DESCRIPTION

THERAPEUTIC AGENT FOR HEART DISEASE

Technical Field

The present invention relates to agents for treating myocardial infarction which comprise an IL-6 inhibitor as an active ingredient and uses thereof. Furthermore, the present invention relates to agents for suppressing left ventricular remodeling after myocardial infarction, which comprise an IL-6 inhibitor as an active ingredient and uses thereof.

Background Art

Myocardial infarction is one of ischemic heart diseases. It is a disorder that causes myocardial necrosis where constriction of cardiac coronary artery occurs due to arteriosclerosis and such, and the bloodstream of the coronary artery becomes dramatically reduced or stopped. The expansion and/or deterioration of infarcted area cause complications such as heart failure and/or ischemia-induced severe arrhythmia, and increase threat to life.

As myocardial infarction progresses, myocardial cells in infarcted areas die and/or slough off, and are displaced with fibrous tissues such as collagen fiber. Such infarcted area lacks contractility, and fails to withstand the intracardiac pressure that rises with cardiac contraction, and then the fibrous wall extends thinly. As a result, to compensate for the hypofunction, hypertrophy of the endocardial cavity in non-infarcted area and dilatation of the whole left ventricle are induced. This phenomenon is called left ventricular remodeling and is known to further decrease the cardiac function and increase the morbidity and mortality thereafter. Therefore, for improving prognosis of myocardial infarction, it is considered important to suppress the progression of left ventricular remodeling as early as possible, and development of effective treatment methods is desired.

IL-6 is a cytokine called B-cell stimulating factor 2 (BSF2) or interferon β2. IL-6 was discovered as a differentiation factor involved in the activation of B-cell lymphocytes (Non-Patent Document 1), and was later revealed to be a multifunctional cytokine that influences the function of various cells (Non-Patent Document 2). IL-6 has been reported to induce maturation of T lymphocyte cells (Non-Patent Document 3).

IL-6 transmits its biological activity via two kinds of proteins on the cell. One of the proteins is the IL-6 receptor which is a ligand binding protein to which IL-6 binds and has a molecular weight of about 80 kDa (Non-Patent Documents 4 and 5). In addition to a membrane-bound form that penetrates and is expressed on the cell membrane, the IL-6 receptor is present as a soluble IL-6 receptor which mainly consists of the extracellular region of the

membrane-bound form.

The other is the membrane protein gp130 which has a molecular weight of about 130 kDa and is involved in non-ligand binding signal transduction. The biological activity of IL-6 is transmitted into the cell through formation of the IL-6/IL-6 receptor complex by IL-6 and IL-6 receptor and binding of the complex with gp130 thereafter (Non-Patent Document 6).

IL-6 inhibitors are substances that inhibit the transmission of IL-6 biological activity. Until now, antibodies against IL-6 (anti-IL-6 antibodies), antibodies against IL-6 receptors (anti-IL-6 receptor antibodies), antibodies against gp130 (anti-gp130 antibodies), IL-6 variants, partial peptides of IL-6 or IL-6 receptors, and such are known.

There are several reports regarding the anti-IL-6 receptor antibodies (Non-Patent Documents 7 and 8; and Patent Documents 1-3). A humanized PM-1 antibody, which had been obtained by transplanting into a human antibody, the complementarity determining region (CDR) of mouse antibody PM-1 (Non-Patent Document 9), which is one of anti-IL-6 receptor antibodies, is known (Patent Document 4).

Until now, it has been suggested that IL-6 affects the function and structure of the heart in view of the facts that it negatively influences the myocontractility (Non-Patent Document 10), that cardiac hypertrophy develops in mice in which gp130 is constantly activated due to overexpression of IL-6 and IL-6 receptors (Non-Patent Document 11), and so on. After myocardial infarction, IL-6 is expressed in the left ventricle, in particular, in the border zone of reperfused myocardial infarction (Non-Patent Document 12), and the expression level is related to the size of the left ventricular (LV) after myocardial infarction (Non-Patent Document 13). Furthermore, it has been reported that myocardial cells generate IL-6 under low oxygen stress (Non-Patent Document 14), and that cytokine expression in non-muscular cells during the post-infarction remodeling plays a regulating role in the changes of extracellular matrix (Non-Patent Document 15). Moreover, regarding the relation between myocardial infarction and IL-6, the JAK/STAT system activated via IL-6 is reported to act protectively on myocardial infarction (Non-Patent Document 16).

On the other hand, according to an experiment using IL-6 knockout mice, it is reported that IL-6 deficiency had no influence on the size of infarcted area, left ventricular remodeling, or such (Non-Patent Document 17). As described above, the role of IL-6 in myocardial infarction and left ventricular remodeling after myocardial infarction was unknown.

Prior art references related to the present invention are shown below.

[Non-Patent Document 1] Hirano, T. et al., Nature (1986) 324, 73-76

[Non-Patent Document 2] Akira, S. et al., Adv. in Immunology (1993) 54, 1-78

[Non-Patent Document 3] Lotz, M. et al., J. Exp. Med. (1988)167, 1253-1258

[Non-Patent Document 4] Taga, T. et al., J. Exp. Med. (1987) 166, 967-981

[Non-Patent Document 5] Yamasaki, K. et al., Science (1988) 241, 825-828
[Non-Patent Document 6] Taga, T. et al., Cell (1989) 58, 573-581
[Non-Patent Document 7] Novick, D. et al., Hybridoma (1991) 10, 137-146
[Non-Patent Document 8] Huang, Y. W. et al., Hybridoma (1993) 12, 621-630
[Non-Patent Document 9] Hirata, Y. et al., J. Immunol. (1989) 143, 2900-2906
[Non-Patent Document 10] Finkel, M. S. et al., Science (1992) 257, 387-389
[Non-Patent Document 11] Hirota, H. et al., Proc. Natl. Acad. Sci. USA (1995) 92, 4862-4866
[Non-Patent Document 12] Gwechenberger, M. et al., Circulation (1999) 99, 546-551
[Non-Patent Document 13] Ono, K. et al., Circulation (1998) 98, 149-156
[Non-Patent Document 14] Yamauchi-Takihara, K. et al., Circulation (1995) 91, 1520-1524
[Non-Patent Document 15] Yue, P. et al., Am. J. Physiol. (1998) 275,H250-H258
[Non-Patent Document 16]Negoro, S. et al., Cardiovasc. Res. (2000) 47, 797-805
[Non-Patent Document 17] Fuchs M. et al., FASEB J. (2003) 17, 2118-2120
[Patent Document 1] WO 95/09873
[Patent Document 2] French Patent Application Publication No. FR 2694767
[Patent Document 3] U.S. Patent No. 5216128
[Patent Document 4] WO 92/19759

Disclosure of the Invention

[Problems to be Solved by the Invention]

Until now, IL-6 has been suggested to be involved in myocardial infarction and left ventricular remodeling thereafter. However, its detailed role has not been clarified. In addition, it has not been revealed what kind of effect the administration of IL-6 inhibitor might show on myocardial infarction and left ventricular remodeling thereafter.

The present invention has been made under such circumstances, and an objective of the present invention is to provide agents for treating myocardial infarction, which comprise an IL-6 inhibitor as an active ingredient. Furthermore, the present invention provides agents for suppressing left ventricular remodeling after myocardial infarction, which comprise an IL-6 inhibitor as an active ingredient. Moreover, other objectives of the present invention are to provide methods for treating myocardial infarction and methods for suppressing left ventricular remodeling after myocardial infarction, both of which comprise the step of administering an IL-6 inhibitor to subjects who have developed myocardial infarction.

[Means for Solving the Problems]

In order to solve the above problems, the present inventors investigated the effects of anti-IL-6 receptor antibodies on improving the condition of an infarcted area in myocardial

infarction, and on suppressing left ventricular remodeling after myocardial infarction.

First, the present inventors produced myocardial infarction models by ligating the left anterior descending branch in male Balb/c mice. Then, 500 μg of an anti-IL-6 receptor antibody (MR16-1) was intraperitoneally administered to the myocardial infarction model mice.

As a result, the increase of myeloperoxidase (MPO) activity in myocardial infarcted area was significantly suppressed. Furthermore, myocardial monocyte chemoattractant protein-1 (MCP-1) expression was suppressed in both the infarcted area and noninfarcted area of the anti-IL-6 receptor antibody administered mice. Moreover, echocardiography and histological examinations both revealed that cardiac hypertrophy was suppressed in anti-IL-6 receptor antibody administered mice.

Thus, the present inventors discovered for the first time that it is possible to improve the condition of an infarcted area in myocardial infarction and suppress left ventricular remodeling after myocardial infarction by administering an anti-IL-6 receptor antibody, and finally completed the present invention.

Specifically, the present invention provides:

[1] an agent for treating myocardial infarction, which comprises an IL-6 inhibitor as an active ingredient;

[2] the agent of [1], wherein the IL-6 inhibitor is an antibody that recognizes IL-6;

[3] the agent of [1], wherein the IL-6 inhibitor is an antibody that recognizes IL-6 receptor;

[4] the agent of [2] or [3], wherein the antibody is a monoclonal antibody;

[5] the agent of [2] or [3], wherein the antibody is an antibody against human IL-6 or a human IL-6 receptor;

[6] the agent of [2] or [3], wherein the antibody is a recombinant antibody;

[7] the agent of [6], wherein the antibody is a chimeric antibody, humanized antibody or human antibody;

[8] an agent for suppressing left ventricular remodeling after myocardial infarction, which comprises an IL-6 inhibitor as an active ingredient;

[9] the agent of [8], wherein the IL-6 inhibitor is an antibody that recognizes IL-6;

[10] the agent of [8], wherein the IL-6 inhibitor is an antibody that recognizes IL-6 receptor;

[11] the agent of [9] or [10], wherein the antibody is a monoclonal antibody;

[12] the agent of [9] or [10], wherein the antibody is an antibody against human IL-6 or a human IL-6 receptor;

[13] the agent of [9] or [10], wherein the antibody is a recombinant antibody;

[14] the agent of [13], wherein the antibody is a chimeric antibody, humanized antibody or human antibody;

[15] the agent of any one of [8] to [14], which is used for treating myocardial infarction;

[16] a method for treating myocardial infarction in a subject, which comprises the step of administering an IL-6 inhibitor to a subject who has developed myocardial infarction;
[17] a method for suppressing left ventricular remodeling after myocardial infarction in a subject, which comprises the step of administering an IL-6 inhibitor to a subject who has developed myocardial infarction;
[18] the method of [16] or [17], wherein the IL-6 inhibitor is an antibody that recognizes IL-6;
[19] the method of [16] or [17], wherein the IL-6 inhibitor is an antibody that recognizes an IL-6 receptor;
[20] the method of [18] or [19], wherein the antibody is a monoclonal antibody;
[21] the method of [18] or [19], wherein the antibody is an antibody against human IL-6 or an antibody against a human IL-6 receptor;
[22] the method of [18] or [19], wherein the antibody is a recombinant antibody;
[23] the method of [22], wherein the antibody is a chimeric antibody, humanized antibody, or human antibody;
[24] use of IL-6 inhibitor for producing an agent for treating myocardial infarction;
[25] use of IL-6 inhibitor for producing an agent for suppressing left ventricular remodeling after myocardial infarction;
[26] the use of [24] or [25], wherein the IL-6 inhibitor is an antibody that recognizes IL-6;
[27] the use of [24] or [25], wherein the IL-6 inhibitor is an antibody that recognizes an IL-6 receptor;
[28] the use of [26] or [27], wherein the antibody is a monoclonal antibody;
[29] the use of [26] or [27], wherein the antibody is an antibody against human IL-6 or an antibody against a human IL-6 receptor;
[30] the use of [26] or [27], wherein the antibody is a recombinant antibody; and
[31] the use of [30], wherein the antibody is a chimeric antibody, humanized antibody, or human antibody.

Best Mode for Carrying Out the Invention

The present inventors discovered that improvement of the condition of infarcted area in myocardial infarction and suppression of left ventricular remodeling after myocardial infarction can be achieved by administering an anti-IL-6 receptor antibody. The present invention is based on these findings.

The present invention relates to agents for treating myocardial infarction and agents for suppressing left ventricular remodeling after myocardial infarction, both of which comprise an IL-6 inhibitor as an active ingredient.

Herein, an "IL-6 inhibitor" is a substance that blocks IL-6-mediated signal transduction

and inhibits IL-6 biological activity. Preferably, the IL-6 inhibitor is a substance that has inhibitory function against the binding of IL-6, IL-6 receptor, or gp130.

The IL-6 inhibitors of the present invention include, but are not limited to, for example, anti-IL-6 antibodies, anti-IL-6 receptor antibodies, anti-gp130 antibodies, IL-6 variants, soluble IL-6 receptor variants, and partial peptides of IL-6 or IL-6 receptors and low molecular weight compounds that show similar activities. Preferable IL-6 inhibitors of the present invention include antibodies that recognize IL-6 receptors.

The source of the antibody is not particularly restricted in the present invention; however, the antibody is preferably derived from mammals, and more preferably derived from human.

The anti-IL-6 antibody used in the present invention can be obtained as a polyclonal or monoclonal antibody via known means. In particular, monoclonal antibodies derived from mammals are preferred as the anti-IL-6 antibody used in the present invention. The monoclonal antibodies derived from mammals include those produced from hybridomas and those produced from hosts transformed with an expression vector that comprises an antibody gene by genetic engineering methods. By binding to IL-6, the antibody inhibits IL-6 from binding to an IL-6 receptor and blocks the transmission of IL-6 biological activity into the cell.

Such antibodies include, MH166 (Matsuda, T. *et al.*, Eur. J. Immunol. (1988) 18, 951-956), SK2 antibody (Sato, K. *et al.*, transaction of the 21st Annual Meeting of the Japanese Society for Immunology (1991) 21, 166), and so on.

Basically, anti-IL-6 antibody producing hybridomas can be prepared using known techniques as follows. Specifically, such hybridomas can be prepared by using IL-6 as a sensitizing antigen to carry out immunization by a conventional immunization method, fusing the obtained immune cells with known parent cells by a conventional cell fusion method, and screening for monoclonal antibody-producing cells by a conventional screening method.

More specifically, anti-IL-6 antibodies can be produced as follows. For example, human IL-6 used as the sensitizing antigen for obtaining antibody can be obtained using the IL-6 gene and/or amino acid sequences disclosed in Eur. J. Biochem. (1987) 168, 543-550; J. Immunol. (1988) 140, 1534-1541; and/or Agr. Biol. Chem. (1990) 54, 2685-2688.

After transforming an appropriate host cell with a known expression vector system inserted with an IL-6 gene sequence, the desired IL-6 protein is purified by a known method from the inside of the host cell or from the culture supernatant. This purified IL-6 protein may be used as the sensitizing antigen. Alternatively, a fusion protein of the IL-6 protein and another protein may be used as the sensitizing antigen.

Anti-IL6 receptor antibodies used for the present invention can be obtained as polyclonal or monoclonal antibodies by known methods. In particular, the anti-IL-6 receptor

antibodies used in the present invention are preferably monoclonal antibodies derived from mammals. The monoclonal antibodies derived from mammals include those produced from hybridomas and those produced from hosts transformed with an expression vector that comprises an antibody gene by genetic engineering methods. By binding to an IL-6 receptor, the antibody inhibits IL-6 from binding to the IL-6 receptor and blocks the transmission of IL-6 biological activity into the cell.

Such antibodies include, MR16-1 antibody (Tamura, T. *et al.*, Proc. Natl. Acad. Sci. USA (1993) 90, 11924-11928); PM-1 antibody (Hirata, Y. *et al.*, J. Immunol. (1989) 143, 2900-2906); AUK12-20 antibody, AUK64-7 antibody and AUK146-15 antibody (WO 92/19759); and so on. Among them, the PM-1 antibody can be exemplified as a preferred monoclonal antibody against the human IL-6 receptor, and the MR16-1 antibody as a preferred monoclonal antibody against the mouse IL-6 receptor.

Basically, hybridomas producing an anti-IL-6 receptor monoclonal antibody can be prepared using known techniques as follows. Specifically, such hybridomas can be prepared by using an IL-6 receptor as the sensitizing antigen to carry out immunization by a conventional immunization method, fusing the obtained immune cells with a known parent cell by a conventional cell fusion method, and screening for monoclonal antibody-producing cells by a conventional screening method.

More specifically, anti-IL-6 receptor antibodies can be produced as follows. For example, a human IL-6 receptor or mouse IL-6 receptor used as the sensitizing antigen for obtaining antibody can be obtained using the IL-6 receptor genes and/or amino acid sequences disclosed in European Patent Application Publication No. EP 325474 and Japanese Patent Application Kokai Publication No. (JP-A) Hei 3-155795, respectively.

There exist two kinds of IL-6 receptor proteins, *i.e.*, protein expressed on the cell membrane and protein separated from the cell membrane (soluble IL-6 receptor) (Yasukawa, K. *et al.*, J. Biochem. (1990) 108, 673-676). The soluble IL-6 receptor consists essentially of the extracellular region of the cell membrane-bound IL-6 receptor, and differs from the membrane-bound IL-6 receptor in that it lacks the transmembrane region or both the transmembrane and intracellular regions. Any IL-6 receptor may be employed as the IL-6 receptor protein so long as it can be used as a sensitizing antigen for producing the anti-IL-6 receptor antibody utilized in the present invention.

After transforming an appropriate host cell with a known expression vector system inserted with an IL-6 receptor gene sequence, the desired IL-6 receptor protein is purified by a known method from the inside of the host cell or from the culture supernatant. This purified IL-6 receptor protein may be used as a sensitizing antigen. Alternatively, a cell expressing the IL-6 receptor or a fusion protein of the IL-6 receptor protein and another protein may be used as

a sensitizing antigen.

Anti-gp130 antibodies used in the present invention can be obtained as polyclonal or monoclonal antibodies by known methods. In particular, the anti-gp130 antibodies used in the present invention are preferably monoclonal antibodies derived from mammals. The monoclonal antibodies derived from mammals include those produced from hybridomas and those produced from hosts transformed with an expression vector that comprises an antibody gene by genetic engineering methods. By binding to gp130, the antibody inhibits gp130 from binding to the IL-6/IL-6 receptor complex and blocks the transmission of IL-6 biological activity into the cell.

Such antibodies include, AM64 antibody (JP-A Hei 3-219894); 4B11 antibody and 2H4 antibody (US 5571513); B-S12 antibody and B-P8 antibody (JP-A Hei 8-291199); and so on.

Basically, Anti-gp130 monoclonal antibody-producing hybridomas can be prepared using known techniques as follows. Specifically, such hybridomas can be prepared by using gp130 as a sensitizing antigen to carry out the immunization by a conventional immunization method, fusing the obtained immune cells with a known parent cell by a conventional cell fusion method, and screening for monoclonal antibody-producing cells by a conventional screening method.

More specifically, the monoclonal antibody can be produced as follows. For example, gp130 used as a sensitizing antigen for obtaining antibody can be obtained using the gp130 gene and/or amino acid sequence disclosed in European Patent Application Publication No. EP 411946.

After transforming an appropriate host cell with a known expression vector system inserted with a gp130 gene sequence, the desired gp130 protein is purified by a known method from the inside of the host cell or from the culture supernatant. This purified gp130 protein may be used as a sensitizing antigen. Alternatively, a cell expressing gp130 or a fusion protein of the gp130 protein and another protein may be used as a sensitizing antigen.

Mammals to be immunized with a sensitizing antigen are not particularly limited, but are preferably selected in consideration of the compatibility with the parent cell used for cell fusion. Generally, rodents such as mice, rats, and hamsters are used.

Immunization of animals with a sensitizing antigen is performed according to known methods. For example, as a general method, it is performed by injecting the sensitizing antigen intraperitoneally or subcutaneously into mammals. Specifically, the sensitizing antigen is preferably diluted or suspended in an appropriate amount of phosphate-buffered saline (PBS), physiological saline or such, mixed with an appropriate amount of a general adjuvant (*e.g.*, Freund's complete adjuvant), emulsified, and then administered for several times every 4 to 21 days to a mammal. In addition, an appropriate carrier may be used for the immunization with a

sensitizing antigen.

Following such immunization, an increased level of the desired antibody in serum is confirmed and then immune cells are obtained from the mammal for cell fusion. Preferred immune cells for cell fusion include, in particular, spleen cells.

For the mammalian myeloma cells to be used as a parent cell, *i.e.* a partner cell to be fused with the above immune cells, various known cell strains, for example, P3X63Ag8.653 (Kearney, J. F. *et al.*, J. Immunol (1979) 123, 1548-1550), P3X63Ag8U.1 (Current Topics in Microbiology and Immunology (1978) 81, 1-7), NS-1 (Kohler, G. and Milstein, C., Eur. J. Immunol. (1976) 6, 511-519), MPC-11 (Margulies, D. H. *et al.*, Cell (1976) 8, 405-415), SP2/0 (Shulman, M. *et al.*, Nature (1978) 276, 269-270), F0 (de St. Groth, S. F. *et al.*, J. Immunol. Methods (1980) 35, 1-21), S194 (Trowbridge, I. S., J. Exp. Med. (1978) 148, 313-323), R210 (Galfre, G. *et al.*, Nature (1979) 277, 131-133), and such are appropriately used.

Basically, cell fusion of the aforementioned immune cell and myeloma cell can be performed using known methods, for example, the method by Milstein *et al.* (Kohler, G. and Milstein, C., Methods Enzymol. (1981) 73, 3-46) and such.

More specifically, the aforementioned cell fusion is achieved in general nutrient culture medium under the presence of a cell fusion enhancing agent. For example, polyethylene glycol (PEG), Sendai virus (HVJ), and such are used as a fusion enhancing agent. Further, to enhance the fusion efficiency, auxiliary agents such as dimethyl sulfoxide may be added for use according to needs.

The ratio of immune cells and myeloma cells used is preferably, for example, 1 to 10 immune cells for each myeloma cell. The culture medium used for the aforementioned cell fusion is, for example, the RPMI1640 or MEM culture medium, which are suitable for the proliferation of the aforementioned myeloma cells. A general culture medium used for culturing this type of cell can also be used. Furthermore, serum supplements such as fetal calf serum (FCS) can be used in combination.

For cell fusion, the fusion cells (hybridomas) of interest are formed by mixing predetermined amounts of the aforementioned immune cell and myeloma cell well in the aforementioned culture medium, and then adding and mixing a concentration of 30 to 60 % (w/v) PEG solution (*e.g.*, a PEG solution with a mean molecular weight of about 1,000 to 6,000) pre-heated to about 37°C. Then, cell fusion agents and such that are unsuitable for the growth of hybridoma can be removed by repeating the steps of successively adding an appropriate culture medium and removing the supernatant by centrifugation.

The above hybridomas are selected by culturing cells in a general selection culture medium, for example, HAT culture medium (a culture medium containing hypoxanthine, aminopterin, and thymidine). Culturing in the HAT culture medium is continued for a sufficient

period of time, generally for several days to several weeks, to kill cells other than the hybridomas of interest (unfused cells). Then, the standard limited dilution method is performed to screen and clone hybridomas that produce the antibody of interest.

In addition to the method of immunizing a non-human animal with an antigen for obtaining the aforementioned hybridomas, a desired human antibody that has the activity of binding to a desired antigen or antigen-expressing cell can be obtained by sensitizing a human lymphocyte with a desired antigen protein or antigen-expressing cell *in vitro*, and fusing the sensitized B lymphocyte with a human myeloma cell (*e.g.*, U266) (see, Japanese Patent Application Kokoku Publication No. (JP-B) Hei 1-59878 (examined, approved Japanese patent application published for opposition)). Furthermore, a desired human antibody can be obtained by administering the antigen or antigen-expressing cell to a transgenic animal that has a repertoire of human antibody genes and then following the aforementioned method (see, International Patent Application Publication Nos. WO 93/12227, WO 92/03918, WO 94/02602, WO 94/25585, WO 96/34096, and WO 96/33735).

The thus-prepared hybridomas which produce monoclonal antibodies can be subcultured in conventional culture medium and stored in liquid nitrogen for a long period.

For obtaining monoclonal antibodies from the aforementioned hybridomas, the following methods may be employed: (1) method where the hybridomas are cultured according to conventional methods and the antibodies are obtained as a culture supernatant; (2) method where the hybridomas are proliferated by administering them to a compatible mammal and the antibodies are obtained as ascites; and so on. The former method is preferred for obtaining antibodies with high purity, and the latter is preferred for large-scale production of antibodies.

For example, the preparation of anti-IL-6 receptor antibody-producing hybridomas can be performed by the method disclosed in JP-A Hei 3-139293. The preparation can be performed by the method of injecting a PM-1 antibody-producing hybridoma into the abdominal cavity of a BALB/c mouse, obtaining ascite, and then purifying PM-1 antibody from the ascite, or the method of culturing the hybridoma in an appropriate medium (*e.g.*, RPMI1640 medium containing 10% fetal bovine serum, and 5% BM-Condimed H1 (Boehringer Mannheim); hybridoma SFM medium (GIBCO-BRL); PFHM-II medium (GIBCO-BRL), *etc.*) and then obtaining PM-1 antibody from the culture supernatant.

A recombinant antibody can be used as a monoclonal antibody of the present invention, wherein the antibody is produced through genetic recombination techniques by cloning an antibody gene from a hybridoma, inserting the gene into an appropriate vector, and then introducing the vector into a host (see, for example, Borrebaeck, C. A. K. and Larrick, J. W., THERAPEUTIC MONOCLONAL ANTIBODIES, published in the United Kingdom by MACMILLAN PUBLISHERS LTD., 1990).

More specifically, mRNA coding for the variable (V) region of an antibody is isolated from a cell that produces the antibody of interest, such as a hybridoma. The isolation of mRNA can be performed by preparing total RNA according to known methods, such as the guanidine ultracentrifugation method (Chirgwin, J. M. *et al.*, Biochemistry (1979) 18, 5294-5299) and the AGPC method (Chomczynski, P. *et al.*, Anal. Biochem. (1987) 162, 156-159), and preparing mRNA using the mRNA Purification Kit (Pharmacia) and such. Alternatively, mRNA can be directly prepared using the QuickPrep mRNA Purification Kit (Pharmacia).

cDNA of the antibody V region is synthesized from the obtained mRNA using reverse transcriptase. The synthesis of cDNA may be achieved using the AMV Reverse Transcriptase First-strand cDNA Synthesis Kit and so on. Furthermore, to synthesize and amplify the cDNA, the 5'-RACE method (Frohman, M. A. *et al.*, Proc. Natl. Acad. Sci. USA (1988) 85, 8998-9002; Belyavsky, A. *et al.*, Nucleic Acids Res. (1989) 17, 2919-2932) using 5'-Ampli FINDER RACE Kit (Clontech) and PCR may be employed. The DNA fragment of interest is purified from the obtained PCR products and then ligated with a vector DNA. Then, a recombinant vector is prepared using the above DNA and introduced into *Escherichia coli* or such, and its colonies are selected to prepare the desired recombinant vector. The nucleotide sequence of the DNA of interest is confirmed by, for example, the dideoxy method.

When a DNA encoding the V region of an antibody of interest is obtained, the DNA is ligated with a DNA that encodes a desired antibody constant region (C region), and inserted into an expression vector. Alternatively, the DNA encoding the antibody V region may be inserted into an expression vector comprising the DNA of an antibody C region.

To produce an antibody to be used in the present invention, as described below, the antibody gene is inserted into an expression vector so that it is expressed under the control of the expression regulating region, for example, enhancer and promoter. Then, the antibody can be expressed by transforming a host cell with this expression vector.

In the present invention, to decrease heteroantigenicity against human and such, artificially modified genetic recombinant antibodies, for example, chimeric antibodies, humanized antibodies, or human antibodies, can be used. These modified antibodies can be prepared using known methods.

A chimeric antibody can be obtained by ligating the antibody V region-encoding DNA obtained as above with a human antibody C region-encoding DNA, inserting the DNA into an expression vector and introducing it into a host for production (see, European Patent Application Publication No. EP 125023; International Patent Application Publication No. WO 92/19759). This known method can be used to obtain chimeric antibodies useful for the present invention.

Humanized antibodies are also referred to as reshaped human antibodies, and are antibodies wherein the complementarity determining regions (CDRs) of an antibody from a

mammal other than human (*e.g.*, mouse antibody) are transferred into the CDRs of a human antibody. General methods for this gene recombination are also known (see, European Patent Application Publication No. EP 125023, International Patent Application Publication No. WO 92/19759).

More specifically, a DNA sequence designed such that the CDRs of a mouse antibody are ligated with the framework regions (FRs) of a human antibody is synthesized by PCR from several oligonucleotides that had been produced to contain overlapping portions at their termini. The obtained DNA is ligated with a human antibody C region-encoding DNA and then inserted into an expression vector. The expression vector is introduced into a host to produce the humanized antibody (see, European Patent Application Publication No. EP 239400, International Patent Application Publication No. WO 92/19759).

The human antibody FRs to be ligated via the CDRs are selected so that the CDRs form a suitable antigen binding site. The amino acid(s) within the FRs of the antibody variable regions may be substituted as necessary so that the CDRs of the reshaped human antibody form an appropriate antigen binding site (Sato, K. *et al.*, Cancer Res. (1993) 53, 851-856).

Human antibody C regions are used for the chimeric and humanized antibodies, and include Cγ. For example, Cγ1, Cγ2, Cγ3, or Cγ4 may be used. Furthermore, to improve the stability of the antibody or its production, the human antibody C regions may be modified.

Chimeric antibodies consist of the variable region of an antibody derived from non-human mammals and a human antibody-derived C region; and humanized antibodies consist of the CDRs of an antibody derived from non-human mammals and the framework regions and C regions derived from a human antibody. Both have reduced antigenicity in human body, and are therefore are useful as antibodies to be used in the present invention.

Preferred specific examples of humanized antibodies used in the present invention include a humanized PM-1 antibody (see, International Patent Application Publication No. WO 92/19759).

Furthermore, in addition to the aforementioned method for obtaining a human antibody, techniques for obtaining human antibodies by panning using a human antibody library are also known. For example, it is possible to express the variable regions of human antibodies on the surface of phages as single chain antibodies (scFv) by the phage display method, and then select antigen-binding phages. By analyzing genes of the selected phages, DNA sequences coding for the human antibody variable regions that bind to the antigen can be determined. Once the DNA sequence of an scFv that binds to the antigen is revealed, an appropriate expression vector comprising the sequence can be constructed to obtain an human antibody. These methods are already known, and the publications of WO 92/01047, WO 92/20791, WO93/06213, WO 93/11236, WO 93/19172, WO 95/01438, and WO 95/15388 can be used as reference.

The above-constructed antibody gene can be expressed according to conventional methods. When a mammalian cell is used, the antibody gene can be expressed using a DNA in which the antibody gene to be expressed is functionally ligated to a useful commonly used promoter and a poly A signal downstream of the antibody gene, or a vector comprising the DNA. Examples of a promoter/enhancer include the human cytomegalovirus immediate early promoter/enhancer.

Furthermore, other promoters/enhancers that can be utilized for expressing the antibody to be used in the present invention include viral promoters/enhancers from retrovirus, polyoma virus, adenovirus, simian virus 40 (SV40), and such; and mammalian cell-derived promoters/enhancers such as human elongation factor 1α (HEF1α).

For example, when the SV40 promoter/enhancer is used, the expression can be easily performed by following the method by Mulligan *et al.* (Mulligan, R. C. *et al.*, Nature (1979) 277, 108-114). Alternatively, in the case of the HEF1α promoter/enhancer, the method by Mizushima *et al.* (Mizushima, S. and Nagata S., Nucleic Acids Res. (1990) 18, 5322) can be used.

When *E. coli* is used, the antibody gene can be expressed by functionally ligating a conventional useful promoter, a signal sequence for antibody secretion, and the antibody gene to be expressed. Examples of a promoter include the lacZ promoter, araB promoter and such. When the lacZ promoter is used, the expression can be performed according to the method by Ward *et al.* (Ward, E. S. *et al.*, Nature (1989) 341, 544-546; Ward, E. S. *et al.*, FASEB J. (1992) 6, 2422-2427); and the araB promoter may be used according to the method by Better *et al.* (Better, M. *et al.*, Science (1988) 240, 1041-1043).

When the antibody is produced into the periplasm of *E. coli*, the pel B signal sequence (Lei, S. P. *et al.*, J. Bacteriol. (1987) 169, 4379-4383) may be used as the signal sequence for antibody secretion. The antibody produced into the periplasm is isolated, and then used after appropriately refolding the antibody structure (see, *e.g.*, WO 96/30394).

As the replication origin, those derived from SV40, polyoma virus, adenovirus, bovine papilloma virus (BPV) and such may be used. In addition, for enhancing the gene copy number in a host cell system, the expression vector may comprise the aminoglycoside phosphotransferase (APH) gene, thymidine kinase (TK) gene, *E. coli* xanthine-guanine phosphoribosyltransferase (Ecogpt) gene, dihydrofolate reductase (dhfr) gene, or such as a selection marker.

Any production system may be used for preparing the antibodies to be used in the present invention. The production systems for antibody preparation include *in vitro* and *in vivo* production systems. *In vitro* production systems include those utilizing eukaryotic cells or prokaryotic cells.

Production systems using eukaryotic cells include those utilizing animal cells, plant cells, or fungal cells. Such animal cells include (1) Mammalian cells, for example, CHO, COS, myeloma, baby hamster kidney (BHK), HeLa, Vero, and such; (2) amphibian cells, for example, *Xenopus* oocyte; and (3) insect cells, for example, sf9, sf21, Tn5, and such. Known plant cells include cells derived from *Nicotiana tabacum*, which may be cultured as callus. Known fungal cells include yeast such as *Saccharomyces* (*e.g.*, *S. cerevisiae*), mold fungi such as *Aspergillus* (*e.g.*, *A. niger*), and such.

Production systems using prokaryotic cells include those utilizing bacterial cells. Known bacterial cells include *E. coli* and *Bacillus subtilis*.

Antibodies can be obtained by introducing an antibody gene of interest into these cells by transformation, and culturing the transformed cells *in vitro*. The culturing is conducted according to known methods. For example, DMEM, MEM, RPMI1640, IMDM may be used as the culture medium, and serum supplements, such as FCS, may be used in combination. Furthermore, a cell introduced with an antibody gene may be transferred into the abdominal cavity or such of an animal to produce an antibody *in vivo*.

On the other hand, *in vivo* production systems include those utilizing animals or plants. Production systems using animals include those that utilize mammals or insects.

Mammals that can be used include goats, pigs, sheep, mice, bovines and such (Vicki Glaser, SPECTRUM Biotechnology Applications, 1993). Further, insects that can be used include silkworms. When using plants, for example, tobacco may be used.

An antibody gene is introduced into these animals or plants, and an antibody is produced in the body of the animals or plants and then recovered. For example, the antibody gene is prepared as a fusion gene by inserting the gene in the middle of a gene encoding a protein, such as goat β casein, which is uniquely produced into milk. A DNA fragment comprising the antibody gene-inserted fusion gene is injected into a goat embryo, and the embryo is introduced into a female goat. The desired antibody is obtained from the milk produced from the transgenic animal born from the goat that received the embryo, or produced from progenies of the animal. To increase the amount of milk that contains the desired antibody produced from the transgenic goat, hormones may by appropriately used on the transgenic goat (Ebert, K. M. *et al.*, Bio/Technology (1994) 12, 699-702).

Furthermore, when a silkworm is used, it is infected with baculovirus inserted with the desired antibody gene, and the desired antibody is obtained from the body fluid of this silkworm (Maeda, S. *et al.*, Nature (1985) 315, 592-594). Moreover, when tobacco is used, the desired antibody gene is inserted into a plant expression vector (*e.g.*, pMON530) and the vector is introduced into bacteria such as *Agrobacterium tumefaciens*. This bacterium is used to infect tobacco (*e.g.*, *Nicotiana tabacum*) to obtain the desired antibody from the leaves of this tobacco

(Julian, K. –C. Ma *et al.*, Eur. J. Immunol. (1994) 24, 131-138).

When producing an antibody in *in vitro* or *in vivo* production systems as described above, DNAs encoding the antibody heavy chain (H chain) and light chain (L chain) may be inserted into separate expression vectors and a host is then co-transformed with the vectors. Alternatively, the DNAs may be inserted into a single expression vector for transforming a host (see, International Patent Application Publication No. WO 94/11523).

The antibodies used in the present invention may be antibody fragments or modified products thereof so long as they can be suitably used in the present invention. For example, antibody fragments include Fab, F(ab')2, Fv, and single chain Fv (scFv) in which the Fvs of the H and L chains are linked via an appropriate linker.

Specifically, the antibody fragments are produced by treating an antibody with an enzyme, for example, papain or pepsin, or alternatively, genes encoding these fragments are constructed, introduced into expression vectors, and expressed in an appropriate host cell (see, *e.g.*, Co, M. S. *et al.*, J. Immunol. (1994) 152, 2968-2976; Better, M. & Horwitz, A. H., Methods in Enzymology (1989) 178, 476-496; Plueckthun, A. & Skerra, A., Methods in Enzymology (1989) 178, 497-515; Lamoyi, E., Methods in Enzymology (1989) 121, 652-663; Rousseaux, J. *et al.*, Methods in Enzymology (1989) 121, 663-666; Bird, R. E. *et al.*, TIBTECH (1991) 9, 132-137).

An scFv can be obtained by linking the H-chain V region and the L-chain V region of an antibody. In the scFv, the H-chain V region and the L-chain V region are linked via a linker, preferably via a peptide linker (Huston, J. S. *et al.*, Proc. Natl. Acad. Sci. USA (1988) 85, 5879-5883). The V regions of the H and L chains in an scFv may be derived from any of the antibodies described above. Peptide linkers for linking the V regions include, for example, an arbitrary single chain peptide consisting of 12 to 19 amino acid residues.

An scFv-encoding DNA can be obtained by using the DNA encoding the H chain or its V region and the DNA encoding the L chain or its V region of the aforementioned antibodies as templates, PCR amplifying the DNA portion that encodes the desired amino acid sequence in the template sequence using primers that define the termini of the portion, and then further amplifying the amplified DNA portion with a peptide linker portion-encoding DNA and primer pairs that link both ends of the linker to the H chain and L chain.

Furthermore, once an scFv-encoding DNA has been obtained, an expression vector comprising the DNA and a host transformed with the vector can be obtained according to conventional methods. In addition, the scFv can be obtained according to conventional methods using the host.

Similarly as above, these antibody fragments can be produced from the host by obtaining and expressing their genes. Herein, "antibody" encompasses these antibody

fragments.

As a modified antibody, an antibody bound to various molecules, such as polyethylene glycol (PEG), may also be used. Herein, "antibody" encompasses these modified antibodies. These modified antibodies can be obtained by chemically modifying the obtained antibodies. Such methods are already established in the art.

The antibodies produced and expressed as above can be isolated from the inside or outside of the cell or from host, and purified to homogeneity. The isolation and/or purification of the antibodies used for the present invention can be performed by affinity chromatography. Columns to be used for the affinity chromatography include, for example, protein A column and protein G column. Carriers used for the protein A column include, for example, HyperD, POROS, SepharoseF.F. and such. In addition to the above, other methods used for the isolation and/or purification of common proteins may be used, and are not limited in any way.

For example, the antibodies used for the present invention may be isolated and/or purified by appropriately selecting and combining chromatographies besides affinity chromatography, filters, ultrafiltration, salting-out, dialysis, and such. Chromatographies include, for example, ion-exchange chromatography, hydrophobic chromatography, gel filtration, and such. These chromatographies can be applied to high performance liquid chromatography (HPLC). Alternatively, reverse phase HPLC may be used.

Concentration of the antibodies as obtained above can be determined by absorbance measurement, ELISA, or such. Specifically, the absorbance is determined by appropriately diluting the antibody solution with PBS(-), measuring the absorbance at 280 nm, and calculating the concentration (1.35 OD = 1 mg/ml). Alternatively, when using ELISA, the measurement can be performed as follows. Specifically, 100 μl of goat anti-human IgG (TAG) diluted to 1 μg/ml with 0.1 M bicarbonate buffer (pH 9.6) is added to a 96-well plate (Nunc) and incubated overnight at 4°C to immobilize the antibody. After blocking, 100 μl of an appropriately diluted antibody of the present invention or an appropriately diluted sample comprising the antibody, and human IgG (CAPPEL) are added as a standard, and incubated for one hour at room temperature.

After washing, 100 μl of 5,000x diluted alkaline phosphatase-labeled anti-human IgG (BIO SOURCE) is added and incubated for one hour at room temperature. After another wash, substrate solution is added and incubated, and the absorbance at 405 nm is measured using MICROPLATE READER Model 3550 (Bio-Rad) to calculate the concentration of the antibody of interest.

IL-6 variants used in the present invention are substances that have the activity to bind to an IL-6 receptor and which do not transmit IL-6 biological activity. That is, the IL-6 variants compete with IL-6 to bind to IL-6 receptors, but fail to transmit IL-6 biological activity, hence

blocking IL-6-mediated signal transduction.

The IL-6 variants are produced by introducing mutation(s) through substitution of amino acid residues in the amino acid sequence of IL-6. The origin of IL-6 used as the base of the IL-6 variants is not limited; however, it is preferably human IL-6 when considering its antigenicity and such.

More specifically, amino acid substitution is performed by predicting the secondary structure of the IL-6 amino acid sequence using known molecular modeling programs (*e.g.*, WHATIF; Vriend *et al.*, J. Mol. Graphics (1990) 8, 52-56), and further assessing the influence of the substituted amino acid residue(s) on the whole molecule. After determining the appropriate amino acid residue to be substituted, commonly performed PCR methods are carried out using the human IL-6 gene-encoding nucleotide sequence as a template to introduce mutations so that amino acids are substituted, and thereby an IL-6 variant-encoding gene is obtained. If needed, this gene is inserted into an appropriate expression vector, and the IL-6 variant can be obtained by applying the aforementioned methods for expression, production, and purification of recombinant antibodies.

Specific examples of the IL-6 variants are disclosed in Brakenhoff *et al.*, J. Biol. Chem. (1994) 269, 86-93, Savino *et al.*, EMBO J. (1994) 13, 1357-1367, WO 96/18648, and WO 96/17869.

Partial peptides of IL-6 and partial peptides of IL-6 receptors to be used in the present invention are substances that have the activity to bind to IL-6 receptors and IL-6, respectively, and which do not transmit IL-6 biological activity. Namely, by binding to and capturing an IL-6 receptor or IL-6, the IL-6 partial peptide or the IL-6 receptor partial peptide specifically inhibits IL-6 from binding to the IL-6 receptor. As a result, the biological activity of IL-6 is not transmitted, and therefore IL-6-mediated signal transduction is blocked.

The partial peptides of IL-6 or IL-6 receptor are peptides that comprise part or all of the amino acid sequence of the region of the IL-6 or IL-6 receptor amino acid sequence that is involved in the binding of IL-6 and IL-6 receptor. Such peptides usually comprise 10 to 80, preferably 20 to 50, more preferably 20 to 40 amino acid residues.

The IL-6 partial peptides or IL-6 receptor partial peptides can be produced according to generally known methods, for example, genetic engineering techniques or peptide synthesis method, by specifying the region of the IL-6 or IL-6 receptor amino acid sequence that is involved in the binding of IL-6 and IL-6 receptor, and using a portion or whole of the amino acid sequence of the specified region.

When preparing an IL-6 partial peptide or IL-6 receptor partial peptide by a genetic engineering method, a DNA sequence encoding the desired peptide is inserted into an expression vector, and then the peptide can be obtained by applying the aforementioned methods for

expressing, producing, and purifying recombinant antibodies.

To produce an IL-6 partial peptide or IL-6 receptor partial peptide by peptide synthesis methods, the generally used peptide synthesis methods, for example, solid phase synthesis methods or liquid phase synthesis methods may be used.

Specifically, the synthesis can be performed following the method described in "Continuation of Development of Pharmaceuticals, Vol. 14, Peptide Synthesis (in Japanese) (ed. Haruaki Yajima, 1991, Hirokawa Shoten)". As a solid phase synthesis method, for example, the following method can be employed: the amino acid corresponding to the C terminus of the peptide to be synthesized is bound to a support that is insoluble in organic solvents, then elongating the peptide strand by alternately repeating (1) the reaction of condensing amino acids whose α-amino groups and branch chain functional groups are protected with appropriate protecting groups one at a time in a C to N-terminal direction; and (2) the reaction of removing protecting groups from the α-amino groups of the resin-bound amino acid or peptide. The solid phase peptide synthesis is broadly classified into the Boc method and the Fmoc method based on the type of protecting group used.

After the protein of interest is synthesized as above, deprotection reaction and reaction to cleave the peptide strand from the support are carried out. For the cleavage reaction of the peptide strand, in general, hydrogen fluoride or trifluoromethane sulfonic acid is used for the Boc method, and TFA for the Fmoc method. According to the Boc method, for example, the above-mentioned protected peptide resin is treated in hydrogen fluoride under the presence of anisole. Then, the peptide is recovered by removing the protecting group and cleaving the peptide from the support. By freeze-drying the recovered peptide, a crude peptide can be obtained. On the other hand, in the Fmoc method, for example, the deprotection reaction and the reaction to cleave the peptide strand from the support can be performed in TFA by a similar method as described above.

The obtained crude peptide can be separated and/or purified by applying HPLC. Elution may be performed under optimum conditions using a water-acetonitrile solvent system, which is generally used for protein purification. The fractions corresponding to the peaks of the obtained chromatographic profile are collected and freeze-dried. Thus, purified peptide fractions are identified by molecular weight analysis via mass spectrum analysis, amino acid composition analysis, amino acid sequence analysis, or such.

Specific examples of IL-6 partial peptides and IL-6 receptor partial peptides are disclosed in JP-A Hei 2-188600, JP-A Hei 7-324097, JP-A Hei 8-311098, and United States Patent Publication No. US 5210075.

The antibodies used in the present invention may also be conjugated antibodies which are bound to various molecules, such as polyethylene glycol (PEG), radioactive substances, and

toxins. Such conjugated antibodies can be obtained by chemically modifying the obtained antibodies. Methods for modifying antibodies are already established in the art. The "antibodies" of the present invention encompass these conjugated antibodies.

The agents for treating myocardial infarction and agents for suppressing left ventricular remodeling after myocardial infarction in the present invention can be used for myocardial infarction treatments.

Herein, "treating myocardial infarction" means suppressing or preventing symptoms of myocardial infarction, and cardiac failure and ischemia-induced severe arrhythmia which occur as complications of myocardial infarction.

Complicating symptoms of myocardial infarction include arrhythmia (extrasystole, ventricular fibrillation, and atrioventricular block), heart failure, papillary muscle rupture, heart rupture, ventricular aneurysm (which is formed in the cardiac apex as a result of infarction in the anterior descending branch of the left coronary artery), and post-myocardial infarction syndrome. The "agents for treating myocardial infarction" of the present invention can suppress and prevent the symptoms described above.

Meanwhile, herein, the term "suppressing left ventricular remodeling after myocardial infarction" means suppressing or preventing myocardial hypertrophy (dilatation of the whole left ventricle) that occurs to compensate for the functional impairment of the infarcted area.

Myocardial hypertrophy occurs when cardiac muscle cells in infarcted areas are displaced with a fibrous tissue such as collagen fibers as a result of the necrosis and/or exfoliation of the cells and the fibrous tissue is thinly extended. Thus, the suppression and prevention of "displacement of the infarcted area with collagen fibers" and "extension of the fibrous tissue", *i.e.*, improvement of the condition of the infarcted area, is also included in the meaning of "suppressing left ventricular remodeling after myocardial infarction" described above.

Whether symptoms of myocardial infarction and left ventricular remodeling after myocardial infarction are suppressed or not can be determined using the activity of myeloperoxidase (MPO) in infarcted and non-infarcted areas of cardiac muscle as an indicator. MPO is an enzyme present in the intracellular granules of neutrophils and its activity is known to be significantly elevated due to coronary artery diseases. MPO activity is increased as infarcted areas spread and aggravate (necrosis and such). That is, when MPO activity is suppressed by administering an agent of the present invention, symptoms of myocardial infarction and left ventricular remodeling after myocardial infarction can be considered to be suppressed. MPO activity can be determined by known methods, which include, for example, measurement methods described in the Examples.

Alternatively, whether symptoms of myocardial infarction and left ventricular remodeling after myocardial infarction are suppressed or not can also be determined using the expression of MCP-1 (monocyte chemoattractant protein-1) in infarcted and non-infarcted areas of cardiac muscle as an indicator. MCP-1 is a chemokine that may cause heart failure by recruiting macrophages to cardiac muscle and enhancing the expression of inflammatory cytokines. MCP-1 is known to activate inflammation and induce the fibrosis of cardiac muscle and perivascular tissues. Spread and/or aggravation (necrosis and such) of infarcted area increase the expression of MPC-1. Specifically, when the MCP-1 expression is suppressed, symptoms of myocardial infarction and left ventricular remodeling after myocardial infarction can be considered to be suppressed. The expression of MCP-1 can be measured by known methods for measuring protein expression, which include, for example, Western blotting and ELISA.

The phrases "suppressing MPO activity" and "suppressing the expression of MCP-1" also mean "improving the condition of infarcted area" mentioned above.

Furthermore, suppression of the symptoms of myocardial infarction and left ventricular remodeling after myocardial infarction can also be determined by measuring the left ventricular end-diastolic dimension and ejection fraction by echocardiography, or quantitative evaluation of the degree of myocardial fibrosis and the hypertrophy of cardiomyocytes by histological examination of cardiac tissues. Such measurements can be achieved using known methods. Such methods include, for example, methods described in the Examples.

In the present invention, the activity of IL-6 inhibitors in inhibiting the transduction of IL-6 signal can be evaluated by conventional methods. Specifically, IL-6 is added to cultures of IL-6-dependent human myeloma cell lines (S6B45 and KPMM2), human Lennert T lymphoma cell line KT3, or IL-6-dependent cell line MH60.BSF2; and the ^{3}H-thymidine uptake by the IL-6-dependent cells is measured in the presence of an IL-6 inhibitor. Alternatively, IL-6 receptor-expressing U266 cells are cultured, and ^{125}I-labeled IL-6 and an IL-6 inhibitor are added to the culture at the same time; and then ^{125}I-labeled IL-6 bound to the IL-6 receptor-expressing cells is quantified. In addition to the IL-6 inhibitor group, a negative control group that does not contain the IL-6 inhibitor is included in the assay system described above. The activity of the IL-6 inhibitor to inhibit IL-6 can be evaluated by comparing the results of both groups.

As shown below in the Examples, administration of an anti-IL-6 receptor antibody was found to suppress the symptoms of myocardial infarction and left ventricular remodeling after myocardial infarction. This finding suggests that IL-6 inhibitors such as anti-IL-6 receptor antibodies are useful as agents for treating myocardial infarction and agents for suppressing left ventricular remodeling after myocardial infarction.

Subjects to be administered with the agents of the present invention for treating

myocardial infarction and agents of the present invention for suppressing left ventricular remodeling after myocardial infarction are mammals. The mammals are preferably humans.

The agents of the present invention for treating myocardial infarction and agents of the present invention for suppressing left ventricular remodeling after myocardial infarction can be administered as pharmaceuticals, and may be administered systemically or locally via oral or parenteral administration. For example, intravenous injection such as drip infusion, intramuscular injection, intraperitoneal injection, subcutaneous injection, suppository, enema, oral enteric tablets, or the like can be selected. An appropriate administration method can be selected depending on the patient's age and symptoms. The effective dose per administration is selected from the range of 0.01 to 100 mg/kg body weight. Alternatively, the dose may be selected from the range of 1 to 1000 mg/patient, preferably from the range of 5 to 50 mg/patient. A preferred dose and administration method are as follows: for example, when an anti-IL-6 receptor antibody is used, the effective dose is an amount such that free antibody is present in the blood. Specifically, a dose of 0.5 to 40 mg/kg body weight/month (four weeks), preferably 1 to 20 mg/kg body weight/month is administered via intravenous injection such as drip infusion, subcutaneous injection or such, once to several times a month, for example, twice a week, once a week, once every two weeks, or once every four weeks. The administration schedule may be adjusted by, for example, extending the administration interval of twice a week or once a week to once every two weeks, once every three weeks, or once every four weeks, while monitoring the condition after transplantation and changes in the blood test values.

In the present invention, the agents for treating myocardial infarction and agents for suppressing left ventricular remodeling after myocardial infarction may contain pharmaceutically acceptable carriers, such as preservatives and stabilizers. The "pharmaceutically acceptable carriers" refer to materials that can be co-administered with an above-described agent; and may or may not itself produce the above-described effect of suppressing symptoms of myocardial infarction and left ventricular remodeling after myocardial infarction. Alternatively, the carriers may be materials that do not have the effect of suppressing symptoms of myocardial infarction and left ventricular remodeling after myocardial infarction, but produce an additive or synergistic stabilizing effect when used in combination with an IL-6 inhibitor.

Such pharmaceutically acceptable materials include, for example, sterile water, physiological saline, stabilizers, excipients, buffers, preservatives, detergents, chelating agents (EDTA and such), and binders.

In the present invention, detergents include non-ionic detergents, and typical examples of such include sorbitan fatty acid esters such as sorbitan monocaprylate, sorbitan monolaurate, and sorbitan monopalmitate; glycerin fatty acid esters such as glycerin monocaprylate, glycerin monomyristate and glycerin monostearate; polyglycerin fatty acid esters such as decaglyceryl

monostearate, decaglyceryl distearate, and decaglyceryl monolinoleate; polyoxyethylene sorbitan fatty acid esters such as polyoxyethylene sorbitan monolaurate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monostearate, polyoxyethylene sorbitan monopalmitate, polyoxyethylene sorbitan trioleate, and polyoxyethylene sorbitan tristearate; polyoxyethylene sorbit fatty acid esters such as polyoxyethylene sorbit tetrastearate and polyoxyethylene sorbit tetraoleate; polyoxyethylene glycerin fatty acid esters such as polyoxyethylene glyceryl monostearate; polyethylene glycol fatty acid esters such as polyethylene glycol distearate; polyoxyethylene alkyl ethers such as polyoxyethylene lauryl ether; polyoxyethylene polyoxypropylene alkyl ethers such as polyoxyethylene polyoxypropylene glycol, polyoxyethylene polyoxypropylene propyl ether, and polyoxyethylene polyoxypropylene cetyl ether; polyoxyethylene alkyl phenyl ethers such as polyoxyethylene nonylphenyl ether; polyoxyethylene hardened castor oils such as polyoxyethylene castor oil and polyoxyethylene hardened castor oil (polyoxyethylene hydrogenated castor oil); polyoxyethylene beeswax derivatives such as polyoxyethylene sorbit beeswax; polyoxyethylene lanolin derivatives such as polyoxyethylene lanolin; and polyoxyethylene fatty acid amides and such with an HLB of 6 to 18, such as polyoxyethylene stearic acid amide.

Detergents also include anionic detergents, and typical examples of such include, for example, alkylsulfates having an alkyl group with 10 to 18 carbon atoms, such as sodium cetylsulfate, sodium laurylsulfate, and sodium oleylsulfate; polyoxyethylene alkyl ether sulfates in which the alkyl group has 10 to 18 carbon atoms and the average molar number of added ethylene oxide is 2 to 4, such as sodium polyoxyethylene lauryl sulfate; alkyl sulfosuccinate ester salts having an alkyl group with 8 to 18 carbon atoms, such as sodium lauryl sulfosuccinate ester; natural detergents, for example, lecithin; glycerophospholipids; sphingo-phospholipids such as sphingomyelin; and sucrose fatty acid esters in which the fatty acids have 12 to 18 carbon atoms.

One, two or more of the detergents described above can be combined and added to the agents of the present invention. Detergents that are preferably used in the preparations of the present invention include polyoxyethylene sorbitan fatty acid esters, such as polysorbates 20, 40, 60, and 80. Polysorbates 20 and 80 are particularly preferred. Polyoxyethylene polyoxypropylene glycols, such as poloxamer (Pluronic F-68® and such), are also preferred.

The amount of detergent added varies depending on the type of detergent used. When polysorbate 20 or 80 is used, the amount is in general in the range of 0.001 to 100 mg/ml, preferably in the range of 0.003 to 50 mg/ml, more preferably in the range of 0.005 to 2 mg/ml.

In the present invention, buffers includes phosphate, citrate buffer, acetic acid, malic acid, tartaric acid, succinic acid, lactic acid, potassium phosphate, gluconic acid, capric acid, deoxycholic acid, salicylic acid, triethanolamine, fumaric acid, and other organic acids; and

carbonic acid buffer, Tris buffer, histidine buffer, and imidazole buffer.

Liquid preparations may be formulated by dissolving the agents in aqueous buffers known in the field of liquid preparations. The buffer concentration is in general in the range of 1 to 500 mM, preferably in the range of 5 to 100 mM, more preferably in the range of 10 to 20 mM.

The agents of the present invention may also comprise other low-molecular-weight polypeptides; proteins such as serum albumin, gelatin, and immunoglobulin; amino acids; sugars and carbohydrates such as polysaccharides and monosaccharides, sugar alcohols, and such.

Herein, amino acids include basic amino acids, for example, arginine, lysine, histidine, and ornithine, and inorganic salts of these amino acids (preferably hydrochloride salts, and phosphate salts, namely phosphate amino acids). When free amino acids are used, the pH is adjusted to a preferred value by adding appropriate physiologically acceptable buffering substances, for example, inorganic acids, in particular hydrochloric acid, phosphoric acid, sulfuric acid, acetic acid, and formic acid, and salts thereof. In this case, the use of phosphate is particularly beneficial because it gives quite stable freeze-dried products. Phosphate is particularly advantageous when preparations do not substantially contain organic acids, such as malic acid, tartaric acid, citric acid, succinic acid, and fumaric acid, or do not contain corresponding anions (malate ion, tartrate ion, citrate ion, succinate ion, fumarate ion, and such). Preferred amino acids are arginine, lysine, histidine, and ornithine. Furthermore, it is possible to use acidic amino acids, for example, glutamic acid and aspartic acid, and salts thereof (preferably sodium salts); neutral amino acids, for example, isoleucine, leucine, glycine, serine, threonine, valine, methionine, cysteine, and alanine; and aromatic amino acids, for example, phenylalanine, tyrosine, tryptophan, and its derivative, N-acetyl tryptophan.

Herein, sugars and carbohydrates such as polysaccharides and monosaccharides include, for example, dextran, glucose, fructose, lactose, xylose, mannose, maltose, sucrose, trehalose, and raffinose.

Herein, sugar alcohols include, for example, mannitol, sorbitol, and inositol.

When the agents of the present invention are prepared as aqueous solutions for injection, the agents may be mixed with, for example, physiological saline, and/or isotonic solution containing glucose or other auxiliary agents (such as D-sorbitol, D-mannose, D-mannitol, and sodium chloride). The aqueous solutions may be used in combination with appropriate solubilizing agents such as alcohols (ethanol and such), polyalcohols (propylene glycol, PEG, and such), or non-ionic detergents (polysorbate 80 and HCO-50).

The agents may further comprise, if required, diluents, solubilizers, pH adjusters, soothing agents, sulfur-containing reducing agents, antioxidants, and such.

Herein, the sulfur-containing reducing agents include, for example, compounds

comprising sulfhydryl groups, such as N-acetylcysteine, N-acetylhomocysteine, thioctic acid, thiodiglycol, thioethanolamine, thioglycerol, thiosorbitol, thioglycolic acid and salts thereof, sodium thiosulfate, glutathione, and thioalkanoic acids having 1 to 7 carbon atoms.

Moreover, the antioxidants in the present invention include, for example, erythorbic acid, dibutylhydroxy toluene, butylhydroxy anisole, α-tocopherol, tocopherol acetate, L-ascorbic acid and salts thereof, L-ascorbic acid palmitate, L-ascorbic acid stearate, sodium hydrogen sulfite, sodium sulfite, triamyl gallate, propyl gallate, and chelating agents such as disodium ethylenediamine tetraacetate (EDTA), sodium pyrophosphate, and sodium metaphosphate.

If required, the agents may be encapsulated in microcapsules (microcapsules of hydroxymethylcellulose, gelatin, poly[methylmethacrylic acid] or such) or prepared as colloidal drug delivery systems (liposome, albumin microspheres, microemulsion, nano-particles, nano-capsules, and such)(see "Remington's Pharmaceutical Science 16th edition", Oslo Ed., 1980, and the like). Furthermore, methods for preparing agents as sustained-release agents are also known, and are applicable to the present invention (Langer *et al.*, J. Biomed. Mater. Res. 1981, 15: 167-277; Langer, Chem. Tech. 1982, 12: 98-105; U.S. Patent No. 3,773,919; European Patent Application No. (EP) 58,481; Sidman *et al.*, Biopolymers 1983, 22: 547-556; and EP 133,988).

Pharmaceutically acceptable carriers used are appropriately selected from those described above or combined depending on the type of dosage form, but are not limited thereto.

The present invention relates to methods for treating myocardial infarction in subjects and methods for suppressing left ventricular remodeling after myocardial infarction, both of which comprise the step of administering an IL-6 inhibitor to subjects who have developed myocardial infarction.

Herein, the "subject" refers to organisms and body parts of the organisms to be administered with an agent of the present invention for treating myocardial infarction or an agent of the present invention for suppressing left ventricular remodeling after myocardial infarction. The organisms include animals (for example, human, domestic animal species, and wild animals) but are not particularly limited.

The "body parts of the organisms" are not particularly limited, but preferably include heart, cardiac muscle, and infarcted and non-infarcted areas in myocardial infarcts.

Herein, "administration" includes oral and parenteral administrations. Oral administration includes, for example, administration of oral agents. Such oral agents include, for example, granule, powder, tablet, capsule, solution, emulsion, and suspension.

Parenteral administration includes, for example, administration of injections. Such injections include, for example, subcutaneous injection, intramuscular injection, and intraperitoneal injection. Meanwhile, the effects of the methods of the present invention can be

achieved by introducing genes comprising oligonucleotides to be administered to living bodies using gene therapy techniques. Alternatively, the agents of the present invention may be administered locally to intended areas of treatment. For example, the agents can be administered by local injection during surgery, use of catheters, or targeted gene delivery of DNA encoding a peptide of the present invention. The agents of the present invention may be administered along with the treatment for occurrence of myocardial infarctions, for example, catheter surgery (percutaneous transluminal coronary angioplasty (PTCA) and percutaneous coronary intervention (PCI)), percutaneous transluminal coronary recanalization (PTCR), coronary artery bypass grafting (CABG) and such.

When the methods of the present invention are conducted, the agents of the present invention may be administered as part of a pharmaceutical composition in combination with at least one known chemotherapeutant. Alternatively, the agents of the present invention may be administered in combination with at least one known immunosuppressant. In one embodiment, the agents of the present invention and known chemotherapeutants may be practically administered at the same time.

All prior art references cited herein are incorporated herein by reference.

[Examples]

Hereinbelow, the present invention will be specifically described with reference to the Examples, but it is not to be construed as being limited thereto.

[Example 1] Preparation of a mouse model of myocardial infarction

Male Balb/c mice (25 to 30 g) were tracheally intubated. The mice were on an artificial respirator and anesthetized by inhalation of 0.5 to 1.0% isoflurane. The left chest was opened. After ligation of the left anterior descending coronary artery, the chest was closed. The mice were grouped into MR16-1-administered group (MR16-1 group) and untreated group (control group). The MR16-1-administered group was subjected to intraperitoneal administration of MR16-1 at a dose of 500 μg/body.

[Example 2] Measurement of MPO activity

Hearts were extracted from mice two days after myocardial infarcts were created (or coronary ligation. The hearts were divided into infarcted area and non-infarcted area, and minced. Then, the minced cardiac muscle was combined with 10 volumes of 50 mM KPO_4 buffer (pH 6.0) containing 0.5% hexadecyltrimethyl ammonium bromide. The minced muscle was homogenized (POLYTRON, KINEMATICAAG, Luzern, Switzerland) and then sonicated.

The resulting extract was centrifuged at 13,000 rpm for 10 minutes at 4°C. After 50 μl of the resulting supernatant was mixed with 1.45 ml of substrate solution (50 mM KPO_4 (pH 6.0), 0.167 mg/ml o-dianisidine dihydrochloride, and 0.005% H_2O_2), changes in the color of the substrate solution were monitored by absorbance at 460 nm (extinction coefficient =2.655).

As a result, the activity of cardiac muscle MPO showed no difference between non-infarcted cardiac muscle and cardiac muscle of the sham-operated group, but significantly increased by about four times in the infarcted area (control-non risk 0.037 ± 0.006; control-risk 0.122 ± 0.035; $p<0.01$). Meanwhile, this increase of MPO activity in the infarcted area was significantly suppressed in the MR16-1-administered group (MR16-1-risk 0.034 ± 0.008; $p<0.05$ vs. control-risk).

[Example 3] MCP-1 expression assay

Hearts were extracted from mice two days after creation of myocardial infarction. The hearts were divided into infarcted area and non-infarcted area, and minced. The minced cardiac muscle was combined with lysis buffer (2x PBS, 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecylsulphate, 1 mM PMSF, 1% protease inhibitor cocktail (Nacalai Tesque), and then homogenized. The extract was centrifuged at 13,000 rpm for 10 minutes at 4°C. The resulting supernatant was used as a total cell lysate to quantify the protein concentration by Lowry method. Equal volumes of the protein solution were separated on a 12% polyacrylamide gel, and the proteins were transferred onto an Immun-Blot™ PVDF membrane. The membrane was then incubated with anti-MCP-1 antibody (1:30; IBL Co.) as the primary antibody at 4°C overnight, and then incubated with goat anti-rabbit IgG (1:400; Cell Signaling) as the secondary antibody at room temperature for 2 hours. The expression of MCP-1 was detected by chemiluminescence using ECL (Amersham Bioscience, Buckinghamshire, U.K.). Image analysis of the photograph was carried out using computer software (Scion Image Frame Grabber Status).

The result showed that the expression of cardiac muscle MCP-1 was increased in both infarcted and non-infarcted areas in the control group, but much higher in the infarcted area. On the other hand, the increase of MCP-1 expression was suppressed in both areas in the MR16-1-administered group.

[Example 4] Echocardiography

Four weeks after myocardial infarction was created, the hearts were examined by echocardiography under anesthesia to determine the left ventricular end-diastolic diameter and fractional shortening (FS).

The result of echocardiography four weeks after the creation of myocardial infarction

showed that the left ventricular end-diastolic diameter in the control group was significantly increased as compared with the sham group. This increase (in the left ventricular diameter) was significantly suppressed by administering MR16-1. Furthermore, while the FS was reduced after myocardial infarction (was created), it was significantly improved by administering MR16-1 (control group 18.5 ± 2.9% vs. MR16-1 group 28.5 ± 1.8%; $p<0.05$).

[Example 5] Histological evaluation

Hearts were extracted from mice four weeks after creation of myocardial infarction, fixed with 4% paraformaldehyde-phosphate buffer and then embedded in paraffin. The hearts were sectioned and then stained with Masson's trichrome to quantitatively evaluate the degree of cardiac fibrosis and hypertrophy of cardiac myocytes in the short-axis section of cardiac muscle in the non-infarcted area.

As a result, hypertrophy of the cardiac myocytes and stromal fibrosis was found in the non-infarcted area in the control group. In contrast, these symptoms were suppressed in the MR16-1-administered group.

Industrial Applicability

Myocardial infarct expansion and/or aggravation may induce complication of heart failure and/or ischemia-induced severe arrhythmia which increase threat to life. The agents of the present invention for treating myocardial infarction, agents for suppressing left ventricular remodeling after myocardial infarction, and methods for treating or preventing myocardial infarction, can suppress complicating symptoms in myocardial infarction and achieve effective treatment.

The occurrence rate of left ventricular remodeling after myocardial infarction is also suggested to be related to the size of infarcted area, and it is deemed to be important for improving the condition and preventing the infarcted area from enlarging at an early stage of myocardial infarction onset. In addition to the complicating symptoms of myocardial infarction, left ventricular remodeling can be suppressed by administering an agent of the present invention that comprises an IL-6 inhibitor as an active ingredient to the patient from an early stage of myocardial infarction.

CLAIMS

1. An agent for treating myocardial infarction, which comprises an IL-6 inhibitor as an active ingredient.

2. The agent of claim 1, wherein the IL-6 inhibitor is an antibody that recognizes IL-6.

3. The agent of claim 1, wherein the IL-6 inhibitor is an antibody that recognizes IL-6 receptor.

4. The agent of claim 2 or 3, wherein the antibody is a monoclonal antibody.

5. The agent of claim 2 or 3, wherein the antibody is an antibody against human IL-6 or a human IL-6 receptor.

6. The agent of claim 2 or 3, wherein the antibody is a recombinant antibody.

7. The agent of claim 6, wherein the antibody is a chimeric antibody, humanized antibody or human antibody.

8. An agent for suppressing left ventricular remodeling after myocardial infarction, which comprises an IL-6 inhibitor as an active ingredient.

9. The agent of claim 8, wherein the IL-6 inhibitor is an antibody that recognizes IL-6.

10. The agent of claim 8, wherein the IL-6 inhibitor is an antibody that recognizes IL-6 receptor.

11. The agent of claim 9 or 10, wherein the antibody is a monoclonal antibody.

12. The agent of claim 9 or 10, wherein the antibody is an antibody against human IL-6 or a human IL-6 receptor.

13. The agent of claim 9 or 10, wherein the antibody is a recombinant antibody.

14. The agent of claim 13, wherein the antibody is a chimeric antibody, humanized antibody or human antibody.

15. The agent of any one of claims 8 to 14, which is used for treating myocardial infarction.

16. A method for treating myocardial infarction in a subject, which comprises the step of administering an IL-6 inhibitor to a subject who has developed myocardial infarction.

17. A method for suppressing left ventricular remodeling after myocardial infarction in a subject, which comprises the step of administering an IL-6 inhibitor to a subject who has developed myocardial infarction.

18. The method of claim 16 or 17, wherein the IL-6 inhibitor is an antibody that recognizes IL-6.

19. The method of claim 16 or 17, wherein the IL-6 inhibitor is an antibody that recognizes an IL-6 receptor.

20. The method of claim 18 or 19, wherein the antibody is a monoclonal antibody.

21. The method of claim18 or 19, wherein the antibody is an antibody against human IL-6 or an antibody against a human IL-6 receptor.

22. The method of claim 18 or 19, wherein the antibody is a recombinant antibody.

23. The method of claim 22, wherein the antibody is a chimeric antibody, humanized antibody, or human antibody.

24. Use of IL-6 inhibitor for producing an agent for treating myocardial infarction.

25. Use of IL-6 inhibitor for producing an agent for suppressing left ventricular remodeling after myocardial infarction.

26. The use of claim 24 or 25, wherein the IL-6 inhibitor is an antibody that recognizes IL-6.

27. The use of claim 24 or 25, wherein the IL-6 inhibitor is an antibody that recognizes an IL-6 receptor.

28. The use of claim 26 or 27, wherein the antibody is a monoclonal antibody.

29. The use of claim 26 or 27, wherein the antibody is an antibody against human IL-6 or an antibody against a human IL-6 receptor.

30. The use of claim 26 or 27, wherein the antibody is a recombinant antibody.

31. The use of claim 30, wherein the antibody is a chimeric antibody, humanized antibody, or human antibody.