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(54) Titre : INHIBITEUR ANTAGONISTE DE LA CROISSANCE DES CELLULES MESENCHYMATEUSES
(54) Title: PROLIFERATION ANTAGONISTIC INHIBITOR OF MESENCHYMAL CELLS

(57) Abrégé/Abstract:
The invention provides an antagonistic inhibitor of the prolifertion of mesenchymal cells, which contains the AP–1 nucleotide comprising TGAGTCA or TGACTCA which is a part of a gene promoter, as a therapeutic agent of collagen diseases including rheumatoid arthritis.

ABSTRACT OF THE DISCLOSURE

The invention provides an antagonistic inhibitor of the prolifertion of mesenchymal cells, which contains the AP-1 nucleotide comprising TGAGTCA or TGACTCA which is a part of a gene promoter, as a therapeutic agent of collagen diseases including rheumatoid arthritis.

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ANTAGONISTIC INHIBITOR FOR THE PROLIFERATION OF MESENCHYMAL CELLS

TECHNICAL FIELD

The present invention relates to the proliferation antagonistic inhibitor of mesenchymal cells and a therapeutic method for collagen diseases by using thereof. In particular, the present invention relates to a novel therapeutic agent useful for the inhibition of the binding of c-Fos protein onto genes thereby inhibiting development of rheumatic diseases, and a therapeutic method using such agent.

BACKGROUND ART

Collagen diseases meaning a group of diseases mainly taking the form of inflammation of connective tissue in a human body has conventionally been known throughout the world as intractable diseases of which it was difficult to clarify the causes and to be successful in therapeutic treatment.

Collagen diseases are believed to include rheumatoid arthritis, rheumatic fever, polymyositis, scleroderma, and the like.

In spite of extensive investigation worldwide, effective therapeutic means have not as yet been found.

Although rheumatoid arthritis, for example, shows a high morbidity rate as 0.3% of the population, it is still one of intractable diseases of which the cause is not as yet known. Therapeutic agents exhibiting a remarkable effect have not as yet been developed, such that the therapeutic level is still on an empirical one. Such current

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circumstances are considered attributable at least partially to the fact that the present development efforts are not always properly based on results of the most advanced research on the cause of diseases.

In rheumatoid arthritis, proliferation of synovial cells in the joints is abnormally accelerated by any of various inflammatory agents, leading to joint destruction. Conventional research and development efforts of therapeutic agents have however been made from the point of view of inhibiting these inflammatory agents (interrheukine-1, adhesion molecules on the vessel), however an effective one is not as yet available.

However, previous studies have shown that rheumatoid arthritis proceeds from joint inflammation to joint destruction and then joint deformation, and although the cause is unknown, demonstrated the following disease conditions:

(1) Some antigen (bacterium or virus) reaches the joint and evokes inflammation in the joint;

(2) Arthritis becomes chronic;

(3) This leads to joint destruction.

Becoming chronic as mentioned under (2) is due to abnormal acceleration of proliferation of synovial cells in the joint.

Synovial cell proliferation is considered to be closely associated with the increase of substances (cytokine, etc.) that stimulate synovial cells.

The process to rheumatoid arthritis conceived in the present studies on causes of rheumatism and other research and development efforts of a therapeutic agent is that first a local immune reaction is started by an unknown etiologic

agent having reached the joint synovial membrane from circulation, then, the infiltration of macrophages, T cells, B cells and neutrophils from blood results in complicated chronic inflammation which finally leads to joint destruction. Important factors participating in this process include specific recognition systems such as T cells which are responsible for immunological memory of specific response to antigen and B cells under the control thereof, as well as nonspecific systems such as cytokines and adhesion molecules broadly associated with chronic inflammation, autoantibodies (rheumatoid factors), and proteases, and the conventional study efforts have been made to inhibit them. Recently in particular, investigators in the world have been competing to inhibit the action of cytokines and adhesion molecules. Current research indicates that, although the immune response system mainly comprising T cells surely constitutes a major portion of the causes of rheumatoid arthritis, what "directly" participate in joint destruction are at least those cells known as nonspecific synovial mesenchymal cells.

Attention given to this mesenchymal cells is noted as a point of view not only toward rheumatoid arthritis, but also toward collagen disease itself.

In spite of the progress in research, however, an effective therapeutic agent or an effective therapeutic method has not as yet been established at present regarding collagen diseases including rheumatoid arthritis.

The present invention is therefore an object to provide a novel therapeutic agent that overcomes previous technical limits as described above and is effective for therapy of collagen diseases including rheumatoid arthritis, and a

therapeutic method using such agent.

DISCLOSURE OF INVENTION

As means to solve the above-mentioned problems, the present invention provides an antagonistic inhibitor of the proliferation of mesenchymal cells, which contains the AP-1 nucleotide comprising TGAGTCA or TGACTCA which is a part of gene promoter.

The present invention provides also a therapeutic method for collagen diseases including rheumatoid arthritis, with the method using the nucleotides containing the AP-1 nucleotide sequence as mentioned above.

BEST MODE FOR CARRYING OUT THE INVENTION

The present invention mentioned above is basically to prevent expression of causes of diseases, for example, joint destruction and the like by inhibiting synovial mesenchymal cells. More specifically, the present invention aims at achieving successful therapy, for rheumatoid arthritis, for example, by inhibiting synovial mesenchymal cells directly related with joint destruction accurately but without entering a complicated bush of T cell responses. This concept is unique to the present inventor based on the results of his study on causes of diseases and is unprecedented in the world at present.

More particularly, in arthritic lesions of rheumatoid arthritis, the T cell-centered immune response responsible for immunological memory and the synovial mesenchymal cell "directly" participating in joint destruction are two basically important factors (Shiozawa et al., Ann. Rheum. Dis.51: 869, 1992). The latter is a major component of pannus and produces cytokines such as IL1, IL6 and TNFα

that are important in synovial lesion of rheumatoid arthritis (Shiozawa et al., Sem. Arthritis Rheum. 21: 267, 1992). While pannus has a proliferation potency just like a tumor, a joint destruction is induced by synovial mesenchymal cells alone without local infiltration of lymphocyte when actually causing experimental arthritis (antigen-induced arthritis) in H2-c-fos transgenic mice prepared by recombination of c-fos gene, a protooncogene, in the downstream of H-2 promoter (Shiozawa et al., J. Immunol. 148: 3100, 1992). The c-fos gene imparts an unique transformation of synovial cells (transformation from dendritic cell to fibroblastic cell) and a proliferation potency (Kuroki, Shiozawa et al., J. Rheumatol. 20: 422, 1993). This c-fos gene is expressed in a large quantity in rheumatoid arthritis synovial membrane, and continuous expression of the human c-fos gene in osteoblast by the transfection method inhibits syntheses of type I collagen and expressions of mRNA (Kuroki, Shiozawa et al., BBRC 182: 1389, 1992).

These facts suggest that over-expression of c-fos gene not only participates in "direct" joint destruction through stimulation of the proliferation of synovial mesenchymal cells, but also participates, as a cause, in osteoporosis of rheumatoid arthritis.

The present invention was developed on the basis of the findings that c-fos gene activates particularly mesenchymal cells, as a result of examination of the mechanism of action thereof.

More specifically, the present invention is based on a premise that c-Fos protein acts on the AP-1 site in the promoter of pathogenic gene of rheumatoid arthritis, thereby

adjusting gene expression. This promoter has a structure represented by the following formula:

5' - GTG TTA CCC TGA GTC AGA GGA GAA - 3'

3' - AAT GGG ACT CAG TCT CCT CTT GGG - 5

and the AP-1 site means TGA GTC A underscored in the formula. Of the two strands of the double-stranded DNA, only one is read upon synthesis of protein, and c-Fos protein complexes bind to the both.

In the present invention, therefore, the AP-1 nucleotide comprising TGAGTCA or TGACTCA which is a part of gene promoter as described above is used as an antagonistic inhibitor of the proliferation of mesenchymal cells.

This permit very effective inhibition of the expression of rheumatoid arthritis and the like. It is needless to mention that the AP-1 nucleotide mentioned above may be used in combination with any of various other additional conditions.

The present invention will now be described further in detail by means of examples.

EXAMPLES

DBA 1/J male mice were subcutaneously immunized with FCA and 200 μg of type II collagen twice at a time interval of three weeks, and after two weeks from the initial immunization, 5 μg of double-stranded AP-1 nucleotide each was intraperitoneally administered at a rate of twice per week. A nonspecific double-stranded nucleotide was used as a control, and upon three weeks from the completion of the second immunization, the foot joints were subjected to a pathologic histological examination.

As a result, the hyperplasta of foot pad (>3.7 mm) was

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6/14 cases (43%) for the treated group and 12/16 cases (75%) for the control group. Cases in which a histologically remarkable inflammatory cell infiltration was observed was 7/14 cases (50%) for the treated group, and 8/16 cases (50%) for the control group. The number of cases in which subjects had arthritis without damage was 12/14 cases (86%) for the treated group and 2/16 cases (13%) for the control group. The change in the body weight of mice between before and after experiment was 151% for the treated group and 144% for the control group: no difference was observed between the two groups. In a synovial culture system in vitro, while the administered AP-1 inhibited the expression system such as interleukin-1 and the like acting through AP-1 site, had no effect on gene expressions not passing through an AP-1 site: the specificity thereof was thus confirmed.

As is clear from the above-mentioned results, the administered AP-1 nucleotide significantly inhibited joint destruction of collagen-induced arthritis in the mice. In contrast to the significant inhibition of joint destruction, there was no difference in the extent of inflammatory cell infiltration to local legions of joints between the two groups. This result suggests that, in arthritis, inflammatory cell in infiltration do not necessarily directly participates in joint destruction. This result is of interest, agreement with the result about the role of synovial mesenchymal cells in joint destruction with synovial mesenchymal cells in joint destruction with H2-c-fos transgenic mice.

INDUSTRIAL APPLICABILITY

According to the present invention, as described above

in detail, it is possible to treat collagen diseases particularly rheumatoid arthritis, thus permitting great expectation in clinical application. It is expected to apply the present invention for the clarification of mechanism and causes of rheumatoid arthritis, other chronic inflammatory diseases and intractable diseases of which proliferation of the cells of mesenchymal origin plays an important role in the pathogenesis and therapeutic application therefor.

WHAT IS CLAIMED IS:

1. An antagonistic inhibitor of the proliferation of mesenchymal cells, which contains the AP-1 nucleotide comprising TGAGTCA or TGACTCA which is part of a gene promoter.

2. A therapeutic agent for collagen disease comprising the antagonistic inhibitor according to claim 1.

3. The therapeutic agent of claim 2, wherein said collagen disease is rheumatoid arthritis.

4. Use of the antagonistic inhibitor of claim 1 for the manufacture of a medicament for the treatment of a collagen disease.

5. The use as claimed in claim 4, wherein said collagen disease is rheumatoid arthritis.