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Fremdragne publikationer:
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FORD K G ET AL: "PROTEIN TRANSDUCTION: AN ALTERNATIVE TO GENETIC INTERVENTION?" GENE THERAPY, MACMILLAN PRESS LTD., BASINGSTOKE, GB, Bd. 8, Nr. 1, Januar 2001 (2001-01), Seiten 1-4, XP001098109 ISSN: 0969-7128
Technical field of the invention

The invention relates to the stimulation and inhibition of the immune system for the prophylaxis, therapy or diagnosis of disorders associated with an immune system which is insufficiently stimulated or is excessively stimulated. These disorders include *inter alia* infectious diseases, neoplastic diseases, allergies, autoimmune diseases, transplant rejection reactions, etc. The core of the invention is a novel method with which the immune system is influenced by administering a MAT molecule that consists of at least the following three constituents:

1. a translocation module which has the effect that the MAT molecule can penetrate into cells from the outside,

2. an intracellular targeting module which has the effect that the MAT molecule is processed within the cell in such a way that there is an altered immune response or an altered presentation of the antigen, and

3. an antigen module which determines the specificity of the modulated immune response.

Combination of these three elements to give a MAT molecule makes it possible for the immune system of the treated individual to be modulated in a targeted and specific manner, or makes it possible for the presentation of the antigen by the antigen-presenting cell to be altered.

Prior art

Processing of antigens by antigen-presenting cells

The processing of antigens by antigen-presenting cells (APC) takes place by two different routes. Antigens occurring inside the cell are presented by MHC I (major histocompatibility complex class I, MHC class I) molecules on the cell surface, whereas extracellular antigens are presented by MHC II (major histocompatibility complex class II, MHC class II) molecules on the cell surface. Both mechanisms
initiate an immune reaction by the host to the antigen. The route taken by the antigen from uptake into the cell until presentation on the cell surface in the form of an MHC II-antigen complex proceeds via various cell organelles, *inter alia* via the endoplasmic reticulum, the Golgi apparatus, the trans-Golgi network, lysosomes, endosomes and via MHC class II compartments (MII/C). The MII/Cs play an important part in the MHC II-mediated antigen presentation. In these organelles of the cell, the MHC II molecules are loaded with low molecular weight antigens or with proteolytic fragments of proteins. In this process, the invariant chain (also called MHC II gamma chain or ii) which is initially bound to the MHC II molecule undergoes proteolytic degradation, and the antigen is bound to the MHC II molecule under the regulation of various proteins which bind directly or indirectly to MHC II [1]. These regulatory molecules include *inter alia* HLA-DM, HLA-DO, LAMP-1, LAMP-2, CD63, CD83, etc. The exact function of these proteins is in part unexplained as yet, but many of them have signal sequences which promote their transport to the lysosomes, to the endosomes, to the trans-Golgi network, to the MII/Cs etc. [2-4]. A number of proteases are involved in the proteolytic reactions necessary so that the antigen can be presented on MHC II molecules. The proteases present in MII/Cs include *inter alia* various members of the cathepsin family such as, for example, cathepsin S and cathepsin L [1].

**Targeting and Targeting Sequences**

Amino acid sequences which have the property of accumulating inside or outside a cell specifically at a particular site or in a particular cell organelle are frequently referred to as targeting sequences. It should be emphasized in this connection that various types of targeting can be distinguished. In particular a distinction is made between intracellular and extracellular targeting. For extracellular targeting, for example, antibodies which bind from outside to directly accessible structures on the cell surface, e.g. to the extracellular portion of membrane proteins, are used. An antibody which binds a protein on the cell surface of tumour cells can be coupled for example to a cytotoxin. This antibody then brings about an extracellular targeting of the cytotoxin for the tumour cell, thus making targeted killing of the latter possible. This type of targeting is fundamentally different from intracellular targeting, in which it is often necessary to overcome intracellular
membranes or in which the targeting sequence is bound by intracellular receptors and thus penetrates for example into particular cell organelles, or in which the targeting sequence can penetrate the specific channels, formed by specific proteins, into the cell organelle for which the targeting sequence is specific. If a targeting sequence or a targeting module is mentioned below in the present patent application, it is in principle always intracellular targeting and not extracellular targeting which is meant thereby. In addition, in the present patent application, targeting does not mean every type of intracellular targeting, but means only intracellular targeting which serves to transport molecules to organelles or intracellular regions involved in the processing, modification and/or binding of antigens to MHC molecules. Examples of such organelles or intracellular regions are the endoplasmic reticulum, the Golgi apparatus, the trans-Golgi network, endosomes, lysosomes and MII Cs. Targeting sequences derived from various proteins are described in the literature [1-5].

Translocation and Translocation Sequences

In addition, numerous amino acid sequences, especially derived from viruses, e.g. HIV tat or the protein VP22 which is derived from herpes simplex virus, which promote the transport of proteins, peptides and other classes of substances, such as, for example, nucleic acids or pharmaceutically active substances, into the interior of cells are known from the literature. For this purpose, these so-called translocation sequences are linked to the molecules to be transported (also called cargo molecules) either via covalent or via non-covalent linkages.

Extracellular addition of the resulting compounds of translocation sequence and cargo molecule to cells is then possible. The translocation sequence then brings about entry of the cargo molecule into the interior of the cell. This principle has likewise been described in numerous studies, especially for the HIV Tat sequence [6-8].

Although it was already possible and intended to use an antigen as cargo molecule, it has as yet not been possible with the aid of the known translocation means alone to generate an immune response in an individual in a sufficiently targeted and dosed manner as appears desirable for certain tasks - for example
allergy desensitization. Hence there is a need for further methods for targeted immunomodulation.

Von Bergen et al., Immunol. Rev., 1999, 172, 87-96, summarises the knowledge in the field of the targeting of antigens up to the year 1999. According to this, two different strategies are used for targeting the antigens to the MHC II molecules, the use of DANN constructs and the targeted receptor-mediated uptake of antigens by APC which express corresponding receptors.

Sheldon K. et al., PNAS, 1995, 92(6), 2056-2060, relates to the use of L-oligomers. The molecules described therein allow the importation of certain sequences from the extracellular region in the cell nucleus.

**Problem of the invention**

The problem of the invention is therefore to provide means for supplying different antigens to their processing site in a targeted manner, such that the latter can be better utilised from both a therapeutic and diagnostic point of view.

**Solution to the problem**

The invention provides for the use of modular antigen transport molecules which enable antigens to be supplied in a very targeted manner to cells in order to achieve an efficient, specific immune reaction. These molecules make it possible firstly to convey antigens efficiently from the extracellular space to the intracellular space of the cell, and secondly for the antigens, when they have arrived in the interior of the cell, to efficiently reach the cell organelles in which they are further processed for antigen presentation. This two-stage process can be used very generally for the targeted, efficient modulation of the immune reaction of an individual.

Special molecules have been developed as a tool for achieving these effects and are referred to hereinafter in this patent application as "modular antigen transporter" molecules or MAT molecules. These MAT molecules, associated
nucleic acids, vectors, cells, cell lines, vesicles, immunoglobulins, and uses and methods which belong to the invention and which relate to these constituents or operate with them are characterised in detail in the claims.

To solve the problem, a combination, not hitherto described, of at least three modules to give a novel class of molecules, which are referred to as MAT molecules (modular antigen transport molecules), is provided. These three modules include at least one translocation module, at least one targeting module and at least one antigen module. The three different modules are coupled to one another via covalent or non-covalent linkages. The MAT molecule thus prepared can be administered directly to an individual whose immune reaction to the antigens present in the MAT molecule is to be influenced. Alternatively, it is also possible for cells to be treated with MAT molecules in vitro, and for the cells thus treated to be subsequently administered to the individual. In this method, the translocation modules have the effect that the MAT molecule can penetrate into the cell, the targeting modules have the effect that the MAT molecule undergoes intracellular processing so that there is an immune response, and the identity of the antigens in the antigen modules determines the antigen against which the immune reaction is directed. A major advantage of this novel method for modulating the immune response of an individual is in particular its universal applicability, i.e. it can operate with a wide variety of antigens, various translocation modules and various targeting modules. In addition, the use of a translocation module results in the method not being tissue- or cell-specific, but being universally suitable for immunomodulation of virtually all types of cells. A further advantage is the modular structure of the MAT molecule. The modular structure enables the MAT molecule to adapt quickly to the particular medical requirements. It is also possible to vary the exact arrangement of the three constituents of the MAT molecule and the nature of their interconnection, as long as at least one module of all three types of modules is present in the MAT molecule. There are no restrictions relating to the antigens on the basis of the method. The method can be used for example for activating the immune system of an individual against pathogens such as, for example, viruses, bacteria, parasites, etc., i.e. in very general terms as a vaccine. In addition, the method can be used to activate the immune system against degenerate cells such as, for
example, tumour cells, etc. However, it can also be used on the other hand for
desensitising the immune system of an individual against allergens such as, for
example, pollen, animal hair, house dust mites, insect toxins, etc. or for targeted
suppression of the immune system, e.g. if autoimmune reactions are present,
such as, for example, arthritis, rheumatism, diabetes, SLE (systemic lupus
erythematosus), etc., and for suppressing transplant rejection reactions. Further
disorders which are not expressly mentioned and which are associated with an
excessively strong or weak immune reaction can likewise be treated with the
MAT molecules of the invention.

In the prior art, it has hitherto been considered to be a severe disadvantage that
translocation sequences are not specific for particular types of cells, but are
equally active in all types of cells [9]. However, in the present invention, the
universal functionality of translocation modules is a great advantage which has
not been recognised in the prior art to date. It has often been attempted in the
prior art to date to protect "cargo molecules", which are inserted into cells with the
aid of translocation sequences, from proteolytic degradation in the cell [9]. In the
present invention, precisely the opposite is expressly desired and advantageous
for the mode of action of the invention. Proteolytic degradation of the antigen
modules in the cell is advantageous for the effect of the invention, i.e. for efficient
antigen presentation. Therefore, the present invention uses targeting modules
which specifically promote the transport of the antigen modules into cell
compartments where they undergo proteolytic degradation.

The best-known amino acid sequences which can be used as translocation
module for the purposes of the invention are the HIV Tat and the VP22
sequence. These sequences are described as bringing about both a
translocation through the cell membrane and transport into the cell nucleus [7].
This transport into the cell nucleus is not wanted in connection with the present
invention, because no antigen processing takes place in the cell nucleus, and
thus efficient antigen presentation does not occur. This hitherto unsolved problem
is solved by the present invention through the use of a targeting module that has
the effect that the MAT molecule is not transported intracellularly into the cell
nucleus, but is transported specifically into those organelles in which antigen
processing or the loading of MHC molecules with antigen take place. The 
intracellular translocation module of the MAT molecules of the invention therefore 
eliminates a major disadvantage associated with Tat antigen fusion proteins 
known from the prior art to date.

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Also known in the prior art to date are fusion proteins consisting of a targeting 
sequence, e.g. the invariant chain of the MHC II molecule, and an antigen. 
However, these fusion proteins are incapable of efficient penetration into antigen-

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presenting cells. For this, when they are used for immunising an individual, an 
adjuvant that promotes uptake of the fusion protein into the cell is additionally 
required. These adjuvants such as, for example, mineral oil, mycobacterial 
extracts or Freund’s adjuvant do, however, have unwanted side effects such as, 
for example, local inflammatory reactions. The MAT molecules used in the 

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present invention have the advantage over conventional vaccines that they are 
directly coupled to a physiologically well-tolerated translocation module that very 
effectively promotes uptake into cells. In this way it is possible in some 
circumstances to dispense wholly or partly with additional adjuvants. The result of 
this is that distinctly fewer unwanted side effects will occur with immunisations 
using MAT molecules.

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It has long been known that extra- or intracellular MHC I presented antigens lead 
to a cytotoxic immune response, but not to a strong projective humoral immune 
response. However, by using MAT molecules, it is possible for the antigens 
present in the antigen module to be added extracellularly, although they act like 

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intracellular antigens, because the translocation module transports the antigen 
into the intracellular space, and the targeting module intracellularly influences the 
transport of the antigen in such a way that there is a humoral immune response. 
Using this novel method it is possible to achieve the strong induction, which has 
been desired for many years, of a humoral immune response with extracellularly 
added antigens (MAT molecules).

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In the summarising article by van Bergen, J., 1999, Immunological REVIEWS, 
172, 87-96, various strategies for targeting antigens in MHC class II molecules 
are described. These strategies are based on the one hand on receptor-
mediated endocytosis of the antigens. On the other hand, molecular biological possibilities for the endogenous targeting of the antigens are discussed. The article by Sheldon, K., et al., PNAS, 1995, 92, 2056-2060, describes new molecules which are referred to as loligomers. These are molecules which describe translocation signals fused with a marker molecule. These constructs are seen as a possible way of permitting controlled intercellular targeting.

For this purpose, in contrast to the prior art to date, two mechanisms known per se are combined in a novel manner in this invention, thus resulting in a substantially improved immunisation with antigens. In both cases, these mechanisms are targeted antigen transport mechanisms which overall lead to a very efficient immune response. Each of these two transport mechanisms is brought about by a defined module of the MAT molecule. They are:

1. transport of the antigen from the extracellular space into the intracellular space (translocation module) in combination with

2. transport of the antigen within the cell specifically to the organelles responsible for antigen processing (targeting module).

The result of this is: very efficient immunisation, IgG only, no IgE

This novel combination of 2 known transport mechanisms makes it possible, for example, to carry out immunisation with much lower antigen concentrations, and additionally has the very great and surprising advantage of predominantly producing an immune response of the Th1 type, i.e. an immune response in the form of IgG antibodies and not in the form of allergy-causing IgE antibodies.
Detailed description of the invention

Translocation Sequences/Translocation Modules

5 The terms translocation sequence and translocation module are used as equivalent and having the same meaning in the text of the present application. The term translocation module was introduced in order to make it clear that translocation modules are only one part of a MAT molecule for the purposes of the invention. In addition, translocation modules represent not only naturally occurring translocation peptide sequences such as, for example, HIV tat, but also, for example, peptidomimetics or other structures which are able to undertake the same function as the naturally occurring translocation peptide sequences.

10 The invention encompasses the use of various translocation modules for preparing MAT molecules which consist of at least one translocation module, at least one targeting module and at least one antigen module. In general, all translocation sequences which are currently known and will be known in future are suitable to be used for the purposes of the present invention. Numerous suitable translocation sequences are described in the literature. These translocation sequences include viral sequences, homeoprotein sequences, leucine zipper sequences, arginine- and lysine-rich sequences, and various other sequences of proteins which are secreted despite the absence of a secretion signal sequence, etc.

20 Viral Peptide Sequences Suitable as Translocation Modules

The peptide sequences suitable as translocation modules for the purposes of this invention include inter alia viral proteins or partial sequences of viral proteins such as, for example, the protein HIV transcriptional activator protein (HIV tat).

25 Besides the Tat protein of the HIV-1 virus, suitable Tat proteins also include the Tat proteins of other lentiviruses [9]. Numerous modified Tat peptides have been described as sequences able to bring about translocation. These include Tat peptides which represent only partial sequences of the Tat protein [10], Tat
peptides which comprise point mutations [10], Tat peptides in which the sequence is reversed (inverted) [10], or Tat peptides which comprise unusual amino acids such as, for example, D isomers of amino acids [10], etc. All these variations of peptide sequences are therefore generally suitable as translocation modules. Peptides also suitable as translocation modules for the purposes of the present invention are those derived from other viruses such as, for example, VP22 (herpes simplex virus-1 VP22 tegument protein) [9]. Commercial expression vectors comprising a VP22 sequence suitable for translocation are now also available. These expression vectors therefore permit VP22 fusion proteins to be prepared (Voyager™ VP22 system, Invitrogen, Breda, the Netherlands). However, no targeting module is present in the fusion protein when this expression system is used. Other viruses, e.g. Marek’s disease virus-1, a virus which causes lymphoma in chickens, also express a protein which is related to VP22 and which is likewise suitable as translocation module [11]. These proteins and partial sequences of these proteins are mentioned only as examples, and numerous further peptides are known at present, and will become known in future, which are suitable as translocation modules for the purposes of the invention.

20 **Homeoproteins Suitable as Translocation Modules**

A further group of translocation modules suitable for the purposes of the present invention are peptides derived from the Drosophila homeotic protein antennapedia (ANTp) [9]. ANTp peptides suitable as translocation module include those comprising an inverted sequence of ANTp [7], those comprising D isomers of amino acids [7], or those comprising point mutations in their sequence [7]. It is additionally expected that numerous further ANTp sequence modifications are also possible which will presumably make translocation possible [7]. ANTp peptide variants are also referred to as transport peptides.

Further homeoproteins such as, for example, engrailed 1 (En1), engrailed 2 (En2), Hoxa-5, Hoxc-8, Hoxb-4 and KNOTTED-1 [7] likewise comprise sequences which can be used as translocation module for the purposes of the present invention. KNOTTED-1 is in fact a plant protein, but is likewise suitable as translocation module in animal cells. These peptides are mentioned only as
examples, and numerous other homeoproteins which comprise peptide sequences which may be suitable as translocation module for the purposes of the invention are known [12]. Other previously undisclosed homeoproteins may also comprise sequences suitable as translocation module.

Leucine Zipper Proteins Suitable as Translocation Module

A further group of sequences suitable as translocation module for the purposes of the present invention are peptides comprising a leucine zipper domain. Examples of proteins whose leucine zipper domains can be used as translocation sequence are, for example, human cFos-(139-164), human cJun-(252-279), or the yeast transcription factor yeast GCN4-(231-252) [8]. Further leucine zipper proteins which are already known or will become known in future are also suitable as translocation module for the purposes of the invention.

Arginine- or Lysine-Rich Peptides Suitable as Translocation Module

Arginine-rich peptides, frequently derived from RNA- and DNA-binding proteins, represent further peptide sequences which can be used as translocation modules for the purposes of the present invention. Examples of such sequences are HIV-1 rev-(34-50), flock house virus coat protein FHV coat-(35-49), BMV Gag-(7-25), HTLV-II Rex-(4-15), CCMV Gag-(7-25), P22 N-(14-30), lambda N-(1-22), phi 21 N-(12-29) and PRP6-(129-144) from yeast [8]. It is also possible, for the purposes of the invention, to use polyarginine peptides having 4 to 16 [8] or else having more than 16 arginine residues. In addition to polyarginine peptides, it is also possible to use, as translocation modules, peptides which also comprise, besides arginine, further amino acids, e.g. the W/R peptide (RRWRRWRRWRRWRRR) [9] or the R9-Tat peptide in which the 9 central amino acid residues of the total of 11 amino acids of a Tat peptide have been replaced by arginine residues (GRRRRRRRRRR) [8]. It has additionally been possible to show that peptides which consist for example of nine lysine residues also have the ability to act as translocation modules for the purposes of the invention [13]. These peptides are mentioned only as examples and numerous further arginine- or lysine-rich peptides are suitable to be used as translocation
modules for the purposes of the invention [8, 13]. Presumably, further arginine- or lysine-rich peptides which are already known at present or will become known in future are also suitable as translocation modules. Sequences comprising guanidino or amidino groups are also suitable as translocation module for the purposes of the present invention [14].

Proteins Without Signal Sequence Which are Suitable as Translocation Modules

A number of further proteins have the ability, without a secretion signal sequence being present, to penetrate the cell membrane from the inside to the outside, i.e. to be secreted. These proteins are frequently also able conversely to penetrate into the interior of the cell from the outside. These proteins or partial sequences of these proteins can thus also be used as translocation modules for the purposes of the present invention. Some representative examples of such proteins are fibroblast growth factor 1 (FGF-1), fibroblast growth factor 2 (FGF-2), caveolin-1, lactoferrin, thioredoxin, interleukin 1 beta and ciliary neurotrophic factor (CNTF) [7], or interleukin 1 alpha, vas deferens protein, platelet-derived endothelial cell growth factor (PR-ECGF), thymosin, para-thymosin, 14.5 kDa lectin (L14), transglutaminase, thioredoxin-like protein (ADF), sciatic nerve growth-promoting activity, factor XIIIa, mammary-derived growth inhibitor, galec tin, rhodanase [15].

These peptides are mentioned solely by way of example, and numerous further peptides are known or will become known in future which are suitable as translocation modules for the purposes of the invention.

Toxins Suitable as Translocation Modules

Many toxins or partial sequences of toxins have the property of acting as translocation modules, such as, for example, the following toxins: complete abrin, complete ricin, complete modeccin, complete pseudomonas exotoxin A, complete diphtheria toxin, complete pertussis toxin, complete Shiga toxin, the A chain of ricin, the A chain of abrin, the A chain of modeccin, the enzymatically active
domain of pseudomonas exotoxin, the A chain of diphtheria toxin A, the
enzymatically active domain of pertussis toxin, the enzymatically active domain of
Shiga toxin, gelonin, pokeweed antiviral protein, saporin, tritin, barley toxin and
snake venom peptides [16]. These toxins mentioned as examples, and many
further other toxins which have not been expressly mentioned or which will
become known in future can be used as translocation modules for the purposes
of the present invention.

Controlling the Efficiency of Translocation Modules

The efficiency of translocation can be controlled by varying the length of, for
example, a poly-arginine chain or by specific selection of, for example, only a
partial sequence of the HIV Tat sequence, so that very efficient translocation of
the corresponding MAT molecule, or a less efficient translocation, takes place,
depending on the particular requirements [8, 13]. A very efficient translocation
may have the advantage that the efficacy of the MAT molecule is increased
and/or that the necessary dose of MAT molecule can be reduced, in turn saving
vaccine costs. A reduced dose of MAT molecule in turn has the advantage that
there are fewer side effects. On the other hand, a reduced efficiency of
translocation makes it possible for the MAT molecules to be distributed widely in
the treated individual, e.g. after intravenous injection, because they do not
immediately penetrate locally and virtually quantitatively into all cells located in
the vicinity.

Examples of Minimal Sequences Acting as Translocation Modules

It is not necessary for all the translocation sequences mentioned as examples to
be in the form of the complete protein as constituent of the MAT molecule in
order to be effective as translocation module for the MAT molecule for the
purposes of the invention. On the contrary, a minimal sequence region which can
be used as translocation sequence is known for many of said proteins. For
example, this sequence region includes the following sequence for HIV Tat, for
example: Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg, the following sequence
for VP22: Asp-Ala-Ala-Thr-Ala-Thr-Arg-Gly-Arg-Ser-Ala-Ala-Ser-Arg-Pro-Thr-Glu-
Arg-Pro-Arg-Ala-Pro-Ala-Ser-Ala-Ser-Arg-Pro-Arg-Pro-Val-Glu and the following sequence for antenapedia: Arg-Gln-Ile-Lys-Ile-Trp-Phe-Gln-Asn-Arg-Arg-Met-Lys-Trp-Lys-Lys [17]. Moreover, the sequences may also be used in the form of fragments which do not correspond to the currently known minimal functional sequence segments, as long as the resulting sequence is still functional in the sense of a translocation module.

Translocation modules therefore need not be in the form of the complete protein or the complete molecule as constituent of the MAT molecule in order to be effective as translocation module for the MAT molecule for the purposes of the invention. On the contrary, a sequence region which can be used as translocation module is known for example for some of the proteins mentioned. In addition, the protein sequences may also be used in the form of fragments which do not correspond to the previously known functional sequence segments, as long as the resulting sequence is still functional as a translocation module.

Testing of the functionality of a translocation module can be ascertained for example by using fluorescence-labelled translocation modules or by using enzyme-labelled translocation modules or by using translocation modules labelled with metal particles. The translocation modules labelled in these ways are administered to an experimental animal or to cells cultivated in vitro, and the fate of the translocation modules is monitored using methods such as FACS (fluorescence activated cell sorting), microscopy, confocal fluorescence microscopy, electron microscopy etc. These techniques for checking the functionality of translocation modules are described in the literature, and some of them have already been used to ascertain the functionality of sequences for translocation [8, 18].

**Targeting Sequences/Targeting Modules**

The terms targeting sequence and targeting module are used as equivalent terms with the same meaning in the text of the present application. In this patent application targeting in principle always means intracellular targeting. The term targeting module was introduced in order to make it clear that translocation modules are only one part of a MAT molecule for the purposes of the invention.
The invention encompasses the use of various sequences as targeting modules for preparing MAT molecules which consist of at least one translocation module, at least one targeting module and at least one antigen module. In general, all amino acid sequences and molecules which are currently known and will become known in future and which are able to mediate targeting are suitable for use as targeting modules for the purposes of the present invention. Numerous suitable sequences are described in the literature. These sequences that are suitable as targeting modules include all those which cause the MAT molecule to be transported intracellularly to the sites or organelles within a cell at which processes involved in the presentation of the antigen modules contained within the MAT molecule take place. These sites and organelles within the cell include, in particular, MHC class II compartments (MIIcs), endosomes, lysosomes, the Golgi apparatus, the trans-Golgi network and the endoplasmic reticulum. These intracellular organelles are involved in processes such as, for example, the transport or processing of antigens, the preparation and loading of MHC II molecules with antigens or processed antigens, and the transport of the MHC II molecules loaded with antigens to the cell surface etc.

**MHC Molecules Comprising Sequences Suitable as Targeting Module**

A number of sequences are suitable in particular as targeting modules for the purposes of the invention. The invariant chain of the MHC II molecule (II, invariant chain, MHC II gamma chain) is the sequence described most often in the literature as being able to mediate targeting. A range of variants of the invariant chain in humans are described, which are also referred to as IIp33, IIp41, IIp35 and IIp43 [1] and which are suitable as targeting modules.

Further sequences suitable as targeting module for the purposes of the invention are the beta chain of the MHC II molecule [19]. Fragments of said sequences are also suitable as targeting module.

**Lysosomal Membrane Proteins Comprising Sequences Suitable as Targeting Module**
A number of membrane proteins which occur in lysosomes and are one of the major protein constituents therein have sequence motifs which bring about targeting for the lysosome. This group of proteins includes *inter alia* Lamp 1 (lysosomal-associated membrane protein-1), Lamp 2, Lamp 3, Limp II (lysosomal integrated membrane protein II) and LAP (lysosomal acid phosphatase) [4]. These and other lysosomal proteins which are either already known or will become known in the future and which have targeting sequence motifs can be used as targeting modules for the purposes of the invention; the complete protein sequence or partial sequences thereof can be used as targeting module.

**Tetraspan Proteins Comprising Sequences Suitable as Targeting Modules**

The members of a family of proteins which have four membrane-spanning domains (tetraspan proteins) are also suitable as targeting modules for the purposes of the invention, because these proteins enter MIICs very efficiently. The mechanism by which "tetraspan" proteins enter MIICs is unclear because they comprise no known amino acid sequences which make targeting possible. Despite this, there are mechanisms which transport these proteins into the MIICs, meaning that they can be used as targeting modules for the purposes of the present invention. The family of "tetraspan" proteins includes CD37, CD53, CD63 (also known as Limp-l and LAMP-3), CD81, CD82 and CD86 [20]. CD63 and CD82 are known to associate with MHC II, HLA-DO and HLA-DM molecules [20]. "Tetraspan" proteins are also present in the membranes of exosomes.

Exosomes are vesicles which are formed after fusion of MIICs with the plasma membrane and are thus released by the antigen-presenting cell. Exosomes comprise MHC II molecules and are able to present antigens and thus stimulate T cells [20]. Further proteins which are very similar to the "tetraspan" protein family, and which are therefore also suitable as targeting modules for the purposes of this invention, are the Schistosoma mansoni membrane protein SM23 and the tumour-associated antigen CO-029 [21]. Further tetraspan proteins, or partial sequences of tetraspan proteins, not expressly mentioned or as yet unknown, may likewise comprise sequences which are suitable as targeting modules.
Further Proteins Having Sequences Suitable as Targeting Modules

Numerous further proteins are to be found in the endosomal/lysosomal compartment of various cell types and therefore reach it via certain mechanisms which are currently known only in part. These proteins or partial sequences of these proteins are accordingly also suitable to be used as targeting modules for the purposes of the present invention. These proteins present in the endosomal/lysosomal compartment include inter alia low density lipoprotein (LDL), insulin, epidermal growth factor (EGF), polymeric immunoglobulin, transferrin, the cation-dependent mannose 6-phosphate receptor, the cation-independent mannose 6-phosphate receptor, CD3, etc. [21], and CD1b [22], and many further proteins or protein sequences which are currently known or will become known in future and which permit targeting into the endosomal/lysosomal compartment. These sequences can be used as targeting module for the purposes of the invention. Various commercial suppliers supply expression vectors comprising nucleic acid sequences which code for sequences suitable for targeting. For example, both BD Bioscience Clontech (Palo Alto, Calif., USA) and Stratagene (La Jolla, Calif., USA) supply expression vectors with targeting modules which guide the resulting fusion protein to the Golgi apparatus or to the peroxisomes. However, these expression vectors do not allow fusion proteins comprising a translocation module to be prepared, so that they differ from the MAT molecules of the invention. In the Clontech vectors for example a sequence of calreticulin (KDEL "retrieval sequence") is used for targeting the fusion protein to the Golgi apparatus. Further proteins which are not expressly mentioned or are currently as yet unknown may likewise comprise sequences suitable as targeting modules for the purposes of the invention.

Sequence Motifs Which Occur in Certain Groups of Targeting Sequences

Certain sequence motifs have been ascertained to be important for the function of various protein sequences as targeting module. For the beta chain of HLA-DM for example the sequence motif tyrosine-threonine-proline-leucine has been identified as targeting motif, with the tyrosine residue and the leucine residue apparently being of particular functional importance [2]. Tyrosine motifs important
for intracellular targeting have been identified for various lysosomal membrane proteins such as LAP (lysosomal acid phosphatase), Lamp 1 (lysosomal associated membrane protein 1), Lamp 2 and Lamp 3 [3, 4] and for CD1b [22]. It was possible to identify a leucine motif important for intracellular targeting for a further lysosomal protein, Limp II (lysosomal integrated membrane protein II) [4].

It was possible to show for LII that two sequence regions are important independently of one another for the intracellular targeting (amino acid position 1 to 11 and position 12 to 29) [23]. Both targeting sequences are functional even on their own, and the sequence 1 to 11 comprises a functionally essential leucine-isoleucine motif at position 7 and 8 [23]. The beta chain of the MHC II molecule likewise comprises a sequence motif which comprises one or two functionally important leucine residues, and a conserved glycine residue is located N-terminally directly preceding this leucine motif [19]. To summarise, accordingly, leucine and tyrosine residues in particular have an important function in targeting sequences and it is therefore possible to design specifically appropriate amino acid sequences as targeting modules. These can be used as targeting modules for the purposes of the invention.

"Non-Amino Acid Structures" Which can be Used as Targeting Modules

Molecules which do not correspond to an amino acid sequence or an amino acid can also be employed as targeting modules for MAT molecules for the purposes of the invention. A long-known example of a structure suitable for targeting in lysosomes is, for example, mannose 6-phosphate [24]. Proteins which contain mannose-6-phosphate residues are transported by various mannose-6-phosphate receptors to the lysosomes. This mechanism can be used within the scope of the invention to transport the MAT molecule into the lysosomes in order thus to achieve efficient presentation of the antigen. For this purpose, mannose 6-phosphate residues can be covalently or non-covalently coupled to the MAT molecule, on their own or as constituents of more complex sugar structures. It is generally possible to use all ligands of mannose 6-phosphate receptors as targeting modules for MAT molecules for the purposes of the present invention. Further structures which are currently known or will become known in future, which make it possible to target MIMCs, endosomes, lysosomes, the Golgi
apparatus, the trans-Golgi network or the endoplasmic reticulum, can be used as targeting modules for the purposes of the invention.

Not all the targeting modules mentioned by way of example need be in the form of the complete protein or the complete molecule as constituent of the MAT molecule in order to be effective as targeting modules for the MAT molecule within the scope of the invention. On the contrary, a sequence region which can be used as targeting module is known for example for some of the proteins mentioned. In addition, the protein sequences mentioned by way of example may also be used in the form of fragments which do not correspond to the previously known functional sequence segments, as long as the resulting sequence is still functional as a targeting module. Testing of the functionality of a targeting module can be ascertained for example by using fluorescence-labelled targeting modules or by using enzyme-labelled targeting modules or by using targeting modules labelled with metal particles. The targeting modules thus labelled are administered to an experimental animal or to cells cultivated in vitro, and the fate of the targeting modules is monitored using methods such as FACS (fluorescence activated cell sorting), microscopy, confocal fluorescence microscopy, electron microscopy etc.

Antigen Modules

In principle all types of antigens able to modulate an immune response can be used as antigen modules for the purposes of the invention. Both antigens that are already known and antigens that will be discovered in the future are suitable. In some circumstances, the antigens used may be of a kind which do not lead to an immune response with conventional immunisation methods currently known in the art but which lead to an immune response by the individual when the novel methods described in the present patent application are used. Not only proteins and peptides but also sugar structures, lipids, e.g. lipopolysaccharides, lipoteichoic acids and other constituents of bacterial membranes (CD1b binds sugar structures and lipids, for example), nucleic acids such as for example DNA containing CpG motifs, organic substances such as latex, for example, or pharmaceutically active substances may be used as antigens within the scope of
the invention. The antigen may be obtained from humans, animals, plants, fungi, parasites, single- or multi-celled micro-organisms, viruses and other life forms. The antigens may have been isolated from biological material, or may have been produced recombinantly or synthetically, e.g. by peptide synthesis. Synthetically prepared antigens may be substances which occur in nature or which do not occur in nature but are obtainable by chemical synthesis. Examples of non-naturally occurring substances which are, however, suitable as antigen in some circumstances are, for example, synthetically prepared substances which are present in medicaments, or synthetic peptides having amino acid sequences which do not occur in nature, or peptidomimetics, etc. Naturally occurring or synthetic or recombinant antigens can be modified by molecular-biological, enzymatic, chemical and other methods in order to confer on them properties which are more advantageous for the particular application. These advantageous properties may be, inter alia, a higher or lower activity as antigen, a broader or a more specific action as antigen, better solubility in hydrophilic or hydrophobic solvents, greater permeability of the antigen modules for cell membranes, for membranes of organelles, for the blood-brain barrier, for the blood-CSF barrier etc., a higher or lower half-life in vivo or in vitro, a lower or higher toxicity, a better detectability of the antigen in vivo or in vitro after application of the antigen in the form of a MAT molecule, etc. It is additionally possible for the purposes of the invention to combine a plurality of antigens in one antigen module [25]. For this it is possible for identical antigens to be present in more than one copy in the antigen module, or different variants of the same antigen may be combined in an antigen module. Combination of antigens, e.g. of antigen 1, and other antigens, e.g. of antigen 2, in an antigen module is also possible, etc. Further combinations such as, for example, antigen 1 in more than one copy and antigen 2 in a single copy may also be combined in an antigen module, etc. It is additionally possible also for one or more different and/or one or more identical antigen modules to be present in a MAT molecule. It is possible in principle for all possible combinations of singly and multiply present identical or altered copies of antigens derived from one or more different antigens to be combined for the purposes of the invention.

**Antigens and Allergens Which can be Used as Antigen Module**
Numerous antigens, particularly allergens, have been described in the literature to date. The allergens specifically known hereinafter can be used as an antigen module for the purposes of the invention. Further allergens and variants of allergens which can likewise be used as antigen module for the purposes of the invention are known in the art [26, 27].

The allergens are categorised into groups such as allergens from plants and grasses, trees, mites, fungi, insects, foodstuffs and other allergens such as latex allergens, for example. In the lists provided below, the following information is given: the scientific name of the organism, a common abbreviation for the allergen, followed immediately by the GeneBank Accession no. of the allergen (given in brackets), where known.

**Plant and grass allergens:**

Ambrosia artemisiifolia, Amb a 1 and Amb a 2; Mercurialis annua, Mer a 1 (Y13271); Parietaria judaica, Par j 1 (X77414), Par j 2 (X95865; X95866);

Cynodon dactylon, Cyn d 1 (S83343); Dactylis glomerata, Dac g 3 (U25343); Holcus lanatus, Hol l 1 (Z27084, Z68893); Lolium perenne, Lol p 1 (M57474), Lol p 2 (X73363) Lol p 5 (M59163); Phalaris aquatica, Pha a 1 (S80654); Phleum pratense, Phil p 1 (X78813), Phil p 2 (X75925), Phil p 3, Phil p 5 (X74735); Artemisia vulgaris, Art v 1 (Z48967)

**Tree Allergens:**

Alnus glutinosa, Aln g 1 (S50892); Betula verrucosa, Bet v 1 (X15877), Bet v 2, Bet v 1d; Carpinus betulus, Car b 1 (X66932, X66918); Corylus avellana, Cor a 1 (X70999, X71000, X70997, X70998, Z72439, Z72440, AF136945, AF323973, AF323974, AF323975); Ligustrum vulgare, Lig v 1 (X77787, X77788); Olea europea, Ole e 1 (S75766), Ole e 9 (AF249675); Syringa vulgaris, Syr v 1 (X76541); Cryptomeria japonica, Cry j 1, Cry j 2 (D29772, D37765); Cupressus arizonica, Cup a 1 (AJ278498); Cupressus sempervirens, Cup s 1 (AF257491); Juniperus ashei, Jun a 2 (AJ404653)

**Mite Allergens:**

Blomia tropicalis, Blo t 5 (U59102); Dermatophagoides farinae, Der f 1, Der f 2, Der f 11; Dermatophagoides pteronyssinus, Der p 1, Der p 2, Der p 5, Der p 7; Lepidoglyphus destructor, Lep d 2 (X81399); P. americana, Cra-A; T. putrescentiae, Tyr p 2
Animal Allergens:
Bos domesticus, Bos d 2 (L42867); Equus caballus, Equ c 1 (U70823); Felis domesticus, Fel d 1 (M74952, M74953)
Fungal Allergens:
5
Alternaria alternata, Alt a 1 (U82633); Alt a 2 (U62442); Aspergillus flavus, Asp f 1 (AF137272); Aspergillus fumigatus, Asp f 1 (M83781, S39330), Asp f/a, Asp f 2 (U56938), Asp f 3 (U20722, U58050), Asp f 4, Asp f 6, Asp f 8; Aspergillus niger, Asp n 18; Aspergillus oryzae, Asp o 13 (X17561); C. comatus, Cop c 1; Penicillium chrysogenum, Pen ch 13 (AF193420), Pen ch 20 (S77837);
10
Penicillium oxalicum, Pen o 18 (AAG44478); Malassezia sympodialis, Mal s 1 (X96486); Cladosporium herbarum, Enolase, Cla h1
Insect Allergens:
Apis mellifera, Api m 1 (X16709), Api m 2 (L10710), Api m 4 (X02007); PLA2 (X16709); Blattella germanica, Bla g 1 (AF0722219, L47595, AF072221,
15
AF072220), Bla g 2 (U28863), Bla g 4 (U40767), Bla g 5 (U92412); Periplaneta americana, Per a 1 (AF072222), Per a 3 (L40819); Dolichovespula maculata, Dol m 1 (X66869), Dol m 2 (L34548), Dol m 5 (J03601); Dolichovespula arenaria, Dol a 5 (M98859), Polistes annularies, Pol a 5 (M98857); Vespuca vulgaris, Ves v 1 (L43561), Ves v 2 (L43562), Ves v 5 (M98858); Myrmecia pilosula, Myr p 1
20
(X70256), Myr p 2 (581785)
Food Allergens:
Salmo salar, Sal s 1 (X97824); Bos domesticus, Bos d 4 (M18780), Bos d 5 (X14712); Gallus domesticus, Gal d 1 (J00902), Gal d 2 (J00992); Metapenaeus ensis, Met s 1 (U08008); Hordeum vulgare, Hor v 15 (X63517); Oryza sativa, Ory s 1 (U31771); Apium graveolens, Api g 1 (Z48967); Daucus carota, Dau c 1
25
(U47087, D88388); Malus domestica, Mal d 1 (X83672); Pyrus communis, Pyr c 1 (AF057030); Persea americana, Pers a 1 (Z78202); Prunus armeniaca, Pru ar 1 (U93165); Prunus avium, Pru av 1 (U66076); Arachis hypogaea, Ara h 1 (L34402), Ara h 2 (L77197); Bertholletia excelsa, Ber e 1 (M17146); Juglans regia, Jug r 1 (U66866), Jug r 2 (AF066055); Ricinus communis, Ric c 1
30
(X54158); Sesamum indicum, Ses i 1 (AF240005); Apium graveolens, Api g 1 (Z48967)
Further Allergens (Latex):
Hevea brasiliensis, Hev b 1 (X56535), Hev b 2, Hev b 3, Hev b 5 (U42640), Hev b 6 (M36986), Hev b 7, Hev b 8

These known allergens are mentioned merely by way of example, and further allergens which can also be used in antigen modules for the purposes of the invention are known in the art.

Besides allergens, there are a number of known pathogens against which no effective or lasting immunisation is available at present. Since the method of the invention is based on a novel immunisation strategy, it may possibly be effective for immunisation against these disorders which have not hitherto been capable of being satisfactorily treated prophylactically by immunisations. These disorders include, in particular, infections with HIV viruses, with hepatitis C viruses, with pathogens of tuberculosis (Mycobacterium tuberculosis), leprosy (Mycobacterium leprae), plague (Yersinia pestis) and with malaria pathogens (Plasmodium species, e.g. falciparum).

Further Modules Which may be Present in MAT Molecules

Besides the three modules already described - translocation module, targeting module and antigen module - which must at least be present in the MAT molecule, it is also possible for further optional modules to be present in the MAT molecule. These optional modules include, for example, modules which make it possible to isolate or detect the MAT molecules. Such modules are often referred to in the art as "tags" and are therefore referred to as tag modules hereinafter in this patent application. Further modules optionally present in the MAT molecules may be spacer modules, i.e. modules which are arranged between the other modules and whose task is to couple these modules to one another. These modules are called spacer modules hereinafter in this patent application. It is also possible for certain modules simultaneously to undertake the function of two or more modules. For example, many tag modules can be used simultaneously for isolation and detection of the MAT molecule, or an antigen module present in a MAT molecule might also be used for detection and/or isolation of the MAT molecule if, for example, an antibody against the antigen module is available, etc.
Tag Modules Which may be Present in MAT Molecules

For the purposes of the invention it is possible for one or more different and/or one or more identical tag modules to be part of a MAT molecule. Tag modules may be short peptides, frequently consisting of not more than 20 amino acid residues, but may also correspond to complete protein sequences or certain domains of proteins. Tag modules may also be functional groups which are not composed of amino acids, such as, for example, biotin or digoxigenin. Almost all tag modules can be used in two different ways. Firstly, they can be used to isolate the MAT molecule, and secondly they can be used to detect the presence of the MAT molecule. In general, all tag modules currently known and all those that will become known in the future are suitable for use for the purposes of the invention. Examples of suitable tag molecules which can be used for the purposes of the present invention are: histidine sequences of 4 to 12 or more, preferably directly consecutive histidine residues, also called His tag, His6 tag, HIS6 tag, penta HiS™, Tetra HiS™, RGS HiS™, etc. (Qiagen, Hilden, Germany), Myc or c-Myc tag, PinPoint™ tag (a signal sequence which has the effect that the corresponding protein is provided with a biotin group by bacteria in vivo), HA tag, 6*HN tag (Promega Biosciences Inc., San Louis Obispo, Calif., USA), Xpress™ tag, myc tag, V5 tag (Invitrogen, Breda, the Netherlands), S tag, CBD tag, GST tag, HSV tag, T7 tag (Novagen Inc., Madison, Wis., USA), FLAG tag, HA tag, c-myc tag, "calmodulin-binding peptide tag" (CBP) tag (Stratagene, La Jolla, Calif., USA), His tag, protein A tag, glutathione S-transferase (GST) tag (Amersham Biosciences, Uppsala, Sweden), Strep-tagII (IBA GmbH, Göttingen, Germany), His tag (Roche Applied Science, Rotkreuz, Switzerland), FLAG tag, GST tag, protein A tag (Sigma, St. Louis, Mo., USA), maltose binding protein (MBP), chitin-binding tag (New England Biolabs, Beverly, Mass., USA), His tag (BD Biosciences Clontech, Palo Alto, Calif., USA). Applications in which more than one tag module are used in a molecule have also been described for some of these tag modules. For example, two tag modules can be coupled to the N terminus or to the C terminus of a protein, or one tag module can be coupled to each of the N terminus and C terminus (Qiagen, Hilden, Germany and Stratagene, La Jolla, Calif., USA). Tag modules may also be introduced internally in the sequence of other proteins, e.g. between two domains of a protein (Strep-
Further tag modules are primarily used to detect the molecule to which they are coupled. However, these tag molecules may also be used in principle for isolating proteins, e.g. by use of affinity chromatography. For this purpose, chromatography materials to which antibodies against these tag modules are coupled may be used, for example. It is also possible for the purposes of the present invention to use tag modules such as, for example, the green fluorescent protein (GFP), the enhanced green fluorescent protein (EGFP), the enhanced cyan fluorescent protein (ECFP), the enhanced yellow fluorescent protein (EYFP), the red fluorescent protein (DsRed2) (BD Bioscience Clontech, Palo Alto, Calif., USA), the renilla green fluorescent protein (hrGFP) (Stratagene, La Jolla, Calif., USA). These tag modules may be located both at the N terminus and at the C terminus of a fusion protein, for example. Besides fluorescent tag modules it is also possible to use enzymes as tag module. Examples of frequently used enzymes are luciferase, beta-galactosidase, alkaline phosphatase, horseradish peroxidase, etc. These enzymes can be detected via their respective enzyme activity, i.e. on the basis of the conversion of substrates of these enzymes. Various types of substrates are suitable for this purpose, such as, for example, substrates which absorb light in the visible range of the spectrum, fluorescent substrates, substrates whose conversion leads to the emission of light, or substrates whose enzymatic conversion can be determined through the decrease in the concentration of the substrate or the increase in the product by use of various detection methods, etc.

A further possible use of tag modules for the purposes of the present invention is the use of, for example, peptide sequences which are suitable as kinase substrates. These peptide sequences can then be radiolabelled by addition of radioactive phosphorus and addition of kinases. Examples of tag modules which can be used in this way for the present invention are: the kemptide tag (a peptide which can be phosphorylated by protein kinase A), the calmodulin-binding peptide tag (CBP), which can likewise be phosphorylated with protein kinase A (Stratagene, La Jolla, Calif., USA), etc.
A further possible use of tag modules for the purposes of the present invention is the use of, for example, proteins, protein domains or peptide sequences which specifically bind other proteins or other structures. Examples of such tag modules known from the literature are: protein A, protein G, protein A/G, protein L, all these proteins binding to antibody structures (Pierce, Rockford, Ill., USA), glutathione S-transferase, which binds to glutathione, the maltose binding protein (MBP) that binds to amylose, streptavidin or avidin, both of which bind to biotin, the calmodulin-binding peptide which binds to calmodulin, the chitin-binding tag which binds to chitin, etc. It is possible in general to use all types of molecules which in each case bind specifically to other molecules as tag module for the purposes of the invention, i.e. receptor-ligand, antibody-antigen, lectin-sugar structure, protein-lipid, protein-nucleic acid, protein-protein, etc., and numerous other examples described in the literature [28].

**Spacer Modules**

Spacer modules which can be used for the purposes of the invention are all types of molecules suitable for coupling other modules, which are a component of the MAT molecule, to one another. The coupling can take place both by covalent and by non-covalent linkages. The spacer modules have the task *inter alia* of spatially separating the various modules of the MAT molecule from one another so that they do not adversely affect one another in terms of their functionality. Modules of the MAT molecule for the purposes of the invention can be coupled by spacer modules which can be cleaved again at a later time by chemical or enzymatic reactions, e.g. by proteases. It is thus possible to separate the modules of the MAT molecule, which are connected by the spacer modules, from one another again as required.

All currently known or future proteases may generally be used for this purpose [29, 30]. Proteases frequently used at present are thrombin, factor Xa, enterokinase or the TAGZyme system (Qiagen, Hilden, Germany) etc. Various chemical reactions suitable for cleaving spacer modules are known to the skilled man or can be found in the information supplied by manufacturers of spacer molecules, such as Pierce, for example.
The spacer modules may be in particular peptide sequences or organic molecules. Numerous spacer molecules which can be used for the purposes of the invention are known in the art. In addition, spacer molecules which will be developed or discovered in the future may be used for the purposes of the invention. Suitable spacer modules are, *inter alia*, peptide spacers, crosslinkers, natural or synthetic polymers such as, for example, nucleic acids, substituted or unsubstituted hydrocarbons, etc. It is additionally possible to use combinations of molecules as spacer modules which are able to form complexes with one another via non-covalent interactions, and can thus join together two or more modules to give a MAT molecule. A known example of such a combination of molecules which bind to one another is biotin/streptavidin.

**Peptide Sequences as Spacer Modules**

Many proteins consisting of a plurality of domains have in their amino acid sequence short sequence regions which are also referred to as spacers in the literature. These spacers have the task of spatially separating the various domains of the protein from one another in such a way that they do not adversely affect one another in terms of their functionality. It is necessary for this purpose to ensure in particular that the spacer sequence is so flexible that the two domains do not sterically hinder one another in their function.

Peptide sequences of this type can be used as spacer modules for the purposes of the present invention. A large number of different spacer peptide sequences are described in the literature. These spacers preferably have a length of between 2 and 60 amino acids, but may also have longer sequences. Spacers may also consist of only one amino acid. Many commercially available expression vectors already comprise sequence regions which code for peptide spacers which, for example, connect a tag sequence to the protein sequence to be introduced into the expression vector. Very short peptide spacers consisting of only two amino acids such as, for example, leucine-glycine, glycine-alanine or serine-alanine (IBA GmbH, Göttingen, Germany) are often used, or short amino acid sequences from 4 to 6 amino acids in length consisting of glycine and/or
alanine (Qbiogene Inc., Carlsbad, Calif., USA). Numerous other spacer sequences are described in the literature and can be used as spacer modules for the purposes of the present invention. It is possible in principle to use any of the spacer molecules which are currently known or may become known in the future as spacer modules in the MAT molecules according to the invention. One method of identifying amino acid sequences that are suitable as spacer modules is the use of databases which search amino acid sequences for protein domains. Short amino acid sequences preferably with a length of from 2 to 60 amino acids, which are present between two protein domains identified in this way in an amino acid sequence, can be used as spacer modules for the purposes of the invention. One of the currently available databases for identifying protein domains and thus also peptide sequences suitable as spacer modules is the "SBASE protein domain library" [31].

Crosslinkers as Spacer Modules

Spacer modules also in the form of crosslinkers can be introduced into the MAT molecule for the purposes of the invention. For this purpose, the individual modules of the MAT molecule are prepared and then covalently coupled to one another by chemical reactions with crosslinkers. Numerous crosslinkers are commercially available for this purpose. For example, Pierce (Pierce Biotechnology, Inc., Rockford, Ill., USA) supplies numerous different crosslinkers. Currently, for example, Pierce offer a choice between crosslinkers which react with amino groups, sulfhydryl groups, sugar structures, carboxyl groups, hydroxyl groups or react non-selectively with the modules which are to be combined to give a MAT molecule. Also currently available, e.g. from Pierce Biotechnology Inc., for preparing MAT molecules are crosslinkers which can be separated again by specific chemical reactions, e.g. using thiols, bases, periodate, hydroxylamine, by the action of light or by non-specific reactions. It is additionally possible by targeted selection of crosslinkers to specifically fix the distance between the individual modules of the MAT molecule. For example, Pierce currently supply crosslinkers which introduce a distance of 1.4 Angström (N-succinimidyl iodoacetate) to 34.7 Angström (bis(beta-(4-azidosalicylamido)ethyl) disulfide), depending on which crosslinker is used. A further possible option in the use of
crosslinkers for coupling various modules to form MAT molecules in the sense of the present invention is the possibility of using the crosslinker sulfo-SBED from Pierce Biotechnology Inc. Sulfo-SBED on the one hand couples two modules by covalent reaction and additionally comprises a biotin group on the introduced spacer molecule. It is then possible to attach a further module of the MAT molecule to this biotin group by non-covalent linkages. For this purpose, the module to be introduced can be coupled to avidin or streptavidin, for example. The streptavidin-coupled module thus produced can then be coupled via the biotin group present in the crosslinker to the other modules. It is possible in principle to use all crosslinkers that are currently known or will become known in future for linking modules to form a MAT molecule for the purposes of the invention.

Further Spacer Modules

Spacer modules for the purposes of the invention may consist for example of L isomers or D isomers of amino acids, unusual amino acids, amino acids with posttranslational modifications, nucleic acids, of PNAs (peptide nucleic acids), lipids, sugar structures, or other natural or synthetic polymers such as, for example, substituted or unsubstituted hydrocarbons, polyacetalte, polyethylene glycol, cyclodextrins, polymethacrylate, gelatin, oligoureia etc., or of other substances or combinations of the above-mentioned or other substances. It is possible in principle to use all currently known substances suitable for joining modules together to give a MAT molecule, as well as molecules which will become known in future and have corresponding properties, as spacer modules for linking modules to form a MAT molecule for the purposes of the invention.

Spacer Modules Which are Linked Together by Non-covalent Interactions

There are very many examples of this class of spacer molecules in the literature. Examples of such combinations of molecules which are linked together via non-covalent interactions and which are commercially available are: biotin/streptavidin or avidin or Strep-tag II (IBA GmbH, Göttingen, Germany) or PinPoint™ tag (Stratagene, La Jolla, Calif., USA), glutathione S-transferase/glutathione and
protein A/constant portion of antibodies (FC part) (Pharmacia Amersham Biosciences, Uppsala, Sweden, Sigma, St. Louis, Mo., USA), maltose-binding protein (MBP)/amylose (New England Biolabs, Beverly, Mass., USA), histidine tag/Ni chelate (Qiagen, Hilden, Germany, BD Bioscience Clontech, Palo Alto, Calif., USA, Invitrogen, Breda, the Netherlands, Novagen Inc., Madison, Wis., USA, Roche Applied Science, Rotkreutz, Switzerland), chitin-binding tag/chitin (New England Biolabs, Beverly, Mass., USA), calmodulin-binding protein/calmodulin (Stratagene, La Jolla, Calif., USA). There is also in addition a number of further molecule combinations such as, for example, receptor/ligand combinations, antibody/antigen combinations, lectin/sugar structure combinations, etc. Numerous currently known protein-protein interactions are to be found in databases and can be used as spacer modules for the purposes of the invention [28]. All currently known and future combinations of molecule capable of entering into non-covalent linkages with one another can in principle be used as spacer modules for the purposes of the invention. A further method for introducing spacer modules into MAT molecules is the use of bispicific molecules which combine two different binding sites in one molecule. Examples of such molecules would be biotin-labelled lectins (Pierce Biotechnology, Inc., Rockford, Ill., USA) which are able to link together for example a streptavidin-labelled module and a further module which has a sugar structure which is bound by the lectin. A further example of a possibility for coupling in this way are bispicific antibodies which recognize two different epitopes, etc.

A further variant for introducing spacer modules into MAT molecules is as follows: first, at least two modules are coupled to one another via a non-covalently linking spacer module, and then the complex is treated with chemical crosslinkers which introduce covalent bonds between modules in spatial proximity. This has the advantage that, in the first step, particular modules are coupled to one another in a targeted and defined manner and then the non-covalent coupling is converted into a more stable covalent coupling. If the modules are treated directly with crosslinkers which produce covalent bonds, the manner in which the modules are coupled to one another is usually random and not specific.
Structure of the MAT Molecules

Generally, any desired arrangement of the individual modules of the MAT molecule is possible. Each module may be present one or more times in the MAT molecule. The minimum requirement is the need for the presence of at least one translocation module, at least one targeting module and at least one antigen module. Additional modules such as tag modules, spacer modules, etc. may optionally be present but need not be present. All modules may be present one or more times in the MAT molecule. If modules are present more than once, they may be present in the form of identical copies, or different versions of a module may be present in each case in a single copy or in more than one copy. It is also possible for entirely different modules of the same class of modules, e.g. a His tag module and a biotin tag module, to be present in a MAT molecule. Both modules undertake functionally the same task (tag module) in the MAT molecule, but need have nothing in common in terms of their molecular structure.

Two or more identical copies of an antigen module in a MAT molecule may serve for example to bring about an enhanced immune response to the relevant antigen. Two or more different antigen modules may for example be combined in one MAT molecule in order to modulate the immune reaction to two or more different antigens simultaneously. Two or more different translocation modules may be used in a MAT molecule. For example, a Tat sequence and a VP22 sequence can serve to make translocation more efficient since the translocation of the MAT molecule then takes place efficiently in a broader spectrum of different cell types or tissue types. It is also possible for example to use two or more tag modules in a MAT molecule, e.g. a His tag and a FLAG tag, in which case for example the His tag is used to isolate the MAT molecule and for example the FLAG tag serves to detect the MAT molecule. It is possible to use two or more different targeting modules in a MAT molecule, e.g. a sequence from the invariant chain of the MHC II molecule and, as a further targeting module, a mannose 6-phosphate group, of which for example the invariant chain acts as targeting module into the MIICs, and the mannose 6-phosphate group brings about a targeting into the lysosome, as a result of which the efficiency of antigen presentation or the number of different epitopes of the antigen presented by the
antigen-presenting cells can be increased.

The position of the individual modules within the MAT molecule can also be varied as desired, provided that there is at least one translocation module, at least one targeting module and at least one antigen module. It is also possible for all or some of the modules of the MAT molecule for example to be present not in the form of a linear sequential arrangement of modules but as a circular or branched module structure or else in the form of dendrimers, or as a combination of linear and/or branched and/or circular and/or dendrimeric molecule portions.

Circular module structures of the MAT molecule can be generated for example by reacting two cysteine residues with one another or by reacting one cysteine residue with a thiol ester group within a chain of modules which originally had a linear structure. There are commercial suppliers of expression vectors which supply specific vectors which make it possible to prepare circular fusion proteins by these mechanisms, such as, for example, the IMPACT™-TWIN system from New England Biolabs, Beverly, Mass., USA. Branched modules might be prepared for example by synthesizing peptides in which, starting from poly L-lysine, a new lysine residue is attached to both free amino groups of each of the subsequent lysine residues. It is possible in this way to create a peptide structure with virtually any extent of branching. It is then possible for example for translocation modules and/or targeting modules subsequently to be synthesized onto the branched peptide basic structure [32]. Further modules can also be coupled to a linear, circular or branched basic peptide structure by protein ligation [33, 34]. It is additionally possible to introduce biotin groups, for example, into the basic peptide structure during the peptide synthesis, and modules can then be attached to these biotin groups via, for example, streptavidin, the Strep tag system or via the PinPoint™ system (IBA GmbH, Göttingen, Germany and Promega Biosciences Inc., San Louis Obispo, Calif., USA, respectively) onto the basic peptide structure. Modules attached in this way are then coupled via non-covalent linkages to the basic peptide structure.
Fig. 1 shows by way of example some examples of possible structures for MAT molecules in terms of their composition from various modules and in terms of the arrangement of the modules within the MAT molecule.

5 Structure of the Modules of MAT Molecules

Peptides, proteins, amino acids, unusual amino acids, posttranslational modifications etc.

10 The terms peptide and protein are used side by side as equivalent in the present patent application. A peptide or a protein for the purposes of the invention means a covalent bonding of at least two amino acids via a peptide linkage. The term "amino acid" and the term "amino acid residue" are used as equivalent in the present application, i.e. the meaning of the two terms is identical. The terms amino acid/amino acid residue and peptide/protein are used in the present application in the form of the widest possible definition.

Amino acids mean for the purposes of the invention, besides the 20 amino acids determined by the genetic code, also the amino acids which can be encoded by stop codons, such as, for example, seleno-cysteine or pyrro-lysine. Additionally included are all known amino acid and peptide derivatives such as, for example, glycosylated, phosphorylated, sulphated amino acids/peptides, and L-isomeric and D-isomeric amino acids, as well as amino acid and peptide derivatives which will be known in future. Amino acid and peptide derivatives can arise, or be deliberately produced, by post-translational modifications, by chemical modifications, by enzymatic modifications or on the basis of other mechanisms. The resulting peptides may comprise modifications which may occur in all regions of the peptide molecule. For example, modifications may occur in the peptide backbone, in the amino acid side chains, at N-terminal ends of the peptide or at C-terminal ends of the peptide. The modifications may be present in single amino acids, in a plurality of amino acids or in all amino acids, and it is possible for no, one or a plurality of types of modifications to be present in any combinations in a peptide. The peptides may be branched, the peptides may be in cyclic form, and any combinations of branched and cyclic peptides are possible. Branched and/or
cyclic peptides may be formed through natural biological processes or be prepared by synthesis. Examples of unusual amino acids which may be mentioned by way of example are, *inter alia*: alpha-aminobutyric acid, beta-aminobutyric acid, beta-aminoisobutyric acid, beta-alanine, gamma-aminobutyric acid, alpha-amino adipic acid, 4-aminobenzoic acid, aminoethylcysteine, alpha-aminopenicillanic acid, allysine, 4-carboxyglutamic acid, cystathionine, carboxyglutamic acid, carboxyamidomethylcysteine, carboxymethylcysteine, cysteic acid, citroline, dehydroalanine, diaminobutyric acid, dehydroamino-2-butyric acid, ethionine, glycine-proline dipeptide, 4-hydroxyproline, hydroxylsine, hydroxyproline, homoserine, homocysteine, histamine, isovaline, lysinoalanine, lanthionine, norvaline, norleucine, ornithine, 2-piperidinocarboxylic acid, pyroglutamic acid, pyrrolysin, proline-hydroxyproline dipeptide, sarcosine, 4-selenocysteine, syndesines, thioproline, etc. All these amino acids can be present in the form of their L isomers or in the form of their D isomers if their structure so permits. In general, all currently known amino acids and amino acid derivatives which occur naturally or are formed or can be prepared enzymatically or chemically or by some other method, as well as modifications of amino acids that may become known in future, are included in the term "amino acid" and may be constituents of MAT molecules for the purposes of the invention.

Examples of postranslational or chemical modifications which may be present in one or more modules of the MAT molecule for the purposes of the invention and which are mentioned are, *inter alia*, modifications of the amino acid sequences by the following structures: binding of free cysteine to a cysteine in the peptide sequence, formation of disulphide linkages between two cysteine residues, methylations, acetylations, acylations, farnesylations, formylations, geranylgeranylations, biotynylations, stearoylations, palmitoylations, lipooylations, C-mannosylations, myristoylations, phosphorylations, sulphatylations, N-glycosilations, O-glycosilations, amidations, deamidations, demethylations, cysteinylations, carboxylations, hydroxylations, iodinations, oxidations, pegylations, prenylations, ADP-ribosylations, 5'adenosylations, 4'-phosphopantheinations, glutathionylations, covalent bonding: of flavin, of heme groups (or other porphyrins), of nucleic acids or of nucleic acid derivatives, of lipids or lipid derivatives, of phosphatidylinositol, of glycosylphosphatidylinositol
anchors (GPI anchors), of pyridoxal phosphate, of mannose 6-phosphate, modifications of cysteine to carboxyamidomethylcysteine or carboxymethylcysteine or pyridylethylcysteine, modification of lysine to lipoic acid, modification of glutamine to pyroglutamic acid, addition of amino acids to peptides by tRNAs, ubiquitin labeling of peptides, branchings of peptides, e.g. in the form of poly-L-lysine, cyclizations of peptides, e.g. by forming disulphide linkages between 2 cysteine residues, etc. Numerous further modifications of proteins which are, inter alia, also archived in databases are described in the literature [35]. In general, the term “peptide” encompasses all currently known modifications of peptides which occur naturally or are formed or can be prepared enzymatically or chemically or in another way, and modifications of peptides which will be known in future, and these may all be constituents of MAT molecules within the scope of the invention.

15 Peptidomimetics

It is additionally possible within the scope of the invention, for one or more amino acids of modules or the complete module or all modules of the MAT molecule to be replaced by structures consisting of peptidomimetics. The term peptidomimetics is used in the present application in the form of the widest possible definition. A peptidomimetic is a substance which comprises non-peptide structural elements and is able to imitate or antagonize the biological effect of the natural parent molecule. There are numerous studies known in the art which deal in detail with possibilities for using peptidomimetics as a replacement for conventional peptide structures are known in the art. It is generally possible for one or more modules of the MAT molecule to be composed entirely or partly of peptidomimetics [36-38]. This may have various advantages. It may enable translocation modules to penetrate more efficiently into cells, it may enable targeting modules to transport the MAT molecule efficiently or less efficiently and/or specifically into the desired intracellular organelle, it may cause antigen modules to lead to an enhanced or reduced immune response relative to the immune response to the conventional antigen, or it may lead to tag modules having better physicochemical properties, improving their suitability for the isolation and/or detection of the MAT molecule, etc. It is additionally possible
through the use of peptidomimetics in some circumstances to reduce or increase the \textit{in vivo} stability of the MAT molecule, to reduce or increase its toxicity, to improve its solubility in hydrophilic or hydrophobic media, and to prolong its \textit{in vitro} stability and possibly to reduce the costs of synthesizing the peptidomimetic relative to the cost of synthesizing the corresponding conventional peptide. One example of peptidomimetics are Spiegelmers\textsuperscript{®} supplied by NOXXON Pharma AG, Berlin, Germany. This type of peptidomimetics has the advantage for example that they do not elicit an immune response and therefore could be employed in a worthwhile manner for example in translocation modules, targeting modules, tag modules, spacer modules, etc. of MAT molecules. However, Spiegelmers\textsuperscript{®} would not be suitable as an antigen module.

\textbf{Preparation and Isolation of MAT Molecules}

The isolation of MAT molecules using recombinant expression systems, chromatography methods and chemical synthesis protocols is known to the skilled man. The MAT molecules isolated in this way can be used \textit{inter alia} for producing medicaments and diagnostic aids and for producing antibodies in experimental animals and in \textit{in vitro} systems.

\textbf{Preparation of MAT Molecules}

Methods known to the skilled worker for preparing MAT molecules include the recombinant expression of peptides. For the expression of the peptides it is possible to use \textit{inter alia} cell systems such as, for example, bacteria such as Escherichia coli, yeast cells such as Saccharomyces cerevisiae, insect cells such as, for example, Spodoptera frugiperda (Sf-9) cells, or mammalian cells such as Chinese hamster ovary (CHO) cells. These cells are obtainable from the American Tissue Culture Collection (ATCC). For the recombinant expression of peptides, for example nucleic acid sequences which code for entire MAT molecules or for individual molecules of MAT molecules are introduced into an expression vector in combination with suitable regulatory nucleic acid sequences such as, for example, selection markers, promoters, etc. using methods of molecular biology. Suitable selection markers are, for example, resistance to
antibiotics such as ampicillin, kanamycin, neomycin or puromycin, or metabolic
defects, e.g. yeast cells unable to produce alanine, leucine, tryptophan etc., or
mammalian cells lacking the enzyme hypoxanthine-guanine
phosphoribosyltransferase and therefore unable to survive in HAT medium
(hypoxantines, aminopterin, thymidine medium), etc. Suitable promoters are, for
example, the cytomegalovirus immediate early promoter (CMV promoter), the
SP1 minimal promoter or the thymidine kinase promoter (TK promoter). When
selecting the promoters it is necessary to choose promoters that are suitable for
the particular cell system. For example, the T7 or the T7/lacO promoter is
suitable for bacteria while, for example, the nmt1 promoter is suitable for yeast
cells. If MAT molecules or modules of MAT molecules are toxic it may be
advantageous or necessary to use expression vectors which make it possible to
control the expression of these molecules from outside, e.g. through the Tet-
On™ and the Tet-Off™ expression system (Promega Biosciences, San Louis,
Calif., USA). In this system, the activity of the promoter of the expression vectors
is regulated by addition of tetracycline to the growth medium of the cells. Further
examples of methods which can be used for external regulation of the expression
of MAT molecules or of modules of MAT molecules is the induction of T7
polymerase by IPTG or the use of ecdysone-inducible expression systems such
as, for example, the Complete Control® Inducible Mammalian Expression
System (Stratagene, La Jolla, Mo., USA). When using vectors which comprise an
IRES (internal ribosome entry site) sequence it is also possible for a plurality of
molecules to be prepared simultaneously through the use of only one expression
vector (e.g. pLP-IRESneo vector, Promega Biosciences, San Louis; CA, USA). It
is thus possible in this way for example for two or more modules of a MAT
molecule which are intended to interact with one another by non-covalent
linkages to be expressed in parallel with one another in appropriate stoichiometric
ratios of amounts, and possibly also purified in parallel. Various companies
supply commercially available expression vectors for different cell systems, e.g.
Invitrogen, Qiagen, Stratagene, Clontech, Novagen, New England Biolabs,
PharMingen, Promega, Pharmacia, etc. The expression vectors isolated in this
way can then be introduced into suitable cells, e.g. by electroporation, calcium
phosphate coprecipitation, liposome-mediated transfection, etc., in a manner
known to the skilled man. Alternatively, it is also possible to use recombinant
viruses produced by methods of molecular biology, which in turn then infect cells and bring about the expression of MAT molecules or modules of MAT molecules by the infected cells. Suitable viral expression systems are, for example, the baculovirus system, e.g. BacculoGold (BD Bioscience Pharmingen, Palo Alto, Calif., USA), adenoviral expression systems such as, for example, ViraPort™ (Stratagene, La Jolla, Calif., USA), retroviral expression systems such as, for example, AdEasy (Stratagene, La Jolla, Calif., USA) etc.

It is also possible, as an alternative to transfection of expression vectors and to viral expression systems, to use in vitro translation systems in which, for example, rabbit reticulocyte lysates or E. coli S30 extracts or wheatgerm extracts are used for the synthesis of MAT molecules or for the in vitro synthesis of modules of MAT molecules without the need to use living cells for the expression.

15 **Cell Systems for Preparing MAT Molecules**

Cell lysates or cell culture supernatants can be used as starting material for preparing MAT molecules or for preparing individual modules of MAT molecules. Cell systems may be obtained for example from bacteria such as, for example, E. coli, bacillus, Caulobacter, Pseudomonas or Streptomyces or yeasts such as, for example, Saccharomyces, Pichia or Hansenula, or insect cells such as, for example, Sf-9, Sf-21 or High Five, or mammalian cells such as, for example, CHO cells, COS cells, 3T3 cells, BHK cells, 293 cells, etc. By using signal sequences which bring about the export of proteins from the interior of the cell into the extracellular space, the protein to be expressed can be deliberately made to accumulate in the cell culture medium or in the periplasmic space of, for example, bacteria. A further source of starting material for preparing MAT molecules or modules of MAT molecules may be transgenic animals, transgenic plants, transgenic fungi or transgenic microorganisms into which nucleic acids which code for MAT molecules or modules of MAT molecules have been introduced stably or transiently. The corresponding nucleic acids may in this case both be integrated directly into the genome of the particular organism and be introduced for example in the form of plasmids or in the form of other DNA or RNA molecules into the organisms. The corresponding MAT molecules or
modules of MAT molecules can then be isolated for example from the milk, the eggs, serum, urine, tissue etc. of the transgenic animals, from, for example, storage tubers, seeds, leaves etc. of transgenic plants, from, for example, the mycelium, the fruiting body etc. of the transgenic fungi or from cells or other organisms cultivated \textit{in vitro}, or from the corresponding cell culture media. All types of organisms are generally suitable for use as an expression system for preparing MAT molecules or modules of MAT molecules.

\textbf{Isolation of MAT Molecules}

The MAT molecules or modules of MAT molecules prepared in this way can be isolated using techniques known to the skilled man. Numerous methods known to the skilled man for isolating proteins can be used for this purpose, such as, for example, precipitation methods, liquid phase separation methods, chromatographic methods, etc. Suitable precipitation methods include \textit{inter alia} immunoprecipitation, ammonium sulphate precipitation, polyethylene glycol precipitation, ethanol precipitation, trichloroacetic acid precipitation (TCA precipitation), thermal precipitation, etc. The liquid phase separation methods include for example extraction with organic solvents such as, for example, alcohols, acetone, chloroform, acetonitrile, etc., and the chromatographic methods include for example cation exchanger chromatography, anion exchanger chromatography, isoelectric focussing, reverse phase chromatography, gel filtration, immobilized metal ion affinity chromatography (IMAC), in which various metal ions such as, for example, nickel, zinc, cobalt etc., can be used, hydroxyapatite chromatography, numerous different affinity chromatography methods such as, for example, immunoaffinity chromatography, affinity chromatography using immobilized nucleic acids, or immobilized protease inhibitors, etc. The chromatographic media used may have structures based on an agarose matrix, based on magnetic particles, in the form of membranes, in the form of hollow fibres, based on various polymers such as, for example, polystyrene, etc. Chromatographic methods can generally be carried out on a wide variety of scales starting from chromatography columns with a volume of a few \(\mu\)l up to large chromatography columns with a volume of several hundred litres. In addition, chromatography procedures can be carried out under normal
atmospheric pressure, under medium pressures in the range from 1 to 50 bar (e.g. the FPLC system, Pharmacia Amersham Biosciences, Uppsala, Sweden) and under very high pressures in the range up to about 400 bar and possibly even greater pressures (HPLC systems). Chromatography procedures can be carried out under conditions which have native and denaturing effects on the MAT molecules. Various interactions between matrix material and a MAT molecule or module of a MAT molecule to be isolated can be used in affinity chromatography. These include numerous tag molecules which have already been mentioned elsewhere in the present patent application and which interact with certain functional groups or ligands and thus allow isolation of MAT molecules or modules of MAT molecules. However, it is possible in principle to use all types of interaction such as, for example, protein-protein interactions, nucleic acid-protein interactions, nucleic acid-nucleic acid interactions, sugar-lectin interactions, sugar-protein interactions, receptor-ligand interactions, antibody-antigen interactions (e.g. anti-FLAG, anti-HA, anti-myc tag antibodies), hapten-antibody interactions, Spiegelmer interactions (NOXXON Pharma AG, Berlin, Germany) etc.

**Affinity Chromatography**

Methods which can be used for the affinity chromatography are in particular those based on selective binding of a tag module to a matrix. Suitable combinations of tag modules and relevant matrix for isolating a MAT molecule are, *inter alia*: histidine tag and nickel chelate matrix (Qiagen, Hilden, Germany), GST tag and glutathione-sepharose (Amersham Biosciences, Uppsala, Sweden), maltose-binding protein tag and amylose matrix (New England Biolabs, Beverly, Mass., USA), biotin tag and streptavidin or avidin matrix (IBA GmbH, Göttingen, Germany), chitin-binding protein tag and chitin matrix (New England Biolabs, Beverly, Mass., USA), calmodulin-binding peptide tag and calmodulin matrix (Stratagene, La Jolla, Calif., USA), protein A or protein G or protein A/G or protein L and particular regions, recognized by the respective proteins, of antibody molecules, such as, for example, the Fc portion of antibodies (Amersham Biosciences, Uppsala, Sweden), FLAG tag, HA tag, myc tag, histidine tag, etc. and a matrix to which an antibody against the particular tag is
coupled (many different companies, including Promega Biosciences Inc., San Louis Obispo, Calif., USA, Invitrogen, Breda, the Netherlands, Qiagen, Hilden, Germany) etc.

5 Protease Recognition Sequences

The MAT molecules or modules of MAT molecules isolated in this way can be separated where appropriate from their tag module and/or other modules. It is possible for this purpose to introduce for example a protease recognition sequence into the particular expression vector at suitable positions. Numerous suitable protease recognition sequences are known to the skilled man, including the recognition sequences of proteases such as, for example, thrombin, factor Xa, enterokinase or the TAGZyme system (Qiagen, Hilden, Germany). The added proteases can be removed again, for example, by means of immobilized protease inhibitors such as, for example, EK-AWAY for enterokinase (Invitrogen, Breda, The Netherlands), Xa Removal Resin (Qiagen Hilden, Germany), benzamidine-Sepharose for removing thrombin, etc. A further possibility for isolating MAT molecules or modules of MAT molecules is the use of inteins, i.e. of proteases which are a constituent of the MAT molecule and which then, under suitable experimental conditions, eliminate themselves proteolytically from the remainder of the MAT molecule or a module of a MAT molecule (Genease™, New England Biolabs, Beverly, Mass., USA). In general, all protease recognition sequences which are currently known [29, 30] and will become known in future are suitable for removing constituents of MAT molecules for the purposes of the invention. The protease recognition sequences may moreover either be naturally occurring or have been designed specifically, and may be composed wholly or partly of natural amino acids, unusual amino acids, peptidomimetics etc.

Inclusion Bodies

A further embodiment of the invention is the preparation of MAT molecules or of modules of MAT molecules in the form of incorrectly folded protein aggregates, which are also referred to as inclusion bodies. Inclusion bodies can be prepared as molecules containing translocation modules which make transport possible
from the extracellular space through the cell membrane into the interior of the cell. This translocation leads to the originally incorrectly folded molecules becoming correctly folded and then acting inside the cell like a MAT molecule which has been correctly folded from the outset. This procedure has the advantage that unfolded proteins can be isolated under denaturing conditions, which is often associated with less technical complexity and thus financial cost. In addition, inclusion bodies are relatively stable structures. This has advantages in some circumstances for the storage and stability of MAT molecules which are kept for later medical use. It is also possible by this method to use unfolded or incorrectly folded MAT molecules or modules of MAT molecules for the purposes of the invention. Certain translocation modules bring about both translocation from the extracellular space into the cell and reverse transport. Transformation of an as yet incorrectly folded MAT molecule can take place directly in vivo in the individual to be treated with the MAT molecule, or the folding of the MAT molecule can be carried out in a cell system in vitro. In addition, translocation of the unfolded MAT molecule into the interior of the cell, and the subsequent translocation of the then correctly folded MAT molecule into the extracellular space, can in some circumstances be brought about by the same translocation module. A mechanism of this type has been described for some sequences which are suitable as translocation modules for the purposes of this invention, such as, for example, the VP22 sequence [39].

**Modification of MAT Molecules**

MAT molecules or modules of MAT molecules can be modified by numerous methods known to the skilled man, enzymatically, chemically or by other methods. For example, peptides can be provided with phosphorus groups by using kinases, phosphorus groups can be removed using phosphatases, sugar structures can be removed using glycosidases, etc. Appropriate kinases, phosphatases and glycosidases etc., and the appropriate protocols, are obtainable from various manufacturers such as, for example, New England Biolabs, Beverly, Mass., USA. Phosphorylation of MAT molecules or of modules of MAT molecules can additionally be used to label MAT molecules or modules of MAT molecules with radioactive phosphorus, thus making them easily detectable.
in vitro and/or in vivo. It is also possible to modify MAT molecules or modules of MAT molecules by chemical reactions. For example, disulphide bridges can be destroyed by reduction, thioester groups can react covalently with cysteine residues, or two cysteine residues can react to form a disulphide bridge, making it possible to prepare circular or branched MAT molecules or modules of MAT molecules (e.g. IMPACT™-TWIN Protein Fusion and Purification System, New England Biolabs, Beverly, Mass., USA). It is also possible to have a specific influence on modifications of the MAT molecule or of modules of MAT molecules by the choice of expression system. For example, no glycosylation takes place in bacteria, and insect cells synthesize only particular types of glycosylations, whereas mammalian cells produce complete glycosylations. The expression can also be carried out using cell lines which have been modified in such a way, or selected in such a way, that they are able specifically to produce, or unable specifically to produce, particular post-translational modifications. These advantageous properties may include inter alia better solubility in hydrophilic or hydrophobic solvents, longer stability of the MAT molecule at room temperature, for example, at 37°C, at 4°C, at -20°C, at -70°C or at other temperatures, longer molecular stability of the MAT molecules if they are present alone or mixed with other solid, liquid or gaseous substances, e.g. in the form of preparations as a medicament or diagnostic aid, higher penetrability of the MAT molecules for cell membranes, for membranes of organelles, for the blood-brain barrier, for the blood-CSF barrier and for other biological membranes and barriers, etc., a higher or lower in vivo or in vitro half-life, lower or higher toxicity, a better in vivo or in vitro detectability of the MAT molecule etc.

**Protein Ligation**

A further possibility for preparing MAT molecules or modules of MAT molecules is protein ligation. By this is meant for example a chemical reaction in which two ends of peptides are linked together covalently by one or more chemical reactions. One example would be the reaction of a thiol ester with a cysteine side chain (e.g. IMPACT™-TWIN Protein Fusion and Purification System, New England Biolabs, Beverly, Mass., USA). Cyclic peptides for example can be prepared in this way. Branched MAT molecules can be prepared for example by
chemical synthesis of polyllysine peptides in which two further lysine residues are attached to each lysine (one lysine to each amino group of the lysine), and thus a branched polyllysine peptide is formed. It is then possible subsequently to synthesize a peptide chain onto each terminal lysine, or to attach a peptide covalently by peptide ligation. Other branched polymers can also be used as carrier structure for MAT molecules or modules of MAT molecules for the purposes of the invention. One example of this is, for example, PEG star molecules, which can be prepared by polymerizing ethylene oxide with crosslinked divinylbenzene.

**Peptidomimetics**

Another possible way of preparing MAT molecules or modules of MAT molecules is chemical synthesis of peptides or peptidomimetics [40] or of combinations of peptides and peptidomimetics. The preparation of MAT molecules or of modules of MAT molecules by chemical synthesis can take place for example by the Merrifield solid-phase synthesis protocol using automatic synthesizers and synthetic chemicals which are obtainable from various manufacturers. One example of a company which supplies syntheses of peptidomimetics is The Peptide Laboratory™, Benicia, Calif., USA. Numerous synthons for conventional peptides and for peptidomimetics can be purchased, for example, from Sigma-Aldrich Co, St. Louis, Mo., USA.

**Composition of Medicaments and Diagnostic Aids**

The peptide portions, amino acid portions, amino acid derivative portions, peptidomimetic portions etc. of the MAT molecules, contained in the medicaments and diagnostic aids, may also be in the form of their salts, provided that these salts are pharmacologically acceptable salts. Medicaments or diagnostic aids intended for injection may be for example sterile aqueous or oily solutions which are mixed according to the prior art with suitable excipients such as, for example, dispersants, humectants and agents which stabilize suspensions. The sterile injectable solutions may be produced using pharmacologically acceptable diluents or solvents such as, for example, 1,3-
butanediol. Among the acceptable solvents and buffers which can be used are, *inter alia*, water, Ringer's solution, isotonic sodium chloride solutions etc. In addition, sterile oils, including synthetic mono- or diglycerides, can be used. It is also possible to use fatty acids such as, for example, oleic acid to prepare the injectable solutions. It is furthermore possible to use dimethylacetamide, detergents, including ionic and nonionic detergents, polyethylene glycols, etc. Mixtures of the abovementioned substances are likewise possible. It is additionally possible for medicaments also to be produced in the form of mixtures with biodegradable polymers which release the medicaments continuously. One example of such a system is, for example, the Atrigel system (Atrix Labs, Fort Collins, Colo., USA).

Preparations which can be used for rectal administrations- are those consisting of mixtures comprising MAT molecules and, where appropriate, further substances with a suitable, non-irritant ointment base or filler such as, for example, cocoa butter, synthetic mono-, di- or triglycerides, fatty acids or polyethylene glycol. It is additionally possible for colorants, preservatives and odorants to be present. These and other suitable substances are solid at room temperature and melt at body temperature, so that they release the contained substances.

It is possible to use for oral administration *inter alia* capsules, tablets, pills, powders, granules etc. In such dosage forms, the active substances of the medicaments and diagnostic aids are often combined with adjuvants suitable for the particular dosage form. The substances may be processed with lactose, sucrose, starch powder, cellulose esters, alkanolic acids, cellulose alkyl esters, stearic acid, magnesium stearate, magnesium oxide, sodium or calcium salts of phosphorous or sulphurous acid, gelatin, gum arabic, sodium alginate, polyvinylpyrrolidones, polyvinyl alcohol etc. to form, for example, tablets, capsules etc. Such capsules, tablets etc. may additionally comprise substances which enable or promote controlled release of the active substances such as, for example, hydroxypropylmethylcellulose. Buffer substances such as sodium citrate, magnesium or calcium carbonates or bicarbonates, etc. may also be present. Further constituents may be colorants, fragrances, flavourings, preservatives and sweeteners. Tablets, pills, capsules etc. may additionally be
provided with coatings which, on the one hand, make them resistant to gastric acid but, on the other hand, cause them to dissolve in the alkaline environment of the intestine.

It is also possible to use liquid, pharmaceutically acceptable emulsions, solutions, syrups and gel-like preparations for oral administration. These preparations may comprise solvents used in medicine, such as, for example, water, ethanol, etc. These preparations may also comprise adjuvants, humectants, emulsifiers and suspending agents, etc., and sweeteners, flavourings, colorants, preservatives and odorants.

Liquid preparations intended for injection purposes can be produced from sterile powders or from granules by dissolving in aqueous or non-aqueous solvents. The powders and granules on which these solutions are based may contain one or more of the substances mentioned for medicaments which can be administered orally. Suitable solvents are, inter alia, water, polyethylene glycol, polypropylene glycol, ethanol, corn oil, cottonseed oil, coconut oil, benzyl alcohol, sodium chloride solutions or various other buffers. Other possible ingredients are colorants, preservatives etc.

The amount of MAT molecules and further ingredients of the medicaments depends on the dosage form, dosage frequency, the chosen administration route, the age, sex, weight and state of health of the patient, etc. An additional factor to be taken into account is whether the treatment is carried out for diagnosis, therapy or for prophylaxis, and whether the aim of the treatment is to enhance the immune reaction or to depress the immune reaction. Numerous works on the formulation and dosage of medicaments are known to the skilled man [41, 42].

**Administration Routes for Medicaments and Diagnostic Aids of the Invention**

The medicaments and diagnostic aids of the invention can be administered by various routes to the patient or to an experimental animal to be immunised. These methods include inter alia oral and sublingual dosage, e.g. in the form of
tablets, coated tablets, capsules, granules, liquid solutions, powders to be
dissolved in liquids, etc.; the coated tablets, tablets, granules and capsules, for
example, optionally being formulated such that the medicaments reach the
intestine without being affected by the acidic environment of the stomach, and
the ingredients of the medicaments are not released until they reach the intestine.
It is additionally possible to administer the medicaments by topical application to
the skin or to mucous membranes for example in the form of ointments, sprays,
dusting powders, tinctures etc. or inhaled as aerosol via the mucous membranes,
e.g. of the respiratory tract. Rectal administration in the form of suppositories,
enemas, etc. is also possible. For transdermal administration of the medicaments
it is also possible to use aids such as, for example, dendritic cells, B
lymphocytes, macrophages, further macrophage-like cells, etc. As a further
example, cells of experimental animals or cell lines can be treated with MAT
molecules. The treated cells can then subsequently be administered to the
patient or the experimental animal. The cells can be administered to the patient
or to the experimental animal as living cells, as inactivated cells no longer
capable of division, or as killed cells. Inactivated or killed cells can be obtained for
example by treatment with suitable substances or by irradiation, e.g. with
radioactive or ultraviolet radiation. A further possibility for administering the
medicaments, in particular the MAT molecules according to the invention, is
stable or transient transfection of animal, human or plant cells with a vector which
leads to expression of a MAT molecule. The cells modified in this way can
optionally be enclosed in a matrix which firstly fixes them locally and secondly
protects them from the patient's or the experimental animal's immune system but
which, on the other hand, allows the MAT molecules released from the cells to
escape into the patient's or experimental animal's body. In some circumstances it
is also possible for the transfected cells to be administered directly to the patient
or experimental animal, in which case the cells are treated where appropriate in
such a way that they are no longer able to divide, e.g. by irradiation or by
treatment with suitable chemicals. The medicaments may additionally be
administered packaged in liposomes or other vesicles such as, for example,
exosomes, or deoxosomes, or the medicaments can be administered in the form
of mixtures with biodegradable polymers which release the medicaments
continuously. One example of such a system is, for example, the Atrigel system
(Arix Labs, Fort Collins, Colo., USA). It is additionally possible for further substances to be administered by the same or by one or more other administration routes simultaneously or sequentially to the dosage of the medicament or diagnostic aid of the invention. These further substances may \textit{inter alia} improve, via their immunostimulating properties, the effect of the medicaments or diagnostic aids of the invention. Such substances given simultaneously or sequentially may be \textit{inter alia} adjuvants, mineral oil, Freund's adjuvant, immunostimulating proteins or mediators such as, for example, cytokines, other vaccines etc. It is additionally worthwhile where appropriate for immunosuppressant substances to be administered simultaneously or sequentially in order for example to reduce or suppress unwanted local immune reactions, while retaining systemic immune reactions. Simultaneous or sequential administration of immunosuppressant substances may, however, also conversely be used to prevent systemic immune reactions, while at the same time preserving the local immune reaction.

**Possibilities for Examining the Efficacy of MAT Molecules**

Various \textit{in vitro} and \textit{in vivo} experiments can be carried out to examine the efficacy of MAT molecules in relation to modulation of the immune response of an individual.

Suitable \textit{in vitro} models include, for example, peripheral blood mononuclear cells (PBMCs), e.g. obtained from the blood of patients suffering from allergic disorders. The advantage of such cells is that the exact antigen against which the particular patient has an allergic response is often known. This knowledge makes it possible for example to simulate a desensitization of the patient \textit{in vitro}, before clinical studies are carried out on the patient or in experimental animals. For this purpose, for example, the PBMCs from allergy sufferers can be treated with the particular antigen against which the allergic person reacts, or with a MAT molecule which comprises the particular allergen in the antigen module. Thus, the immunological reaction of the primary patient's cells to various dosage forms (complete MAT molecule, molecules with/without translocation module, molecules with/without targeting module etc.) of the allergen can be investigated.
Suitable measurement parameters are *inter alia* cytokine determinations in the cell culture supernatant. Various types of T cells are involved in the immune response to an antigen, such as, for example, T-helper cells of type 1 (Th1 cells) or of type 2 (Th2 cells) or of type 0 (Th0 cells). The type of T cells involved in each case has a great effect on whether the immune response induced against the antigen primarily consists of immunoglobulins of class E (IgE) or of immunoglobulins of class G (IgG). It is known from the literature that, in particular, IgE immune responses are responsible for allergic reactions and asthma, whereas IgG immune responses are usually associated with a tolerance to the antigen. The particular T cell type activated by the treatment of the PBMCs with an antigen can also be determined for example by determining the expression of surface antigens on the cell surface or by determining messengers such as, for example, cytokines which are released by the PBMCs. Markers of a Th1 immune response which can be determined are, for example, interferon gamma (INF-γ) or interleukin-1 (IL-1) in the cell culture medium, whereas IL-4, IL-5, IL-6, IL-10 and IL-13 indicate a Th2 immune response. These cytokines can be detected by standard methods known to the skilled man, such as, for example, ELISA determinations from, for example, the cell culture supernatants or FACS analyses of the messengers present on the surface or inside the cells of the PBMCs, or by Western blots of cell culture supernatants or cell lysates etc.

Numerous other methods suitable for detecting these or other messengers are known in the literature [43, 44]. Besides the messengers released by the PBMCs, it is also possible to use intracellular or membrane-associated messengers or further proteins for immunological characterization of the T cells in PBMCs.

Numerous antibodies suitable for such investigations are supplied *inter alia* by Pharmingen, San Diego, Calif., USA, Beckmann Coulter Inc., Fullerton, Calif., USA, Santa Cruz Biotechnology Inc., Santa Cruz, Calif., USA, etc. Corresponding investigations can, however, be carried out not only with patients' primary cells but also with cells obtained from appropriately treated experimental animals, e.g. mice, rats, guinea pigs, etc. Experiments with experimental animals have the advantage that not only can the cells of these animals be studied *in vitro*, but that the immune system can be investigated *in vivo* in the context of an intact organism. It is thus also possible to investigate the effect of the dosage, composition and administration form of the MAT molecules and of corresponding
controls such as, for example, molecules which consist only of an antigen or which consist only of a translocation module and an antigen module or which consist only of a targeting module and of an antigen module. It is possible to investigate *inter alia* whether there are differences in the nature of the immune response if MAT molecules or corresponding controls are injected subcutaneously in a conventional way or if the injection is given directly into lymph nodes, for example. It is also possible to investigate the effect of non-invasive administrations such as, for example, oral or sublingual administration of the MAT molecules and of corresponding controls. A further investigation which can be carried out in experimental animals is administration of MAT molecules or corresponding controls with and without simultaneous administration of adjuvants such as, for example, mineral oil, mycobacterial extracts or Freund's adjuvant. It is also possible to ascertain the most effective or best-tolerated time schedule for the immunisations to be carried out, and the dose and number of immunisations.

Different antigen modules can moreover be tested for their efficacy. It is possible in this way to ascertain the best immunisation strategy for later studies on human patients.

Besides messengers, intracellular proteins and surface proteins, immunoglobulins are also suitable in particular for characterizing the immune reaction of cells cultivated *in vitro* or for characterizing the immune reaction of experimental animals or of human patients in clinical studies. It is possible for example by using ELISAs to investigate and quantify whether the antibodies released as a result of the administration of the allergen or antigen are of the IgE or IgG type. This information would indicate the type of immune reaction involved.

Since the messengers released by the T cells also have an effect *inter alia* on the proliferation of cells of the immune system which are present *inter alia* in PBMC preparations, it is also possible to characterize the immune response by determining the proliferation of cells such as, for example, PBMCs. It is possible for this purpose to carry out for example *in vitro* investigations such as, for example, DNA incorporation studies with tritium-labelled thymidine to detect cell growth. Numerous other methods known to the skilled man for determining cell proliferation can also be used in these investigations. Proliferation of certain cells
of the immune system can also be determined in vivo by for example carrying out FACS investigations with blood samples from experimental animals or human patients taking part in trials. It is possible by selecting suitable antibodies to quantify for example different subpopulations of cell types present in the blood.

The effect of a treatment with MAT molecules or corresponding controls can be investigated in this way.

Exemplary Embodiments

The following exemplary embodiments are intended to illustrate the invention by way of example but are by no means intended to restrict the scope of protection of the invention.

EXAMPLE 1

Cloning of Expression Vectors for MAT Molecules

All the molecular-biological methods described below were carried out in accordance with the standard methods known to the skilled man [43]. The vector pQE-30 (Qiagen, Hilden, Germany) was used to clone a vector for expression of MAT molecules (modular antigen transport molecules).

In a first step, a nucleic acid sequence which codes for a translocation module was introduced into a bacterial expression vector. The DNA sequence which codes for the amino acids GYGRKKRRQRRR of HIV Tat was introduced via synthetic oligonucleotides into the vector pQE-30. The oligonucleotides comprised in addition to the HIV Tat sequence at the 5’ end a recognition sequence of the restriction endonuclease Bgl II and at the 3’ end recognition sequences for BamH I, Spe I, Pst I and Hind III. The synthetically prepared HIV Tat sequence was subsequently cut with Bgl II and Hind III and the vector pQE-30 was cut with the restriction endonucleases Bam HI and Hind III. The Tat sequence and the vector pQE-30 were then isolated using NucleoSpin extract 2 in 1 (Macherey-Nagel, Oensingen, Switzerland), joined together using ligase, transformed into competent bacteria by electroporation, and plated out on
ampicillin-containing agar plates. Some of the resulting bacterial colonies were selected, and vector DNA was isolated therefrom. The vectors obtained in this way were sequenced using standard methods to confirm the nucleic acid sequence. Bacterial clones which comprised vectors having the correct sequence were used for further studies. In a second step, a targeting module was introduced into the vector. For this purpose, mRNA from human peripheral mononuclear cells (PBMCs) was isolated using standard methods known to the skilled man and transcribed by reverse transcriptase PCR into complementary DNA (cDNA). The nucleic acid sequence of the human invariant chain of the MHC II molecule which codes for amino acids 1 to 110 of the invariant chain of MHC II was obtained by using the cDNA obtained in this way and by using various PCR primers which introduced a Bgl II recognition sequence at the 5' end of the PCR product and introduced Spe I, Bam HI, Pst I and Hind III recognition sequences at the 3' end of the PCR products. This sequence region of the invariant chain of MHC II was introduced in accordance with the first step behind the 3' end of the Tat sequence already introduced into the pQE-30 vector. The sequence of the resulting vector was confirmed by sequencing. Standard methods of site-directed mutagenesis known to the skilled man were used to replace cytosine by thymidine at position 292 and guanine by adenine at position 318. The two point mutations do not lead to changes in the amino acid sequence of the corresponding protein, but merely remove unwanted recognition sequences of restriction endonucleases. In a third step, finally, an antigen module was introduced into the vector. Nucleic acid sequences of various antigens were obtained by using the pQE-30 vector into which the coding sequence of the particular antigen had previously been introduced and by using PCR primers which introduce an Spe I and/or BamHI recognition sequence at the 5' end of the PCR product and a stop codon and a Pst I or a Hind III recognition sequence at the 3' end of the PCR product. Inter alia, the nucleic acid sequence which codes for amino acids 1 to 222 of the antigen Der p 1 (based on the amino acid sequence of the mature Der p 1 protein) was obtained as antigen module. This sequence region of the antigen Der p 1 was introduced, in accordance with the first two steps, behind the 3' end of the sequence of the invariant chain of MHC II which had already been introduced into the pQE-30 vector. The correct sequences of the resulting vectors were confirmed by
sequencing. For subsequent studies, the expression vectors were isolated using QIAfilter plasmid midi kits (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions.

5 EXAMPLE 2

Obtaining the Coding Sequences of the Antigens Bet v 1, Asp f 1, Asp f 6 and Der p 1

10 The coding sequences of the various antigens in the antigen modules were isolated by various methods known to the skilled man [43]. The Bet v 1 sequence was obtained via synthetic oligonucleotides, the Asp f1 and Asp f6 sequences were obtained in earlier studies and isolated from the vectors used in these studies [45, 46] and subsequently introduced into the pQE-30 vector, and the Der p 1 sequence was isolated by reverse transcriptase PCR using mRNA obtained from house dust mites (Dermatophagoides pteronyssinus).

EXAMPLE 3

20 Expression and Isolation of MAT Molecules in Bacteria

The expression and isolation of MAT molecules was carried out in accordance with the manufacturer's information (Qiagen, Hilden, Germany). In detail, a preculture of E. coli M15 bacteria transfected with the particular expression vector (pQE-30-MAT molecule) was set up in 20 ml of medium (2x YT medium, 100 μg/ml ampicillin, 25 μg/ml kanamycin) in a bacterial shaker at 37°C overnight. The preculture was then cultivated in 2000 ml of medium (2x YT medium, 100 μg/ml ampicillin, 25 μg/ml kanamycin) at 37°C in a bacterial shaker until it reached an optical density of 0.6 at a wavelength of 600 nm. After addition of IPTG in a final concentration of 1 mM and a renewed growth phase of from 4 to 15 h, the bacteria were separated from the culture medium by centrifugation at 2000 g for 20 minutes, and the bacterial pellet was stored at -20°C. Cell lysates were produced by thawing the bacterial pellet, resuspending in 8 M urea solution (5 ml of urea solution per gram wet weight of the bacterial pellets), cautiously
stirring for 1 to 2 h and subsequently centrifuging for 30 min at 48,000 g. The clear supernatant was used for preparative nickel chelate chromatography of the MAT molecules. The MAT molecules were isolated by using 49 or 18 ml columns (Bio-Rad Laboratories Inc., Hercules, Calif., USA), packed with 5 to 10 ml of Ni-NTA Superflow matrix (Qiagen) and a chromatography system from Bio-Rad (Econo pump and UV monitor). The chromatography column is initially washed with 5 column volumes of buffer A (100 mM NaH2PO4, 10 mM Tris/HCl, 6 M guanidine HCl, pH adjusted to 8.0 with HCl) and then the bacterial lysate is loaded onto the column at a flow rate of 1.4 ml/min. Then 5 to 10 column volumes each of buffer A and buffer B (buffer B: 100 mM NaH2PO4, 10 mM Tris/HCl, 8 M urea, pH 8.0) are pumped onto the column at a flow rate of 1.4 ml/min, and the absorption of the flow-through at a wavelength of 280 nm (A280) is observed. As soon as the flow-through has reached a stable A280 of less than about 0.01, the column is washed with 5 to 10 column volumes of buffer C (100 mM NaH2PO4, 10 mM Tris/HCl, 8 M urea, pH adjusted to 6.3 with HCl) until finally a stable A280 of less than about 0.01 is reached. The MAT molecule is then eluted with buffer E (100 mM NaH2PO4, 10 mM Tris/HCl, 8 M urea, pH adjusted to 4.5 with HCl) and collected.

EXAMPLE 4

Detection of MAT Molecules with SDS Polyacrylamide Gels, Coomassie Staining and with Anti-His Western Blotting

SDS Polyacrylamide Gel Electrophoresis:

Buffer, NuPAGE® gels and an Xcell SureLock™ electrophoresis chamber from Invitrogen (Invitrogen Life Technologies, Breda, The Netherlands) were used for the electrophoresis in accordance with the manufacturer's instructions. 5 or 10 µg of the isolated MAT molecules per lane underwent electrophoretic separation in 12% NuPAGE® Novex bis-Tris gels (Invitrogen) at a constant voltage of 200 V using 1x concentrated NuPAGE® SDS sample buffer under reducing conditions over the course of 35 to 50 min. The eluent buffer used was MES or MOPS buffer (MES buffer: 50 mM MES (morpholinoethanesulphonic acid), 50 mM Tris/HCl,
3.5 mM SDS, 1 mM EDTA, pH 7.3, MOPS buffer: 50 mM MOPS (3-(N-morpholino)propanesulphonic acid), 50 mM Tris/HCl, 3.5 mM SDS, 1 mM EDTA, pH 7.7).

Coomassie Blue Staining:

The gels are stained by incubating them in staining solution (200 ml of methanol, 50 ml of acetic acid, 250 ml of water, 0.5 g of Coomassie blue R-250) for 1 h and then destaining with multiple changes of the destaining solution (200 ml of methanol, 50 ml of acetic acid, 250 ml of water) until the background of the gels is clear. The Xcell II™ blot module (Invitrogen) is used for electrotransfer of the proteins from the NuPAGE® gel onto a blotting membrane in accordance with the manufacturer's instructions. The blotting apparatus is set up in accordance with the manufacturer's instructions using 1×NuPAGE® transfer buffer with 10 or 20% methanol. PVDF membranes were used as blotting membrane, and the electrotransfer took place at a constant voltage of 30 V for 1 h.

Immunological Detection of MAT Molecules:

Immunological detection of MAT molecules took place using anti-His antibodies in accordance with the manufacturer's instructions (anti-RGS(4His)4 antibodies, Qiagen, Hilden, Germany). All the experimental steps take place at room temperature. The PVDF membrane is initially dried and then incubated directly, without previous blocking of free protein-binding sites, with the anti-His antibody (Qiagen) in a dilution of from 1:1000 to 1:2000 in TBS (50 mM Tris/HCl, 150 mM NaCl, pH 7.5) with 3% BSA (bovine serum albumin) for 1 h. The membrane is then washed 3x with TBS, 0.05% Tween 20, 0.2% Triton X-100 for 10 min each time and subsequently washed once with TBS without further additions. The secondary antibody used is anti-mouse Ig-HRP conjugate (Amersham, Buckinghamshire, England) in a dilution of from 1:10,000 to 1:20,000 in TBS with 10% milk powder, incubating for 1 h. Finally, the blot is washed 4x with TBS, 0.05% Tween 20, 0.2% Triton X-100 for 10 min each time. The conjugate is detected using the ECL™ system (Amersham) in accordance with the manufacturer's instructions. The chemiluminescence signal is detected using
autoradiography films, which are developed in accordance with standard protocols.

EXAMPLE 5

Translocation of MAT Molecules into Cell Lines and Primary Human Cells

PBMCs (peripheral blood mononuclear cells) were obtained from fresh, heparin-treated human blood from volunteer subjects by standard methods of density gradient centrifugation known to the skilled man using Ficoll-Paque (Pharmacia, Uppsala, Sweden). The Jurkat cell line was obtained from the ATCC (American Type Culture Collection, Manassas, Va., USA). The cells were cultivated in RPMI-1640 medium containing the following additions: 10% foetal calf serum, 200 units/ml penicillin, 200 µg/ml streptomycin, MEM vitamins, MEM non-essential amino acids, 1 mM sodium pyruvate, 2 mM L-glutamine and 0.001% 2-mercaptoethanol. To determine the translocation, the cells were resuspended in a concentration of 1*10^6/ml RPMI medium with all additions. The cells were incubated according to the respective data contained in the explanations of the Figures with concentrations of from 0.01 to 5 mM of the recombinant molecules or MAT molecules over a period of 5 min at 4, 22 or 37°C. The cells were then centrifuged, and the cell pellet was incubated in 40 µl of lysis buffer (8 M urea, 100 mM sodium phosphate, 10 mM Tris/HCl, 100 mM ammonium chloride) at room temperature for 5 min. Insoluble cell constituents were removed by centrifugation (15,000 g, 15 min) and the clear lysate was further investigated by Western blotting in accordance with example 4. An anti-His antibody was used to detect the proteins.

EXAMPLE 6

Stimulation of Peripheral Blood Mononuclear Cells (PBMC) from Patients with Allergies

The methods described below for stimulating PBMCs and for determining the proliferation of the stimulated PBMCs are described in the literature [44]. PBMCs were isolated from fresh, heparin-treated blood from volunteer subjects by density gradient centrifugation using Ficoll-Paque (Pharmacia, Uppsala,
Sweden). The volunteer subjects were patients allergic to a known allergen for
the individual patient. The patients were informed in accordance with Swiss laws
about the experiments carried out on their blood samples and gave their consent
to take part in this study. After the PBMCs had been obtained they were taken up
in RPMI medium with the additions according to Example 5, and in each case
from 0.01 to 100 nM of the recombinantly prepared antigen to which the particular
patient shows an allergic reaction were added. In each case, the unmodified
antigen and the antigen coupled to a translocation module and a targeting
module (MAT molecule) were investigated. Experimental approaches in which no
targeting module but only a translocation module and the antigen module were
coupled were also carried out in some experiments (Fig. 7 A). As controls, some
experiments used only the translocation module or a construct consisting of
translocation module and targeting module, but no antigen module. After an
incubation period of 5 days, 10 µCi/ml tritium-labelled thymidine was added to the
medium. The thymidine incorporation of the PBMCs was ascertained as a
measure of the efficiency of antigen presentation and the associated proliferation
of the treated cells. For this purpose, the radioactive cell culture medium was
removed after 8 to 10 h, the cells were washed and the amount of incorporated
radioactive thymidine was determined by measuring the radioactivity. A 1205
Betaplate liquid scintillation counter from Wallac ADL AG, Hünenberg,
Switzerland, was used for this. As control, cells not stimulated with antigen were
also incubated with tritium-labelled thymidine and then analysed in the same way.
The result obtained is a measurement of the incorporation of radioactive
thymidine as a measure of the proliferation and hence as a measure of the
efficiency of antigen presentation. The higher the measured thymidine
incorporation, the more efficient the antigen presentation. Since antigen
concentrations of from 0.01 nM to 100 nM were investigated in each experiment,
it is additionally possible to determine the antigen concentration at which
maximum antigen presentation takes place. The lower this concentration, the
more effective the antigen as modulator of an immune response. As a further
control, cells were treated with 0.5 µg/ml each of an anti-CD3 and of an anti-
CD28 antibody: this represents a very strong proliferation stimulus. It was thus
possible to determine the maximum possible thymidine incorporation due to the
proliferation of PBMCs for each experiment.
EXAMPLE 7
MAT molecule-stimulated cytokine release

PBMCs were isolated from the blood of allergy sufferers as described in Example 5 and diluted 10^6 per ml of medium. 100 μl batches of this cell suspension were seeded in 96-well plates and treated with from 0.01 to 1000 nM of the isolated antigens for 5 days. The cell culture medium was not replaced during this time. After centrifugation of the 96-well plates, the supernatants were removed and stored at -20°C until the cytokines were analysed. The PBMCs were treated in each case with the antigen to which the patient from whom the PBMCs were isolated shows an allergic reaction. The following cytokines were investigated in the supernatants obtained in this way: interferon gamma (INFγ), interleukin-10 (IL-10) and interleukin-5 (IL-5). Figs. 8A and 8B show results obtained with PBMCs from patients showing an allergic reaction to Bet v 1. The INFγ, IL-10 and IL-5 immunoassays (ELISAs = enzyme-linked immunosorbent assays) were carried out by methods known to the skilled man using DuoSet® ELISA Development Systems from R & D Systems Inc., Minneapolis, USA.

In parallel with the obtaining of the supernatants for the cytokine measurements, stimulation experiments were also carried out with PBMCs from the same donors in accordance with Example 6. The results of these cell proliferation assays are likewise depicted in Figs. 8A and 8B.

EXAMPLE 8
In Vivo Effect of Unmodified Antigens and Antigens Present in the Antigen Module of MAT Molecules

Immunization of Mice

In order to test the efficacy of MAT molecules, CBA/2 mice were immunised 3x at an interval of 2 weeks each time with recombinant MAT molecules together with aluminium hydroxide as adjuvant in a manner known to the skilled man. The recombinant MAT molecules were produced as described in Example 3. Three
different routes were used for the immunisation. A series of experiments was
carried out in each case with subcutaneous, intraperitoneal and intranodal
injection of the antigens or of the controls. In the case of intranodal injection, the
tissue was exposed surgically so that direct injection was possible. The MAT
molecule used was a protein consisting of the HIV Tat sequence as translocation
module, the human invariant chain as targeting module and the PLA2
(phospholipase A2 from bee venom) peptide as antigen module (designation:
trans-target-PLA2). PLA2 (designation: PLA2) was used as control, and 0.9%
strength saline solution (designation: control) was used as a further control. The
intranodal immunisation was carried out with 0.1 μg, the subcutaneous and
intraperitoneal immunisation were carried out with 10 μg of MAT molecule or

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Determination of the PLA2-Specific IgG2a Titres in Mouse Sera

In order to determine the PLA2-specific IgG2a titre, microtitre plates (96 cavities)
were coated with 100 μl/cavity of a solution of 5 μg/ml PLA2 (Sigma-Aldrich,
Buchs DG, Switzerland) in carbonate buffer at 4°C overnight. After washing 2x
with phosphate-buffered sodium chloride solution (PBS), 0.05% Tween, free
protein-binding sites were blocked by incubation with blocking buffer (PBS, 2.5%

skim milk powder), 200 μl/cavity, at room temperature for 1 to 2 h. The plates
were washed a further two times, and then serial 1:2 dilution series in blocking
buffer (50 μl/cavity) of the serum samples to be tested (1:2 to 1:64 dilutions) were
incubated at room temperature for 3 h or at 4°C overnight. Incubations without
serum or with serum from untreated animals were carried out as negative controls. The plates were then washed 5 times and incubated with a 1:500 dilution in blocking buffer of a biotin-labelled anti-mouse IgG2a (PharMingen GmbH, Hamburg, Germany) at room temperature (100 µl/cavity) for 2 h, and washed a further five times. Finally, 100 µl/cavity of a horseradish peroxidase diluted 1:1000 in blocking buffer were incubated at room temperature for 1 h and then washed 6x. The staining reaction was carried out with 100 µl/cavity of a solution of ABTS (2,2'-azinodi-(3-ethylbenzothiazolinesulphonic acid) in ABTS buffer in accordance with the manufacturer's instructions with 0.1% (v/v) of a 30% strength hydrogen peroxide solution. After about 30 minutes, the absorption at a wavelength of 405 nm (reference filter: 595 nm) was measured. The results of these tests are shown in Fig. 9.

**Determination of the PLA2-Specific IgE Titers in Mouse Sera**

The PLA2 IgE ELISA is carried out in accordance with the method for the PLA2 IgG2a ELISA. The procedure differs from the method described above in the following respects: the microtitre plates are coated with 5 µg/ml of an anti-mouse IgE antibody. After the serum samples have been incubated and the plates have been washed, a 1:333 dilution of biotin-labelled PLA2 (Pierce Biotechnology Inc., Rockford, USA) is incubated. The development of the stain with ABTS takes about 1 h. The results of these tests are depicted in Fig. 9.

**DESCRIPTION OF THE DRAWINGS**

**Fig. 1**
Theoretical Structure of MAT Molecules

Fig. 1 schematically shows by way of example how the individual modules of a MAT molecule of the invention may be constructed. In the Figure, "Trans" denotes a translocation module, "Target" denotes a targeting module, "AG" denotes an antigen module, "Tag" denotes a tag module and a dash (-) denotes a linker module. The linker module may connect the other modules together by
covalent and/or non-covalent linkages. Fig. 1 A shows a number of examples of linear arrangements of the various modules. Fig. 1 B shows various examples of arrangements of branched MAT molecules, with dendrimeric structures also being included, and Fig. 1 C depicts some examples of arrangements of circular MAT molecules, while circular arrangements may be combined with linear and/or branched arrangements. In general, all the arrangements shown are only examples intended to illustrate the fact that a wide variety of arrangements is possible. The examples of MAT molecule diagrammatically shown are by no means to be understood as limiting the scope of protection of the present invention.

Fig. 2
Expression, Purification and Detection of Fusion Proteins

Various MAT molecules consisting of tag module (His6 tag), translocation module (Tat sequence), targeting module (human invariant chain of MHC II) and antigen module (Asp f1=Aspergillus fumigatus antigen 1) were expressed in E. coli and isolated under denaturing conditions using immobilised metal ion affinity chromatography. The steps were carried out in accordance with the instructions from the manufacturer of the expression system (Qiagen, Hilden, Germany) [47, 48]. 10 μg or 5 μg samples of the isolated proteins were fractionated by electrophoresis in SDS polyacrylamide gels and then either stained with Coomassie blue (Fig. 2 A) or analysed in a Western blot using antibodies which recognize the tag module (Fig. 2 B). The positions of the expressed proteins are marked by arrows.

Fig. 3
Temperature-Independent Translocation of Proteins and MAT Molecules

Primary human peripheral mononuclear cells (PBMCs) were incubated with the particular proteins or MAT molecules in a concentration of 1 μM at 4, 22 or 37°C for 5 minutes, washed, taken up in urea-containing sample buffer and lysed. The lysates were then fractionated by electrophoresis in SDS polyacrylamide gels and electrotransferred to PVDF membranes, and the proteins or MAT molecules were
detected using a specific antibody (anti-RGS(His)4 antibodies). The arrows show
the position of the proteins or MAT molecules. The fusion proteins were
detectable in the lysates of the cells at all temperatures, indicating successful
translocation to the interior of the cells.

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Fig. 4
Translocation of MAT Molecules in Cell Lines and Primary Cells

The result depicted in Fig. 4 was carried out with virtually the same methods as
the experiment in Fig. 3, but with two differences: Fig. 4 A shows that
translocation of the MAT molecules was successful both in primary human cells
(PBMCs) and in human tumour cell lines (Jurkat cells). The arrows show the
position on the Western blot at which the protein or the MAT molecule was
detected. Figs. 4 A and 4 B additionally show that various antigen modules (Asp f
1=Aspergillus fumigatus allergen, Der p 1=house dust mite allergen, Bet v
1=birch pollen allergen) can enter primary human PBMC cells by translocation.

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Fig. 5
Dose Kinetics of the Translocation of MAT Molecules

The result depicted in Fig. 5 was obtained with virtually the same methods as the
experiment in Fig. 3, but in this case three or four different concentrations of
proteins or MAT molecules were used (0.01 mM, 0.1 mM, 1 mM and 5 mM). It
was possible to show a clear dose dependency for the translocation reaction.
20 Larger amounts of the particular molecule were detected in the interior of the
cells as the concentration of added MAT molecules increased. The positions of
the respective molecules are marked with arrows.

Fig. 6 Various Functional Structures of MAT Molecules

The result depicted in Fig. 6 was obtained with virtually the same methods as the
experiment in Fig. 3, but in this case three different arrangements of translocation
module (Tat sequence) and antigen module (Asp f 1=Aspergillus fumigatus
allergen) were investigated. The arrow in Fig. 6 A indicates the position at which
the fusion protein was detected in the Western blot. Fig. 6 B depicts the structure of the three different fusion proteins. Fusion proteins with an N-terminal, inverted C-terminal and C-terminal translocation module were investigated. All three fusion proteins were successfully transported into the interior of the cells by translocation.

Fig. 7
Antigen/MAT Molecule-Mediated In Vitro Cell Proliferation of PBMCs of Allergy Sufferers

Fig. 7 depicts investigations on PBMCs (peripheral blood mononuclear cells) from allergy sufferers, which were combined with a MAT molecule in vitro. The MAT molecule comprised as antigen module the particular allergen to which the allergy patient shows an allergic reaction. An antigen presentation of the antigen-presenting cells of the allergic person which is more efficient owing to the addition of the MAT molecule leads to stimulation of growth of the PBMCs. The cell proliferation resulting therefrom was quantified via the incorporation of radiolabelled DNA building blocks (thymidine) (y axis). The x axis indicates the MAT molecule concentration used in the particular cell stimulation test, or the antigen molecule concentration, in nM. When MAT molecules are used at relatively low concentrations, cell proliferation (= immunostimulation = increase in thymidine incorporation = increase in radioactivity) occurs in all the patients investigated with all the antigen modules investigated (as part of the MAT molecule). As control, in each case the same PBMCs were treated only with water containing no MAT molecules or antigens (= control in the legend). In addition, the thymidine incorporation by cells treated with a strong growth stimulus (anti-CD3 and anti-CD28 antibodies, 0.5 μg/ml each) for 5 days was measured as a positive control for each experiment. The growth obtained by this stimulus represents a check of the quality of the prepared PBMCs in relation to their ability to proliferate. The value resulting therefrom in the experiment in Fig. 7A was 77,864 ± 5,347 cpm, the value resulting in the experiment in Fig. 7B was 100,374 ± 11,678 cpm and the value resulting in the experiments in Figs. 7C and D was 112,205 ± 5,958 cpm.
Figs. 7 A to 7 G show that, with all 7 antigens tested, incorporation of the antigen into the antigen module of a MAT molecule leads to the onset of proliferation of the PBMCs of the allergy sufferer even at relatively low concentrations. The antigens tested were Der p 1 (house dust mite allergen; Fig. 7 A), Bet v 2 (birch pollen allergen, Fig. 7 B), Asp f 1 (Aspergillus fumigatus allergen 1, Fig. 7 C), Asp f 6 (Aspergillus fumigatus allergen 6, Fig. 7 D), Asp f 3 (Aspergillus fumigatus allergen 3, Fig. 7 E), PLA2 (phospholipase A2 allergen from bee venom, Fig. 7F) and Fel d 1 (Felis domesticus, Fig. 7G). Fig. 7 H additionally shows, for the antigen Bet v 1, that when MAT molecules with Bet v 1 in the antigen module are used, in 4 different patients (I. to IV.) allergic to Bet v 1, a proliferation of the PBMCs of the individual patients occurs in all 4 cases at lower concentrations than when Bet v 1 is used as antigen.

Fig. 8

Antigen/MAT Molecule Mediated In Vitro Cytokine Secretion by PBMCs from Allergic People

Figs. 8A and 8B show, for two independent patients showing an allergic reaction to Bet v 1, that PBMCs cultivated in vitro and stimulated with the Bet v 1 antigen (Bet v 1 alone or as module of a MAT molecule) display a defined cytokine secretion pattern. I. shows in each case the result of a cell proliferation test (as in Fig. 7), II. shows the interferon gamma (INFg) levels, III. shows the interleukin-10 (IL-10) values and IV. shows the interleukin-5 (IL-5) levels in the cell culture supernatants. In a desensitizing PBMC stimulation, the PBMC proliferation and an increase in INFg release should take place even at lower antigen doses. In addition, the IL-5 production should be low. This cytokine secretion pattern indicates a desensitization of the immune cells of the allergy sufferers (Th1 instead of Th2 immune response). The increase in the IL-10 concentration explains why the cell proliferation (Fig. 1.) falls at higher antigen doses. This cytokine secretion pattern occurs in both cases (Fig. 8A and Fig. 8B).

Fig. 9

Antigen/MAT Molecule-Mediated In Vivo Immune Response in Mice
CBA/2 mice were immunised with isolated PLA2, with MAT molecules having PLA2 in the antigen module or with control buffer 3x at intervals of 2 weeks and then the PLA2-specific serum titres of IgG2a and IgE antibodies were measured. If there is desensitization, only PLA2-specific IgG, but not PLA2-specific IgE, antibodies should appear. IgE antibodies are responsible for allergic reactions.

Three different immunisation routes were investigated, subcutaneous, intraperitoneal and intranodal injection of the antigens. It is found with all three immunisation routes that immunisation with PLA2 leads to a distinct IgE immune response (allergy) (Fig. 9, left-hand column). On immunisation with a MAT molecule which comprises PLA2 as antigen module, however, there is no IgE immune response (no allergy). By contrast, both immunisation with PLA2 and with MAT molecules which comprise PLA2 in the antigen module lead to a desired IgG immune response which does not induce allergic reactions.

All the examples and listings in the present patent application are intended in principle to explain the subject matter but not to restrict the claims. In particular, the examples of translocation modules, targeting modules, antigen modules, spacer modules and tag modules are to be understood as being only examples, but not an exhaustive list of all the possible constituents of the MAT molecule.

The basic idea of the invention does not consist of a particular combination of particular translocation modules, particular targeting modules and particular antigen modules. On the contrary, the basic idea of the present invention is to use a combination of at least these three modules to form a MAT molecule for immunisation. The example specifically used in each individual module is therefore immaterial to the concept of the invention, and it is consequently also possible to use other examples, including those not currently known, for the respective modules for the purposes of the invention.

If a term is not unambiguously defined in this patent, or is not known to the skilled man in the particular art, or a term cannot be unambiguously defined from the context, then the definition given for the respective term in the following standard works applies in each case. If a term is mentioned in more than one of the works cited below with different definitions, the definition which applies in each case is that mentioned in the first work mentioned in the following list. The following
publications are cited for this purpose:

The Merck Manual [49]
Molecular Cloning - A Laboratory Manual [43]
Current Protocols in Immunology [44]
Current Protocols in Protein Science [50]
Current Protocols in Pharmacology [51]
Current Protocols in Cell Biology [52]
Bibliography


SEQUENCE LISTING

[0121]

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for Modulating Immune Reactions

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<151> 2002-10-11

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Lys Leu 210

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| Met | Arg | Gly | Ser | His | His | His | His | His | Gly | Ser | Met | Gly | Val | Phe |   |
| 1  |     | 5   |     | 10  |     |     |     |     |     |     | 15  |     |     |     |   |
| Asn | Tyr | Glu | Thr | Glu | Thr | Thr | Ser | Val | Ile | Pro | Ala | Ala | Arg | Leu | Phe |
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| Gln | Ala | Ile | Ser | Ser | Val | Glu | Asn | Ile | Glu | Gly | Asn | Gly | Gly | Pro | Gly |
|     | 50  |     | 55  |     |     |     |     |     |     |     |     |     |     |     | 60 |
| Thr | Ile | Lys | Lys | Ile | Ser | Phe | Pro | Glu | Gly | Phe | Pro | Phe | Lys | Tyr | Val |
|     | 65  |     | 70  |     | 75  |     |     |     |     |     |     |     |     |     | 80 |
| Lys | Asp | Arg | Val | Asp | Glu | Val | Asp | His | Thr | Asn | Phe | Lys | Tyr | Asn | Tyr |
|     | 85  |     | 90  |     |     |     |     |     |     |     |     |     |     |     | 95 |
| Ser | Val | Ile | Glu | Gly | Gly | Pro | Ile | Gly | Asp | Thr | Leu | Glu | Lys | Ile | Ser |
|     | 100 |     | 105 |     |     |     |     |     |     |     |     |     |     |     | 110 |
| Asn | Glu | Ile | Lys | Ile | Val | Ala | Thr | Pro | Asp | Gly | Gly | Ser | Ile | Leu | Lys |
|     | 115 |     | 120 |     | 125 |     |     |     |     |     |     |     |     |     |     |
| Ile | Ser | Asn | Lys | Tyr | His | Thr | Lys | Gly | Asp | His | Glu | Val | Lys | Ala | Glu |
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35 40 45
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50 55 60

Asn His Leu Ser Asp Leu Ser Leu Asp Glu Phe Lys Asn Arg Phe Leu
65 70 75 80

Met Ser Ala Glu Ala Phe Glu His Leu Lys Thr Gln Phe Asp Leu Asn
85 90 95

Ala Glu Thr Asn Ala Cys Ser Ile Asn Gly Asn Ala Pro Ala Glu Ile
100 105 110

Asp Leu Arg Gln Met Arg Thr Val Thr Pro Ile Arg Met Gln Gly Gly
115 120 125

Cys Gly Ser Cys Trp Ala Phe Ser Gly Val Ala Ala Thr Glu Ser Ala
130 135 140

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

Val Asp Cys Ala Ser Gln His Gly Cys His Gly Asp Thr Ile Pro Arg
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Gly Ile Glu Tyr Ile Gln His Asn Gly Val Val Gln Glu Ser Tyr Tyr
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Ile Arg Glu Ala Leu Ala Gln Thr His Ser Ala Ile Ala Val Ile Ile
225 230 235 240

Gly Ile Lys Asp Leu Asp Ala Phe Arg His Tyr Asp Gly Arg Thr Ile
245 250 255

Ile Gln Arg Asp Asn Gly Tyr Gln Pro Asn Tyr His Ala Val Asn Ile
260 265 270

Val Gly Tyr Ser Asn Ala Gln Gly Val Asp Tyr Trp Ile Val Arg Asn
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Ser Trp Asp Thr Asn Trp Gly Asp Asn Gly Tyr Gly Tyr Phe Ala Ala
290 295 300

Asn Ile Asp Leu Met Met Ile Glu Glu Tyr Pro Tyr Val Val Ile Leu
305 310 315 320
PATENTKRAV


2. Anvendelse ifølge krav 1, hvor translokationsmodulet er en aminosyresekvens, målretningsmodulet er en aminosyresekvens, og antigenmodulet er et protein eller peptid.

3. Anvendelse ifølge et af kravene 1 eller 2, hvor enkeltmodulerne er bundet til hinanden med kovalente bindinger.


5. Anvendelse ifølge et af kravene 1 til 4, hvor MAT-molekylet indeholder op til to antigenmoduler, op til to målretningsmoduler og op til to translokationsmoduler.


7. Anvendelse ifølge et af kravene 1 eller 6, hvor mindst et målretningsmodul indeholder en af følgende sekvenser eller strukturer eller mindst en funktionel delsekvens af denne sekvens: invariant MHC-kæde (IIP33, IIP41, IIP35, IIP43) MHC II-β-kæde, mannose-6-phosphatereceptort, membranboundet immunoglobulin (mIg), HLA-DM-β-kæde, LAMP-1, LAMP-2, LAMP-3, Limp 1, CD63, CA82, CD1b eller sekvenser, der er inverterede i forhold til de i det foregående nævnte.

8. Anvendelse ifølge et af kravene 1 til 7, hvor mindst et antigenmodul er et protein, en nukleinsyre, en sukkerstruktur eller et lipid.

9. Anvendelse ifølge krav 8, hvor antigenet kan fremstilles under anvendelse af kombinatoriske fremgangsmåder.

10. Anvendelse ifølge et af kravene 1 til 9, hvor der yderligere er mindst et markør-modul til stede.

11. Anvendelse ifølge krav 10, hvor mindst et markør-modul udvælges fra gruppen His-markør med 4 til 6 på hinanden følgende histidinrester, Myc-markør, FLAG-markør, HA-markør, Strep-markør, Xpress-markør, S-markør, CBD-markør, GST-markør, HSV-markør, T7-markør, protein A-markør, V5-markør, chitinbindende markør, maltosebindende markør,
calmodulinbindende markør eller en biotingruppe.

12. Anvendelse ifølge et af kravene 1 til 11, hvor modulerne er forbundet med hinanden via afstandsskabende moduler.


14. Anvendelse ifølge krav 13, hvor antigenmodulet mindst er et allergen, som er udvalgt fra gruppen Feld 1, Bet v 1, Bet v 2, Der p 1, PLA 2.

15. Anvendelse ifølge et af de forrige krav, hvor lægemidlet yderligere indeholder mindst en af følgende bestanddele og/eller andre farmaceutisk acceptable hjælpe- og tilsætningsstoffer:
   a) en fysiologisk natriumchloridopløsning
   b) en farmaceutisk acceptabel adjuvans
   c) en farmaceutisk acceptabel buffersubstans
   d) et farmaceutisk acceptabelt bærerprotein
   e) et farmaceutisk acceptabelt konserveringsstof
   f) et farmaceutisk acceptabelt farvestof.


17. Anvendelse ifølge et af de foregående krav, hvor lægemidlet er en vaccine.

18. Anvendelse ifølge et af de foregående krav, k e n d e t e g n e t ved, at præparatet udformes til injektion i en lymfeknude eller til indgift over slimhinder.
Figure 1
Figure 3
Figure 4
Figure 5
Figure 7A - B
Figure 7C-D
Figure 7E-7F
Figure 7G
Figure 7H
Figure 8A
Figure 8B
Figure 9