# AUTOANTIGEN AND PROTEINS STRUCTURALLY RELATED THERETO FOR USE IN IMMUNOTHERAPY OF AUTOIMMUNE DISEASES

The invention relates to a novel autoantigen and proteins related thereto, their use in treatment of chronic destruction of articular cartilage in autoimmune diseases, pharmaceutical compositions comprising said autoantigen and/or proteins, a diagnostic method for the detection of autoreactive T cells in a test sample and test kits to be used in said method.

The immune system is established on a principle of discrimination between foreign antigens (non-self antigens) and autoantigens (self antigens, derived from the individuals own body) achieved by a build in tolerance against the autoantigens.

The immune system protects individuals against foreign antigens and responds to exposure to a foreign antigen by activating specific cells such as T- and B lymphocytes and producing soluble factors like interleukins, antibodies and complement factors. The antigen to which the immune system responds is degraded by the antigen presenting cells (APCs) and a fragment of the antigen is expressed on the cell surface associated with a major histocompatibility complex (MHC) class II glycoprotein. The MHC-glycoprotein-antigen-fragment complex is presented to a T cell which by virtue of its T cell receptor recognizes the antigen fragment conjointly with the MHC class II protein to which it is bound. The T cell becomes activated, i.e. proliferates and/or produces interleukins, resulting in the expansion of the activated lymphocytes directed to the antigen under attack (Grey et al., Sci. Am., 261:38-46, 1989).

Self antigens are also continuously processed and presented as antigen fragments by the MHC glycoproteins to T cells (Jardetsky et al., Nature 353:326-329, 1991). Self recognition thus is intrinsic to the immune system. Under normal circumstances the immune system is tolerant to self antigens and activation of the immune response by these self antigens is avoided.

When tolerance to self antigens is lost, the immune system becomes activated against one or more self antigens, resulting in the activation of autoreactive T cells and the production of autoantibodies. This phenomenon is referred to as autoimmunity. As the immune response in

general is destructive, i.e. meant to destroy the invasive foreign antigen, autoimmune responses can cause destruction of the body's own tissue.

The contribution of T cells to autoimmune diseases has been established by several studies. In mice, experimental autoimmune encephalomyelitis (EAE) is mediated by a highly restricted group of T cells, linked by their specificity for a single epitope of myelin basic protein (MBP) complexed to an MHC class II molecule. In the Lewis rat, a species with high susceptibility to various autoimmune diseases, disease has been shown to be mediated by T cells. In humans autoimmune diseases are also thought to be associated with the development of auto-aggressive T cells.

A destructive autoimmune response has been implicated in various diseases such as rheumatoid arthritis (RA), in which the integrity of articular cartilage is destroyed by a chronic inflammatory process resulting from the presence of large numbers of activated lymphocytes and MHC class II expressing cells. The mere presence of cartilage appears necessary for sustaining the local inflammatory response: it has been shown that cartilage degradation is associated with the activity of cartilage-responsive autoreactive T cells in RA (Sigall et al., Clin. Exp. Rheumat. 6:59, 1988; Glant et al., Biochem. Soc. Trans. 18:796, 1990; Burmester et al., Rheumatoid arthritis Smolen, Kalden, Maini (Eds) Springer-Verlag Berlin Heidelberg, 1992). Furthermore, removal of cartilage from RA patients by surgery was shown to reduce the inflammatory process (G.S. Panayi et al, Clin. Exp. Rheumatol. 11 (suppl.8): S1-S8, 1993). The cartilage proteins are therefore considered to be target autoantigens which are competent of stimulating T cells. Activation of these autoreactive T cells leads to development of autoimmune disease. However, the identification of the autoantigenic components that play a role in the onset of rheumatoid arthritis has so far remained elusory.

The inflammatory response resulting in the destruction of the cartilage can be treated by several drugs, such as for example steroid drugs. However, these drugs are often immunosuppressive drugs that are nonspecific and have toxic side effects. The disadvantages of nonspecific immunosuppression makes this a highly unfavourable therapy.

The antigen-specific, nontoxic immunosuppression therapy provides a very attractive alternative for the nonspecific immunosuppression. This antigen-specific therapy involves the treatment of patients with the target autoantigen or antigens having an amino acid sequence which exhibits sequence homology with the amino acid sequence of the target autoantigen, or synthetic T cell-reactive peptides derived from said autoantigen or said antigen. These synthetic peptides correspond to T cell epitopes of the autoantigen and can be used to induce specific T cell tolerance both to themselves and to the autoantigen. Although it seems paradoxical to desensitize the immune system with the very same antigen responsible for activating the immune system, the controlled adminstration of the target (auto)antigen can be very effective in desensitization of the immune system. Desensitization or immunological tolerance of the immune system is based on the long-observed phenomenon that animals which have been fed or have inhaled an antigen or epitope are less capable of developing a systemic immune response towards said antigen or epitope when said antigen or epitope is introduced via a systemic route.

To effectively use the tolerance therapy to treat the T cell mediated cartilage destruction, there is a great need to identify the responsible autoantigen or antigens having an amino acid sequence which exhibits sequence homology with the amino acid sequence of the target autoantigen, to desensitize patients against the autoantigen that is activating the T cells responsible for the inflammatory process.

It is an object of the invention to provide the autoantigen, and proteins having an amino acid sequence which exhibit sufficient homology with the amino acid sequence of the autoantigen, said autoantigen and/or protein being suitable to induce specific T cell tolerance to the responsible cartilage antigen in patients suffering from T cell-mediated cartilage destruction. It is a further object of the invention to provide arthritic animal testmodels which are suitable for use in screening for novel drugs to suppress arthritic symptoms. It is another object of the invention to provide a method for detecting autoreactive T cells involved in the destruction of articular cartilage and test kits to be used in said method.

It was surprisingly found that Human Cartilage glycoprotein 39 (herein after referred to as HC gp-39) is a target autoantigen in RA patients which activates specific T cells, thus causing or mediating the inflammatory process. The arthritogenic nature of HC gp-39 was

substantiated in the Balb/c mouse. A single, subcutaneous injection of said protein in Balb/c mice was able to initiate arthritic signs in the animals. The course of the HC gp-39- induced disease was characterized by relapses occuring periodically in fore paws and/or hind paws and gradually developed from a mild arthritis into a more severe form. Also, a symmetrical distribution of afflicted joints was observed which is, together with the observation of recurrent relapses and nodule formation, reminiscent of disease progression in arthritis, especially RA.

Even more surprisingly it was found that administration of HC gp-39 resulted in immunological tolerance and, more importantly, in delayed and/or suppressed arthritic development.

It was furthermore surprisingly found that proteins comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence of HC gp-39 are able to induce arthritis when injected to animals. In particular proteins comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPDALDRFLCTHIIYSFANISND (SEQ ID NO: 1) were able to induce arthritis in animals, in the same way as described for HC gp-39.

Preferably, these arthritogenic proteins comprise an amino acid sequence which exhibits at least 70%, more preferably 80%, most preferably 90% homology with the amino acid sequence of HC gp-39, more in particular with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPDALDRFLCTHIIYSFANISND (SEQ ID NO: 1).

The percentage of sequence homology of the arthritogenic proteins according to the invention with the amino acid sequnece of SEQ ID NO: 1 is understood to be measuered via generally used sequence comparison programmes such as FASTA (W.R. Pearson and D.J. Lipman, Proc. Natl. Acad. Sci. USA, 85 , 2444-2448, 1988).

Suitable arthritogenic proteins according to the invention are for example pig heparine-binding 38kDa protein, bovine 39 kDa whey protein, human YKL-39 protein, murine breast regressing 39kDa protein (brp39), human oviduct-specific glycoprotein, murine oviduct-specific glycoprotein, hamster oviduct-specific glycoprotein, bovine oviduct-specific glycoprotein, human chitotriosidase precursor protein and murine secretory protein YM-1 precursor. The arthritogenic proteins according to the invention are very suitable for inducing systemic tolerance of the immune system to homologous autoantigens and can be used to delay and/or suppress arthritic development in mammals.

HC gp-39 is present in serum of both patients and healthy adults, although the serum concentration of the protein is about twice as much in patients as compared to healthy adults. Furthermore, mRNA coding for HC gp-39 can be found in synovial specimens or cartilage obtained from RA patients, whereas cartilage of healthy adults, obtained at surgery, does not contain a significant amount of said mRNA. When articular chondrocytes and synovial cells are cultured, their major secretory product becomes HC gp-39 (Hakala et al., J. Biol. Chem., Vol. 268, 34:25803, 1993). The arthritogenic nature of HC gp-39 was neither described nor suggested in the Hakala et al publication, nor in any other publication.

Proteins of which the amino acid sequence exhibits at least 50% homology with the amino acid sequence of HC gp-39, more in particul;ar with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPDALDRFLCTHIIYSFANISND (SEQ ID NO: 1) have been described. The identification of pig heparine-binding 38kDa protein is described in Shackelton et al. (1995), J.Biol.Chem. Vol. 270, No. 22, 13076-13083, however no function of the protein was identified. The isolation and characterization of bovine 39 kDa whey protein is described in J.J. Rejman et al. (1988), Biochem.Biophys.Res.Comm. Vol. 150, No. 1, 329-334. Murine breast regressing 39kDa protein (brp39) is described in Morrison and Leder, 1994, Oncogene 9, 3417. Cloning of the cDNA encoding human oviduct-specific glycoprotein and the corresponding amino acid sequence is described in Arias et al. (1994), Biology of Reproduction 51, 685-694. Other mammalian oviduct-specific glycoproteins such as murine- and hamster oviduct-specific glycoprotein are disclosed in JP-A-07107979, Kinosei Peptide Kenkyusho KK. The purification and molecular cloning of the bovine oviduct-specific glycoprotein is described in Y. Sendai et al, 1994, Biol. of Reprod. 50, 927-934. Human chitotriosidase precursor protein is secreted by activated human marcophages and the cloning of the corresponding cDNA and amino acid sequence is described in Boot et al. (1995), J.Biol.Chem. Vol. 270, No. 44, 26252-26256. The amino acid sequence of murine secretory protein YM-1 precursor was submitted to EMBL Data Library, June 1992, Accession No. M94584 by Chang et al., unpublished. The amino acid sequence of human chondrocyte protein YKL-39 is described in Hu et al. (1996), J.Biol.Chem. Vol 271, No. 32, 19415-19420. None of the afore-mentioned publications however hint or suggest towards the arthritogenic nature of the proteins according to the invention nor to the fact that these proteins can be used as a medical substance in a therapy to

induce specific T-cell tolerance to HC gp-39 in mammals, more specifically man, suffering from T-cell mediated cartilage destruction, such as for example arthritis, more specifically rheumatoid arthritis.

Thus, according to the invention HC gp-39, and proteins comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence of HC gp-39, more in particular with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPDAL-DRFLCTHIIYSFANISND (SEQ ID NO: 1) are very suitable for use in a therapy to induce specific T-cell tolerance to HC gp-39 in patients suffering from T-cell mediated cartilage destruction, such as for example arthritis, more specifically rheumatoid arthritis. Also within the scope of the invention are fragments of HC gp-39 and the arthritogenic proteins according to the invention, which are able to induce T-cell specific tolerance to the HC gp-39 autoantigen in the cartilage under attack.

WO 95/01995 and WO 95/02188 describe the diagnostic use of HC gp-39 as a marker for RA, however the arthritogenic nature of HC gp-39 is neither disclosed nor suggested. Nowhere do they hint or suggest towards the use of HC gp-39, or fragments thereof or T-cell reactive peptides according to the present invention in the antigen specific therapy to induce T-cell specific tolerance to the HC gp-39 in the cartilage under attack.

HC gp-39, arthritogenic proteins comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence of HC gp-39, more in particular with the amino acid sequence YKLVCYYTSWSQYREGDGSC-FPDALDRFLCTHIIYSFANISND (SEQ ID NO: 1), and fragments derived from said arthritogenic proteins according to the invention can be prepared with the aid of recombinant DNA techniques. For this purpose, a nucleic acid sequence which codes for HC gp-39 or a protein comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence of HC gp-39, more in particular with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPDALDR-FLCTHIIYSFANISND (SEQ ID NO: 1) or a fragment derived from the proteins according to the invention or a multimer of said fragment is inserted into an expression vector. Suitable expression vectors are, amongst others, plasmids, cosmids, viruses and YAC's (Yeast Artificial

Chromosomes) which comprise the necessary control regions for replication and expression. The expression vector can be brought to expression in a host cell. Suitable host cells are, for instance, bacteria, yeast cells and mammalian cells. Such techniques are well known in the art (Sambrook et al., Molecular Cloning: a Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1989).

Using these recombinant DNA techniques bovine whey protein, the sequence of which has been disclosed herein, can be prepared in a host cell.

Thus, the present invention also provides for isolated cDNA encoding the bovine whey protein as indicated by SEQ ID NO: 3 as well as the bovine whey protein according to SEQ ID NO: 2. It will be clear that also fragments can be prepared once given the teachings of the present invention. The term "fragment" refers to any sequence of amino acids that is part of the protein, having common elements of origin, structure and mechanism of action that are within the scope of the present invention.

Suitable fragments derived from the arthritogenic proteins according to the invention can also be prepared by means of one of the known organic chemical methods for peptide synthesis. The organic chemical methods for peptide synthesis are considered to include the coupling of the required amino acids by means of a condensation reaction, either in homogeneous phase or with the aid of a so-called solid phase. The most common methods for the above condensation reactions are: the carbodiimide method, the azide method, the mixed anhydride method and the method using activated esters, such as described in The Peptides, Analysis, Synthesis, Biology Vol. 1-3 (Ed. Gross, E. and Meienhofer, J.) 1979, 1980, 1981 (Academic Press, Inc.).

Preparation of suitable fragments of above-mentioned HC gp-39 and arthritogenic proteins according to the invention using the "solid phase" is for instance described in J. Amer. Chem. Soc. 85:2149 (1963) and Int. J. Peptide Protein Res. 35:161-214 (1990).

A particulary suitable solid phase is, for example, the p-alkoxybenzyl alcohol resin (4-hydroxy-methyl-phenoxy-methyl-copolystrene-1% divinylbenzene resin), described by Wang (1974) J. Am. Chem. Soc. 95:1328. After synthesis the peptides can be split from this solid phase under mild conditions.

After synthesis of the desired amino acid sequence, detaching of the peptide from the resin follows, for example, with trifluoroacetic acid, containing scavengers, for example triisopropyl silane, anisole or ethanedithiol,thioanisol.

The reactive groups which may not participate in the condensation reaction are, as stated effectively protected by groups which can be removed agains very easily by hydrolisis with the aid of acid, base or reduction. A more extensive account of possible protecting groups can be found in The Peptides, Analysis, Synthesis, Biology, Vol. 1 - 9 (Eds. Gross, Udenfriend and Meienhofer) 1979 - 1987 (Academic Press, Inc.). The protective groups can be split off by various conventional methods, depending on the nature of the particular group, for example with the aid of trifluoroacetic acid or by mild reduction, for example with hydrogen and a catalyst, such as palladium, or with HBr in glacial acetic acid.

Although it seems paradoxical to desensitize the immune system with the very same antigen responsible for activating the immune system, this desensitization is based on the long-observed phenomenon that animals which have been fed or have inhaled an antigen or epitope are less capable of developping a systemic immune response towards said antigen or epitope when said antigen or epitope is introduced via a systemic route. Hence, controlled administration of HC gp-39, and/or proteins comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence of HC gp-39, more in particular with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPDALDRFLCTHIIYSFANISND (SEQ ID NO: 1) can be effective in desensitization of the immune system. Fragments of the proteins according to the invention that are capable to desensitize patients against HC gp-39 are also within the scope of the invention.

According to the invention, patients in which the cartilage is under attack of autoresponsive T cells can be treated with a pharmaceutical composition comprising HC gp-39, one or more proteins comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence of HC gp-39, more in particular with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPDALDRFLCTHIIYSFANISND (SEQ ID NO: 1) or one or more fragments derived from a protein according to the invention and a pharmaceutical

acceptable carrier in order to make the specific autoreactive T cells of these patients tolerant to the HC gp-39 in the cartilage under attack and to diminish the inflammatory response.

Suitable proteins to be used in a pharmaceutical composition according to the invention are proteins comprising an amino acid sequence which exhibits at least 70%, preferably 80%, more preferably 90% homology with the amino acid sequence of HC gp-39, more in particular with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPDALDRFLCT-HIIYSFANISND (SEQ ID NO: 1).

Very suitable proteins to be used in a pharmaceutical composition according to the invention are for example pig heparine-binding 38kDa protein, bovine 39 kDa whey protein, murine breast regressing 39 kDa protein (brp39), murine oviduct-specific glycoprotein, hamster oviduct-specific glycoprotein, bovine oviduct-specific glycoprotein, human YKL-39, human oviduct-specific glycoprotein, human chitotriosidase precursos protein and murine secretory proteinYM-1 precursor.

Also very suitable to be used in a pharmaceutical composition according to the invention are DNA (expression)vectors comprising DNA which encodes for HC gp-39 or a protein comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence of HC gp-39, more in particular with the amino acid sequence YKLVCYYTSWS-QYREGDGSCFPDALDRFLCTHIIYSFANISND (SEQ ID NO: 1) or one or more fragments derived from the proteins according to the invention. Upon delivery the DNA (expression)vector can provide by expression a level of the recombinant HC gp-39 or protein comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence of HC gp-39, more in particular with the amino acid sequence YKLVCYYTSWSQYREGDGSCFP-DALDRFLCTHIIYSFANISND (SEQ ID NO: 1) or fragments according to the invention which is similar to the level which would be achieved by direct administration of a pharmaceutical composition comprising the HC gp-39 protein or peptides.

The autoantigen HC gp-39, arthritogenic proteins comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence of HC gp-39, more in particular with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPDALDR-FLCTHIIYSFANISND (SEQ ID NO: 1) and fragments derived from said arthritogenic proteins according to the invention have the advantage that they have a specific tolerizing effect on the

autoreactive T cells thus leaving the other components of the immune system intact as compared to the nonspecific suppressive effect of the immunosuppressive steroid drugs. Treatment with the autoantigen or the proteins comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence of HC gp-39, more in particular with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPDALDRFLCTHIIYSFANISND (SEQ ID NO: 1) or fragments according to the invention will be safe and no toxic side effects will occur.

Tolerance can be attained by administering high or low doses of the autoantigen or proteins according to the invention. The amount of autoantigen or protein will depend on the route of administration, the time of administration, the age of the patient as well as general health conditions and diet.

In general, a dosage of 0.01 to 1000 μg of protein per kg body weight, preferably 0.5 to 500 μg, more preferably 0.1 to 100 μg of protein can be used.

Pharmaceutical acceptable carriers are well known to those skilled in the art and include, for example, sterile salin, lactose, sucrose, calcium phosphate, gelatin, dextrin, agar, pectin, peanut oil, olive oil, sesame oil and water. Other carriers may be, for example MHC class II molecules, if desired embedded in liposomes.

In addition the pharmaceutical composition according to the invention may comprise one or more adjuvants. Suitable adjuvants include, amongst others, aluminium hydroxide, aluminium phosphate, amphigen, tocophenols, monophosphenyl lipid A, muramyl dipeptide and saponins such as Quill A. The amount of adjuvant depends on the nature of the adjuvant itself.

Furthermore the pharmaceutical composition according to the invention may comprise one or more stabilizers such as, for example, carbohydrates including sorbitol, mannitol, starch, sucrosedextrin and glucose, proteins such as albumin or casein, and buffers like alkaline phosphates.

Suitable administration routes are intramuscular injections, subcutaneous injections, intravenous injections or intraperitoneal injections, oral and intranasal administration. Oral and intranasal administration are preferred administration routes.

Due to its arthritogenic nature, HC gp-39 as well as the proteins comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence of HC gp-39, more in particular with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPDALDR-FLCTHIIYSFANISND (SEQ ID NO: 1) can be used to induce clinical arthritis in animals. Upon administration of small amounts of HC gp-39 or one or more proteins comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence of HC gp-39, more in particular with the amino acid sequence YKLVCYYTSWSQYREGD-GSCFPDALDRFLCTHIIYSFANISND (SEQ ID NO: 1) or one or more frgaments of the proteins according to the invention, arthritic signs will develop in said animals resulting in a disease pattern reminiscent of disease progression in arthritis, especially rheumatoid arthritis. When Balb/c mice were injected subcutaneously with HC gp-39 protein or a protein comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence of HC gp-39, more in particular with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPDALDRFLCTHIIYSFANISND (SEQ ID NO: 1), the animals developed arthritic signs. The course of the HC gp-39-induced disease as well as the disease induced by the protein comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence of HC gp-39, more in particular with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPDALDRFLCTHIIYSFANISND (SEQ ID NO: 1) was characterized by relapses occuring periodically in fore paws and/or hind paws and the gradually development from a mild arthritis into a more severe form. Also, a symmetrical distribution of afflicted joints was observed which is, together with the observation of recurrent relapses and nodule formation, reminiscent of disease progression in arthritis, especially RA.

Thus, these afflicted animals provide an adequate animal model to study the mechanism underlying the initiation and progression of arthritic development. Additionally, said afflicted animals can be used to search for new drugs to treat arthritis and to study the effect of these drugs upon the arthritic development. Preferably mice are used as animal model for arthritis, especially rheumatoid athritis.

To induce arthritis in said animals, suitable amounts of HC gp-39 or one or more of the proteins according to the invention have to be administered. Suitable amounts are 0.1-1000 μg, preferably 1-100 μg, more preferably 10-50 μg per kg body weight. The amount of HC gp-39 or proteins comprising an amino acid sequence which exhibits at least 50% homology with the

amino acid sequence of HC gp-39, more in particular with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPDALDRFLCTHIIYSFANISND (SEQ ID NO: 1) or fragments thereof will depend on the route of adminstration, time of administration and the type of animal used. Suitable adminstration routes are the same as described before. To induce the effect of arthritis inducton, the HC gp-39 protein, or proteins comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence of HC gp-39, more in particular with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPDALDRFLC-THIIYSFANISND (SEQ ID NO: 1) or peptides according to the invention may comprise one or more stabilizers or adjuvants as described before.

HC gp-39 , the proteins comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence of HC gp-39, more in particular with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPDALDRFLCTHIIYSFANISND (SEQ ID NO: 1) or fragments thereof according to the invention are also very suitable for use in a diagnostic method to detect the presence of activated autoreactive T cells involved in the chronic inflammation of the articular cartilage.

The diagnostic method according to the invention comprises the following steps:

a) isolation of the peripheral blood mononuclear cells (PBMC) from a blood sample of an individual,

b) culture said PBMC under suitable conditions,

c) incubation of said PBMC culture in the presence of the autoantigen or proteins according to the invention and fragments thereof, and

d) detection of a response of T cells, for example a proliferative response, indicating the presence of activated autoreactive T cells in the individual.

In case of detection of a response by measuring the proliferative response of the autoreactive T cells, the incorporation of a radioisotope such as for example $^{3}$H-thymidine is a measure for the proliferation. A response of the autoreactive T cells present in the PBMC can also be detected by measuring the cytokine release with cytokine-specific ELISA, or the cytotoxicity with $^{51}$Chromium release. Another detection method is the measurement of expression of activation markers by FACS analysis, for example of Il-2R. A diagnostic

composition comprising one or more of the peptides according to the invention and a suitable detecting agent thus forms part of the invention. Depending on the type of dection, the detection agent can be a radioisotope, an enzyme, or antibodies specific for cell surface or activation markers.

Also within the scope of the invention are test kits which comprise one or more peptides according to the invention. These test kits are suitable for use in a diagnostic method according to the invention.

Thus, the present invention provides for a method to detect whether autoaggressive T cells reactive towards HC gp-39 are present in patients suffering from T-cell mediated cartilage destruction such as for example arthritis, in particular rheumatoid arthritis. If HC gp-39-specific T cells are present, tolerization of these T cells with a pharmaceutical compostion comprising HC gp-39 or one or more arthritogenic proteins comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence of HC gp-39, more in particular with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPDALDRFLCTHIIYS-FANISND (SEQ ID NO: 1) or peptides according to the present invention or combinations thereof can delay or suppress arthritis development.

The following examples are illustrative for the invention and should in no way be interpreted as limiting the scope of the invention.

LEGENDS TO THE FIGURES

Figure 1: Initiation and progression of arthritis in HC gp-39 tolerized and non-tolerized Balb/c mice. AS = total arthritic score of the afflicted animals per day following sensitization. N = number of afflicted animals per day following sensitization.

Figure 2: Initiation and progression of arthritis in Balb/c mice upon administration of 0.2 µg, 1 µg, 5 µg and 25 µg bovine 39 kDa whey protein respectively. Initiation/progression is presented as accumulative score (total arthritic score of afflicted animals) per day following sensitization.

## EXAMPLE 1

### METHODS

**Purification of HC gp-39 from the MG63 osteosarcoma cell line**

MG63 cells (human osteosarcoma ATCC CRL 1427) were cultured in cell factories in DMEM/HAM's F12 serum free medium. HC gp-39 was purified from the culture supernatant by heparin affinty chromatography folllowed by super dex 75 chromatography. Purity was checked by SDS-PAGE. In addition, N-terminal amino acid sequencing confirmed that the purified protein was identical to the protein described by Hakala et al.

**Arthritogenicity of HC gp-39 in Balb/c mice**

10 or 50 µg of purified HC gp-39 in a 100 µl volume PBS (0.5 M NaCl, 0.01 M sodium phosphate buffer, pH 7.5) mixed 1:1 in incomplete Freunds adjuvant (IFA) was injected subcutaneously in the chest region in 2 x 4 female Balb/c mice (Harlan CPB, Zeist, The Netherlands) whereas 4 controls were injected with PBS (1:1 in IFA). Mice were examined daily for clinical signs of arthritis. Severity of arthritis was assessed by scoring each paw from 0-3 (according to the article by Glant et al). In short, score 0 = no changes, score 1 = erythema and swelling, score 2 = swelling and appearance of deformities, score 3 = immobility due to loss of flexion and extension.

**Tolerance induction by intranasal administration of HC gp-39**

Twenty eight µg of protein was administered intranasally (2 x 10 µl) to 10 female Balb/c mice (anesthetized lightly with Enflurance) using a PT45 micro conduit and a Hamilton syringe. Antigen was administered on day -15, -10 and -5 prior to arthritis induction. Controls (n = 10) were submitted to the same procedure but recieved the vehicle (PBS) only (Table 1).

Immunological tolerance was evaluated by measuring delayed type hypersensitivity (DTH) responses following sensitization as described above on day 0, using 10 µg of protein. Sensitization on day 0 was followed by an injection of 10 µg HC gp-39 in 50 µg volume in the left hind footpad on day 8 (challenge). DTH reactions were measured as the increase in footpad ([swelling left (mm x $10^{-3}$) -swelling right (mm x $10^{-3}$)]/swelling right (mm x $10^{-3}$)) x 100%.

The footpad swelling was measured using an inhouse designed μmeter at 0, 24 and 48 hr after challenge.

Tolerance to arthritis induction in these same mice was then further monitored until day 31 following sensitization and mice were examined daily for clinical signs. Severity of arthritis was assessed as mentioned above.

*Table 1. Tolerization scheme.*

| DAY | HC gp-39 tolerized | non-tolerized |
|---|---|---|
| -15 | 28 μg HC gp-39 intra nasal | PBS |
| -10 | 28 μg HC gp-39 intra nasal | PBS |
| -5 | 28 μg HC gp-39 intra nasal | PBS |
| 0 | 10 μg HC gp-39 subcutaneous | 10 μg HC gp-39 subcutaneous |
| 8 | 10 μg HC gp-39 footpad | 10 μg HC gp-39 footpad |
| 9 | 24 hour DTH | 24 hour DTH |
| 10 | 48 hour DTH | 48 hour DTH |
| 0-31 | score arthritic signs | score arthritic signs |

*DTH = delayed type hypersensitivity. PBS = 0.5 M NaCl, 0.01 M.sodium phosphate buffer, pH 7.5*

RESULTS

**Arthritogenicity of HC gp-39**

Following one injection of 50 μg HC gp-39 mixed with IFA, all mice gradually developed a severe arthritis (Table 2). Signs of arthritis were observed first at day 15-20 after sensitization, in the fore paws of 3 out of 4 animals. The mouse that did not show any signs in the fore paws developed arthritis in the hind paws by day 34 upon sensitization. The course of HC gp-39-induced disease was characterized by relapses occuring periodically in fore paws or hind paws and gradually develloped from mild arthritis into a more severe arthritis (disease progression was followed for 62 days). Very often (>50%) a symmetrical distribution of afflicted joints was observed, meaning that both fore paws or both hind paws showed arthritic signs at the same time.

Arthritis induction was similarly achieved using 10 µg instead of 50 µg HC gp-39 mixed with IFA. Arthritic scores, however, were somewhat lower (data not shown). Three out of 4 mice developed a severe arthritis. One mouse showed only mild signs during the duration of the experiment. All through the length of the experiment control mice showed no signs of arthritis.

In aggregate, both 10 and 50 µg of protein were sufficient to induce a progressive arthritis in Balb/c mice. The chronic nature of arthritis induction by HC gp-39, characterized by recurrent relapses in addition to symmetrical affliction of joints is reminiscent of disease progression in rheumatoid arthritis (RA).

*Table 2. Initiation and progression of arthritis in HC gp-39 sensitized Balb/c mice.*

| sensitization (n = 4) | arthritis onset (day) | | arthritis signs | | |
|---|---|---|---|---|---|
| | FP | HP | none | mild | severe |
| PBS contr. | - | - | 4 | 0 | 0 |
| 10 µg HC gp-39 | -, 13, 15, 57 | 32, 34, 36, 43 | 0 | 1 | 3 |
| 50 µg HC gp-39 | 13, 15, 15, 48 | 29, 34, 34, 53 | 0 | 0 | 4 |

*FP = fore paw. HP = hind paw. contr. = controls. Arthritis signs: none: score = 0, mild: score per animal does not exceed 2, severe: score per animal is ≥ 3.*

**Immunological tolerance measured in the DTH assay**

Control mice injected with HC gp-39 at day 0 showed a strong, antigen-specific DTH response, which suggests that a cellular immune response to HC gp-39 was elicited upon sensitization (Table 3). Intranasal administration of HC gp-39, however, completely abrogated DTH responses upon challenge with the autoantigen, thereby showing that the HC gp-39-specific T-cells were indeed tolerized.

Notably, 4 out of 10 animals of the non-tolerized group developed arthritis in the ankle adjacent to the site of challenge. In contrast, the tolerized group did not develop an arthritis in the joints neighbouring the challenged site, thereby suggesting that immunological tolerance to HC gp-39 results in protection against arthritis development.

*Table 3. DTH responses to HC gp-39 following tolerization by nasal administration.*

| teatment | mean % swelling | | | arthritis ankle |
|---|---|---|---|---|
| | 0 hr | 24 hr | 48 hr | |
| controls (n = 10) | -1.3 | 31.4 | 38.6 | 4/10 |
| tolerized (n = 9) | -0.05 | 3.7 | 1.5 | 0/9 |

**Tolerance to arthritis induction or progression**

The HC gp-39 tolerized and the non-tolerized Balb/c mice were then further monitored for initiation and progression of arthritis.

In all mice of the control (non-tolerized) group, disease was initiated upon sensitization with HC gp-39 (Table 4). Seven mice gradually developed a severe arthrtis whereas three mice showed only mild signs (highest score 2). In contrast, five mice of the HC gp-39-tolerized group were protected against disease development during the course of the experiment. Furthermore, two animals of the tolerized group showed only mild signs for brief periods of time. Three animals developed a more severe arthritis with scores of 4.

Interestingly, in tolerized animals, arthritis onset was delayed in both hind and fore paws by a minimum of 7-9 days respectively (Figure 1). Although fewer animals were affected in the tolerized group, the arthritic score per animal of the fore paws was comparabe to the arthritic score in the non-tolerized animals. The arthritic score per animal of the hind paws however, was somewhat lower in the tolerized animals (Figure 1).

*Table 4. Initiation and progression of arthritis in HC gp-39-tolerized and non-tolerized Balb/c mice.*

| animals | arthritis onset (day) | arthritis signs | | |
|---|---|---|---|---|
| | | none | mild | severe |
| controls (n = 10) | 13-16 | 0 | 3 | 7 |
| tolerized (n = 10) | 23-24 | 5 | 2 | 3 |

*Onset arthritis: first signs appear - highest number of animals affected. Arthritis signs: no signs: score = 0, mild: score per animal does not exceed 2, severe: score per animal is $\geq 4$.*

The experiments above demonstrate the arthritogenic nature of HC gp-39 in Balb/c mice. The course of HC gp-39-induced disease was characterized by relapses occuring periodically in fore paws and/or hind paws and gradually developed from a mild arthritis into a more severe form. Also, a symmetrical distribution of afflicted joints was observed which is together with the observation of recurrent relapses, reminiscent of disease progression in rheumatoid arthritis. The fierce arthritogenic nature of HC gp-39 was illustrated by a single, subcutaneous injection of 10 or 50 µg of protein which initiated arthritic signs in all animals. That HC gp-39 specific T cells are indeed elicited in response to HC gp-39 sensitization was shown by induction of HC gp-39-specific DTH responses. These data were further confirmed by the demonstration of HC gp-39-specific in vitro proliferative responses in animals immunized in the footpad with HC gp-39 (data not shown). Importantly, non-tolerized animals developed arthritis in the ankle neighbouring the injection site, thereby indeed suggesting an involvement of HC gp-39-specific T cells in arthritis induction.

Intranasal administration of peptide antigen has been used to induce antigen-specific immune tolerance. The experiments showed that intranasal administration of HC gp-39 leads to immunological non-responsiveness. DTH responses following sensitization were completely abrogated in HC gp-39-tolerized mice whereas control mice showed an antigen-specific swelling. These observations indicate that adminstration of HC gp-39 leads to peripheral immune tolerance.

In non-tolerized animals DTH responses were accompanied by arthritis in the ankle (adjacent to the challenged site) in four out of ten mice. In contrast, the ankles of HC gp-39-tolerized animals were indeed fully protected, thereby suggesting that autoreactive T-cells have been effectively silenced. The notion that tolerization with HC gp-39 protects against disease developement was taken further by the observation that 5 out of 10 animals in the tolerized group were entirely protected throughout the length of the experiment. Although the other five animals in the group did eventually develop clinical signs, the onset of arthritis was considerably delayed. Hence it can be concluded that HC gp-39-specific T cells are involved in the arthritogenic process and more importantly, that by tolerization of these T cells with a pharmaceutical composition according to the present invention arthritis development can be delayed or suppressed.

## EXAMPLE 2

## METHODS

**Arthritogenicity of Bovine 39 kDa whey protein in Balb/c mice**

0.2, 1, 5 or 25 µg of purified Bovine 39 kDa whey protein in a 100 µl volume (0.5 M NaCl, 0.01M sodium phosphate buffer, pH 7.5) mixed 1:1 in incomplete Freunds adjuvant (IFA) was injected subcutaneously in the chest region in 4 x 10 female Balb/c mice (Charles River, Sulzfeld, Germany) whereas 10 controls were injected with PBS (1:1 in IFA). Mice were examined every other day for clinical signs of arthritis. Severity of arthritis was assessed by scoring each paw from 0-3 (according to the article by Glant et al). In short, score 0 = no changes, score 1 = erythema and/or mild swelling, score 2 = severe swelling and/or appearance of deformities, score 3 = immobility due to loss of flexion and extension.

**Cloning of the bovine whey gp39 cDNA**

A Cow mammary gland Lambda Bluemid(-) cDNA library (oligo dT and random-primed, custom made by Clontech Laboratories Inc.) was amplified using the XL1 Blue MRF strain as a host and library plating and preparation of filter replicas were performed according to the manufacturers instructions. Filters were hybridised with a PvuII fragment from HC gp-39 cDNA after $^{32}P$ labelling using an oligolabelling kit (Pharmacia). Positive plaques were amplified, rescreened and insert DNA of the corresponding phages were sequenced using a Thermosequenase kit (Amersham). The sequence is indicated in SEQ ID NO: 3. At nucleotide position 954 one clone contained a C whereas another contained a T.

## RESULTS

**Arthritogenicity of Bovine 39 kDa whey protein**

Following one injection of 25 µg Bovine 39 kDa whey protein mixed with IFA, all mice gradually developed arthritis (Table 5). Signs of arthritis were observed first at 8-20 days after sensitization in fore- or hind paws. The course of Bovine 39 kDa whey protein-induced arthritis was characterized by relapses occurring periodically in hind paws and gradually developed from

mild arthritis into a more severe arthritis (disease progression was followed for 70 days). Very often a symmetrical distribution of afflicted joints was observed, meaning that both fore paws or both hind paws showed arthritic signs at the same time.

Arthritis induction was similarly achieved using 5, 1 or 0.2 instead of 25 µg Bovine 39 kDa whey protein mixed with IFA.

Mice induced with either 1 or 0.2 µg Bovine 39 kDa whey protein developed a more mild form of arthritis: 7 and 6 mice respectively developed a mild arthritis, whereas only 3 and 4 mice developed severe arthritis.

At the end of the experiment, cumulative scores per scoring day for each group of mice were calculated (Figure 2). Following immunization with 25µg Bovine 39 kDa whey protein, a maximum score of 27 (25 µg) was reached after 30 days, followed by distinct relapses. When lower dosages of Bovine 39 kDa whey protein were used for immunization, animals showed a somewhat lower arthritis score compared with mice induced with the 25µg dose. Although lower scores were achieved, a relapsing pattern of arthritis was found in all groups.

In control, PBS treated mice sometimes a slight and transient swelling of hind paws was observed. This phenomenon was also observed in naive, non-injected animals. This mean cumulative swelling per scoring day for these groups of mice had a mean value of 3.4 and was considered as biological background variation.

In conclusion, Bovine 39 kDa whey protein (both 25, 5, 1 and 0.2 µg) is capable to induce a progressive arthritis in female Balb/c mice. The chronic nature of arthritis induction by Bovine 39 kDa whey protein, characterized by recurrent relapses in addition to symmetrical affliction of joints, is reminiscent of disease progression in rheumatoid arthritis (RA).

*Table 5: Initiation and progression of arthritis in Bovine 39 kDa whey protein sensitized Balb/c mice.*

| sensitization (n=10) | arthritis onset (day) | | arthritis signs | | |
|---|---|---|---|---|---|
| | FP | HP | none | mild | severe |
| 0.2 µg B 39 kDa whey protein | -,-,-,-,-, -,-,-,14,18 | 8,14,14,14,18, 18,18,18,30,54 | 0 | 6 | 4 |
| 1 µg B 39 kDa whey protein | -,-,-,-,-, 8,12,12,16,18 | 8,8,8,8,8, 8,14,16,18,18 | 0 | 7 | 3 |
| 5 µg B 39 kDa whey protein | -,-,-,-,-, 8,10,10,12,14 | 6,6,8,10,10 16,16,18,20,30 | 0 | 0 | 10 |
| 25 µg B 39 kDa whey protein | -,-,-,-,-, -,-,-,-,12 | 8,8,10,12,12 14,14,18,18,20 | 0 | 1 | 9 |

*FP = fore paw, HP = hind paw. Arthritis signs: none: score = 0, mild: score per animal does not exceed 2, severe: score per animal ≥ 3.*

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: AKZO NOBEL N.V.
(B) STREET: Velperweg 76
(C) CITY: Arnhem
(E) COUNTRY: The Netherlands
(F) POSTAL CODE (ZIP): 6824 BM
(G) TELEPHONE: 0412 666376
(H) TELEFAX: 0412 650592
(I) TELEX: 37503 akpha nl

(ii) TITLE OF INVENTION: Novel peptides derived from autoantigen for use in immunotherapy of autoimmune diseases

(iii) NUMBER OF SEQUENCES: 3

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Tyr Lys Leu Val Cys Tyr Tyr Thr Ser Trp Ser Gln Tyr Arg Glu Gly
1 5 10 15

Asp Gly Ser Cys Phe Pro Asp Ala Leu Asp Arg Phe Leu Cys Thr His
20 25 30

Ile Ile Tyr Ser Phe Ala Asn Ile Ser Asn Asp
35 40

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 383 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Gly Leu Arg Ala Ala Gln Thr Gly Phe Val Val Leu Val Leu Leu
1 5 10 15

Gln Ser Cys Ala Ala Tyr Lys Leu Ile Cys Tyr Tyr Thr Ser Trp Ser
20 25 30

Gln Tyr Arg Glu Gly Asp Gly Ser Cys Phe Pro Asp Ala Ile Asp Pro
35 40 45

Phe Leu Cys Thr His Val Ile Tyr Ser Phe Ala Asn Ile Ser Asn Asn
50 55 60

Glu Ile Asp Thr Trp Glu Trp Asn Asp Val Thr Leu Tyr Asp Thr Leu
65 70 75 80

Asn Thr Leu Lys Asn Arg Asn Pro Asn Leu Lys Thr Leu Leu Ser Val
85 90 95

Gly Gly Trp Asn Phe Gly Ser Gln Arg Phe Ser Lys Ile Ala Ser Lys
100 105 110

Thr Arg Ser Arg Arg Thr Phe Ile Lys Ser Val Pro Pro Phe Leu Arg
115 120 125

Thr His Gly Phe Asp Gly Leu Asp Leu Ala Trp Leu Tyr Pro Gly Trp
130 135 140

Arg Asp Lys Arg His Leu Thr Thr Leu Val Lys Glu Met Lys Ala Glu
145 150 155 160

Phe Val Arg Glu Ala Gln Ala Gly Thr Glu Gln Leu Leu Leu Ser Ala
165 170 175

Ala Val Pro Ala Gly Lys Ile Ala Ile Asp Arg Gly Tyr Asp Ile Ala
180 185 190

Gln Ile Ser Arg His Leu Asp Phe Ile Ser Leu Leu Thr Tyr Asp Phe
195 200 205

His Gly Ala Trp Arg Gln Thr Val Gly His His Ser Pro Leu Phe Arg
210 215 220

Gly Gln Glu Asp Ala Ser Ser Asp Arg Phe Ser Asn Ala Asp Tyr Ala
225 230 235 240

Val Ser Tyr Met Leu Arg Leu Gly Ala Pro Ala Asn Lys Leu Val Met
245 250 255

Gly Ile Pro Thr Phe Gly Arg Ser Tyr Thr Leu Ala Ser Ser Lys Thr
260 265 270

Asp Val Gly Ala Pro Ile Ser Gly Pro Gly Ile Pro Gly Gln Phe Thr
275 280 285

Lys Glu Lys Gly Ile Leu Ala Tyr Tyr Glu Ile Cys Asp Phe Leu His
290 295 300

Gly Ala Thr Thr His Arg Phe Arg Asp Gln Gln Val Pro Tyr Ala Thr
305 310 315 320

Lys Gly Asn Gln Trp Val Ala Tyr Asp Asp Gln Glu Ser Val Lys Asn
325 330 335

Lys Ala Arg Tyr Leu Lys Asn Arg Gln Leu Ala Gly Ala Met Val Trp
340 345 350

Ala Leu Asp Leu Asp Asp Phe Arg Gly Thr Phe Cys Gly Gln Asn Leu
355 360 365

Ala Phe Pro Leu Thr Ser Ala Ile Lys Asp Val Leu Ala Glu Val
370 375 380

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1152 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ATGGGGCTGA GGGCGGCTCA GACAGGTTTT GTGGTCCTGG TGCTGCTCCA GAGCTGTGCT 60

GCATACAAGC TGATCTGCTA CTACACCAGC TGGTCCCAGT ACCGGGAGGG TGATGGGAGC 120

TGCTTCCCAG ACGCCATCGA CCCCTTCCTG TGCACCCATG TCATCTACAG CTTTGCCAAC 180

ATAAGCAACA ATGAGATCGA CACCTGGGAG TGGAATGACG TGACGCTCTA TGACACACTG 240

AACACACTCA AGAACAGGAA CCCCAACCTG AAGACCCTCC TATCTGTTGG AGGATGGAAC 300

TTCGGTTCTC AAAGATTTTC CAAGATAGCT TCCAAGACCC GGAGTCGCAG GACTTTCATC 360

AAGTCGGTGC CACCATTTCT GCGGACCCAT GGCTTTGATG GACTGGACCT AGCATGGCTC 420

TACCCCGGGT GGAGAGACAA GCGGCATCTC ACCACTCTGG TCAAGGAAAT GAAGGCTGAG 480

TTTGTAAGGG AAGCCCAAGC AGGCACAGAG CAGCTTCTGC TCAGTGCAGC AGTACCTGCA 540

GGGAAGATTG CTATTGACAG AGGCTATGAC ATCGCCCAGA TATCCCGACA CCTGGACTTC 600

ATCAGCCTTT TGACCTATGA CTTTCACGGA GCCTGGCGCC AGACAGTCGG ACACCACAGC 660

CCCCTGTTTC GAGGCCAGGA AGATGCAAGT TCTGACAGAT TCAGTAACGC TGACTACGCT 720

GTGAGCTACA TGCTGAGGCT GGGGGCTCCA GCCAATAAGC TGGTGATGGG TATCCCCACT 780

TTTGGGAGGA GCTACACTCT GGCCTCTTCC AAGACAGATG TGGGAGCCCC CATCTCAGGG 840

CCAGGAATTC CAGGCCAGTT CACCAAGGAG AAAGGGATCC TTGCCTATTA TGAGATCTGT 900

GACTTCCTCC ACGGAGCCAC CACCCACAGA TTCCGTGACC AGCAGGTCCC CTATGCCACC 960

AAGGGCAACC AGTGGGTGGC GTATGACGAC CAGGAGAGTG TCAAAAACAA GGCACGGTAC 1020

CTGAAGAACA GGCAGCTGGC TGGCGCCATG GTGTGGGCCC TGGACTTGGA TGACTTCCGG 1080

GGCACCTTCT GTGGGCAGAA CCTGGCCTTT CCTCTCACAA GTGCCATCAA AGATGTGCTT 1140

GCTGAGGTGT AG 1152

**Claims**

1. Protein comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPDALDRFLCTHIIYS-FANISND (SEQ ID NO: 1) for use as a therapeutical substance.

2. Use according to claim 1, characterized in that said protein is bovine 39 kDa whey protein or human YKL-39 protein.

3. Pharmaceutical composition comprising one or more proteins comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPDALDRFLCTHIIYSFANISND (SEQ ID NO: 1), and a pharmaceutical acceptable carrier.

4. Parmaceutical composition according to claim 3, characterized in that said protein is bovine 39 kDa whey protein or human YKL-39 protein.

5. Use of the protein comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPDALDR-FLCTHIIYSFANISND (SEQ ID NO: 1) for the manufacture of a pharmaceutical preparation for the induction of specific T-cell tolerance to the homologous autoantigen in mammals suffering from T-cell mediated cartilage destruction.

6. Use according to claim 5, characterized in that said protein is bovine 39 kDa whey protein or human YKL-39 protein.

7. One or more proteins comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPDALDR-FLCTHIIYSFANISND (SEQ ID NO: 1) for use in a method to induce arthritis in animals, preferably mice.

8. A method according to claim 7, characterized in that said protein is bovine 39 kDa whey protein or human YKL-39 protein.

9. Animals which suffer from arthritis, whereby said arthritis was induced by the method according to claim 7 or 8.

10. Use of the animals according to claim 9 for use in the screening of novel drugs for the treatment of arthritis, especially rheumatoid arthritis.

11. Protein having the amino acid sequence of SEQ ID NO: 2.

12. Isolated DNA encoding the amino acid sequence according to claim 11.

13. Isolated DNA according to claim 12 having the DNA sequence of SEQ ID NO: 3.