said polypeptide for use in therapy.

2. The use of a polypeptide having the amino acid sequence of SEQ ID NO 1 or a fragment, analog or derivative in the manufacture of a medicament for treating diseases associated with PAF.

3. A method of treating a disease associated with PAF which comprises administering to a patient in need thereof an effective amount of a polypeptide having the amino acid sequence of SEQ ID NO 1 or a fragment, analog or derivative.

4. A pharmaceutical composition comprising the polypeptide of claim 1 and a pharmaceutically acceptable carrier or excipient.