COMPOSITION FOR TREATING INFLAMMATORY DISEASE INDUCED BY HYPERIMMUNE RESPONSE

Technical Field

[0001] The present invention relates to a cell therapeutic agent, more specifically, it relates to a cell therapeutic agent for treating an inflammatory disease induced by a hyperimmune response, which comprises (i) a chondrocyte or a cell capable of differentiating into a chondrocyte; and (ii) a cell into which a gene encoding TGF-β (transforming growth factor-beta) is introduced, wherein the cell in (ii) is hChonJb#7.

Description of the Related Art

[0002] Degenerative arthritis is a disease caused by gradual loss of cartilage which protects the joints due to aging, and it is apt to occur in the knee joint which supports a significant portion of the body weight.

[0003] In contrast, rheumatoid arthritis of an autoimmune disease is a disease that our body's immune cells attack the joints to cause inflammation and inflammation of the joints continuously infiltrates into the articular cartilage and bone tissue, resulting in tissue erosion. Rheumatoid arthritis often occurs in fingers and wrists, and it is especially characterized by protruded middle nodes of the fingers. Unlike degenerative arthritis, which occurs mainly at the age of fifties or elder, rheumatoid arthritis often occurs at young ages.

and Remicade (ingredient name: infliximab), Humira (ingredient name: adalimimab) of TNF-α antagonists as biological agents. Most of the therapeutic agents which have been so far developed for rheumatoid arthritis have adverse effects such as gastrointestinal disorders, and cannot significantly suppress the progress of joint damage.

[0008] TGF-β1, one of the cell therapeutic agent components of the present invention, is a multifunctional modulator for cell growth and differentiation. It has been reported that the neuropathic pain due to nerve injury is significantly reduced by intrathecal injection of recombinant TGF-β1 by targeting various types of cells with multi-differentiating potencies.

[0009] It has been recently reported that chondrocytes or fibroblasts expressing TGF-β1 are effective in treating degenerative arthritis (Lee KH et al., Hum Gene Ther 2001; 12: 1805-1813, SUN U. SONG et al. Tissue Engineering 2005; 11: 1516-1526), but the possibility of treating rheumatoid arthritis which has different causes and pathologies from those of degenerative arthritis has not been reported.

[0010] Accordingly, the present inventors have made efforts to develop a cell therapeutic agent having anti-inflammatory action and therapeutic effect for rheumatoid arthritis, and as a result, it has been confirmed that the symptoms of rheumatism are improved when a mixed cell composition of a chondrocyte and a cell expressing TGF-β1 is treated to a rheumatoid arthritis-induced animal model, whereby the present invention has been completed.

SUMMARY

[0011] An object of the present invention is to provide a therapeutic use of a chondrocyte or a cell capable of differentiating into a chondrocyte and a cell expressing TGF-β1, which is not a chemically synthesized drug, for treating an inflammatory disease induced by a hyperimmune response, wherein the cell in (ii) is hChonJb#7.

[0012] In order to accomplish the above object, an aspect of the present invention provides a composition for treating an inflammatory disease induced by a hyperimmune response, which comprises (i) a chondrocyte or a cell capable of differentiating into a chondrocyte; and (ii) a cell into which a gene encoding TGF-β is introduced, wherein the cell in (ii) is hChonJb#7.

[0013] Another aspect of the present invention provides a method of treating an inflammatory disease induced by a hyperimmune response, which comprises administering to a subject with a composition comprising (i) a chondrocyte or a cell capable of differentiating into a chondrocyte; and (ii) a cell into which a gene encoding TGF-β is introduced, wherein the cell in (ii) is hChonJb#7.

[0014] Further, still another aspect of the present invention provides a use of a composition comprising (i) a chondrocyte or a cell capable of differentiating into a chondrocyte; and (ii) a cell into which a gene encoding TGF-β is introduced as a therapeutic agent for treating an inflammatory disease induced by a hyperimmune response, wherein the cell in (ii) is hChonJb#7.

[0015] Yet still another aspect of the present invention provides

a method of inhibiting the expression or activity of a cytokine selected from the group consisting of IL-6, IL-17A, IL-1β, and TNF-α, which comprises administering to a subject with a composition comprising (i) a chondrocyte or a cell capable of differentiating into a chondrocyte; and (ii) a cell into which a gene encoding TGF-β is introduced, wherein the cell in (ii) is hChonJb#7.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] The above and other aspects, features and other advantages of the present disclosure will be more clearly understood from the following detailed description taken in conjunction with the accompanying drawings, in which:

Figure 1 is a diagram illustrating the mechanism of a therapeutic action of a mixed cell composition of the present invention for rheumatoid arthritis and degenerative arthritis;

Figure 2 illustrates the results for the pain relief effect after treatment of a mixed cell to an animal model suffering with degenerative arthritis confirmed through the von Frey filament test;

Figure 3 illustrates the results for H&E staining performed to confirm the cartilage status of the knee joint after treatment of a mixed cell to an animal model suffering with degenerative

arthritis-induced animal model; and

Figure 11 illustrates the results for a change in cytokine measured by harvesting the synovial lavage fluid from the articular cavity on the 28th day after treatment of a mixed cell to a rheumatoid arthritis-induced animal model.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0017] A mixed cell comprising an allogeneic (non-autologous) chondrocyte; and a cell expressing TGF-β(hChonJb#7) is known as a cell-mediated gene therapeutic agent for degenerative arthritis. In a phase 2 clinical study for patients suffering with osteoarthritis, it has been confirmed that the mixed cell improves pain, activity and cartilage structures of patients suffering with osteoarthritis. However, a mechanism by which the mixed cell acts is not yet found.

[0018] The present inventors observed effects on pain and structural transformation after injection of the mixed cell to an osteoarthritis-induced animal model and confirmed whether the mixed cell induces an anti-inflammatory environment, and as a result, it has been confirmed that anti-inflammatory cytokines and M2 macrophages are increased in the arthritic knee joint treated with mixed cell to induce an anti-inflammatory environment that alleviates pain and induces joint regeneration.

[0019] Accordingly, it has been confirmed that the mixed cell induces an M2 macrophage-rich environment with immunosuppressive properties, and under the assumption that the mixed cell has an effect even on an inflammatory disease induced by a hyperimmune

response, in a rheumatoid arthritis animal model, a chondrocyte and a cell expressing TGF-β(hChonJb#7) are mixed at a predetermined ratio and treated to knee joints. As a result, in a group treated with the mixed cell, it was confirmed that the metatarsal edema was reduced and further, it was confirmed that expression of IL-6, IL-17A, IL-1β, and TNF-α as cytokines that induced and promoted an inflammatory response in the synovial lavage fluid was reduced. A therapeutic effect by a single dose of the mixed cell lasted for 26 days.

[0020] Conventionally, materials such as methotrexate (MTX) used as a therapeutic agent for rheumatoid arthritis may cause digestive system and mouth ulcers and are at risk for causing hepatotoxicity, renal failure, and the like. Further, therapeutic agents such as anti-TNF-α antibodies comprising Remicade and the like are known to disturb a patient's innate immune system, resulting in the expression of tuberculosis (TB) from a tuberculosis carrier. In contrast, a cell therapeutic agent according to the present invention has an advantage in that the above adverse effects are not shown in a clinical test result for degenerative arthritis. Accordingly, the present inventors have confirmed the possibility that the mixed cell may be a novel therapeutic agent which overcomes disadvantages of conventional therapeutic agents for rheumatoid arthritis.

[0021] Therefore, an aspect of the present invention relates to a composition for treating an inflammatory disease induced by a hyperimmune response, which comprises (i) a chondrocyte or a cell capable of differentiating into a chondrocyte; and (ii) a cell into

which a gene encoding TGF-β is introduced, wherein the cell in (ii) is hChonJb#7.

[0022] Another aspect of the present invention relates to a method of treating an inflammatory disease induced by a hyperimmune response, which comprises administering to a subject with a composition comprising (i) a chondrocyte or a cell capable of differentiating into a chondrocyte; and (ii) a cell into which a gene encoding TGF-β is introduced, wherein the cell in (ii) is hChonJb#7.

[0023] Still another aspect of the present invention relates to a use of a composition comprising (i) a chondrocyte or a cell capable of differentiating into a chondrocyte; and (ii) a cell into which a gene encoding TGF-β is introduced as a therapeutic agent for treating an inflammatory disease induced by a hyperimmune response, wherein the cell in (ii) is hChonJb#7.

[0024] In the present invention, the inflammatory disease induced by the hyperimmune response comprises rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, myasthenia gravis, colitis, type 1 diabetes, encephalitis, and the like, and among the diseases, it is particularly effective in the treatment for rheumatoid arthritis.

[0025] In the present invention, the cell capable of differentiating into the chondrocyte may be a cartilage precursor cell or a stem cell.

[0026] In the present invention, (i) the chondrocyte or the cell capable of differentiating into a chondrocyte and (ii) the cell into which a gene encoding TGF-β is introduced may be mixed at

various mixing ratios, and the ratio of (i) and (ii) may be preferably 1 : 1 to 100 : 1 and more preferably 3 : 1, wherein the cell in (ii) is hChonJb#7.

[0027] In an aspect of the present invention, the composition for treating the inflammatory disease induced by the hyperimmune response comprises a non-transduced human chondrocyte (hChonJ cell) and a cell expressing TGF-β1(hChonJb#7) , more specifically, a human allogeneic chondrocyte (hChonJ) and a cell expressing human TGF-β1. The two types of cells may be mixed at various mixing ratios. For example, these two cells may be mixed at a ratio of 3 : 1, that is, a ratio at which the hChonJ cell is 3 and the hChonJb#7 is 1. The mixed cell at the ratio of 3 : 1 and a production method thereof are disclosed in a thesis [Cytotherapy, 2012 Feb; 14(2): 247-256] and US Patent Registration Nos. 7,005,127 and 7,282,200.

[0028] In an aspect of the present invention, in an arthritis rat model treated with the mixed cell, the pain started to be reduced from 15 days after treatment and a pain relief effect was continued up to the time (an experiment end date) after 56 days after treatment. It has been confirmed that the pain relief was associated with a structural improvement of arthritic knee joints, and the treated mixed cell was found to be effective for both pain relief and cartilage regeneration. That is, the mixed cell used in the present invention is a cell-mediated gene therapy for pain relief and regeneration of cartilage tissue.

[0029] In another aspect of the present invention, with respect to the cartilage tissue in the arthritis rat model treated with the mixed cell, staining for type 1 collagen and type 2 collagen

was performed and histologically analyzed, and as a result, it was confirmed that the mixed cell regenerated a hyaline cartilage tissue which was type 2 collagen-positive.

[0030] The macrophages which are innate immune cells are classified into activated "M1 macrophages" having a pro-inflammatory property and activated "M2 macrophages" having an immunosuppressive property according to an activation phenotype. IFN-γ is a key cytokine involved in the activation of the M1 macrophages and a TLR signal induced by pathogen recognition contributes to differentiation of the M1 macrophages. It has been known that the M1 macrophages increase expression of TNFα, IL-12 and IL-23 and decrease expression of IL-10 to induce Th1 and Th17 immune responses which cause tissue damage. In contrast, the M2 macrophages increase the expression level of IL-10 and decrease expression of IL-12, thereby reducing inflammation and promoting tissue regeneration.

[0031] In an aspect of the present invention, TGF-β expressed in the hChonJb#7 which is a cell expressing TGF-β1 and IL-10 induced through the hChonJ which is a human chondrocyte induces generation of the M2 macrophages and it is determined that the generated M2 macrophages secrete IL-10 to create an anti-inflammatory environment in the joints treated with the mixed

[0042] Example 1: Induction of pain relief and regeneration of Col II positive cartilage by treatment of mixed cell in arthritis animal model

[0043] In the case of treating a mixed cell in a clinical phase 2 for patients suffering with arthritis, it was observed that pain was reduced and an environment of joints was structurally improved. In order to confirm a mechanism therefor, in a degenerative arthritis animal model induced by MIA, a mixed cell was treated and a joint histological examination and a von Frey filament test were performed.

[0044] 1-1: Preparation of MIA-induced arthritis animal model

[0045] In preparation of an animal model, a 6-week-old male rat (Spargue-Dawley, 200 to 225 g, Nara Biotech, Korea) were used. An animal experiment was conducted under a veterinary control after approval of the Kolon Life Science (Korea) Institutional Animal Care and Use Committee (IACUC No. KLS IACUC 2013-04).

[0046] In order to induce arthritis, 50 μl of an MIA (monosodium iodoacetate, Sigma, USA) solution having a concentration of 60 mg/mL was treated to the articular cavity of the left knee of a rat by using a 31G syringe.

[0047] 1-2: Preparation of cells for cell therapy

[0048] The hChonJ used in the present invention is a human-derived chondrocyte. Preparation methods and mixing ratios of the cells are disclosed in a thesis [Cytotherapy, 2012 Feb; 14(2): 247-256] and US Patent Registration Nos. 7,005,127 and 7,282,200.

[0049] The hChonJb#7 may be prepared by injecting a cDNA of TGF-β into cells according to a known method. For example, a promoter

such as methallothionein and a cDNA of TGF-β are included in a known vector [for example, pUC19 (including an ampicillin resistance gene) by Gibco-BRL Corporation] having a resistance gene such as ampicillin and neomycin to prepare a vector including the cDNA of TGF-β. Thereafter, the vector including the cDNA of TGF-β is injected into a cell according to a known method such as a calcium phosphate method or a lipofectin method to prepare the hChonJb#7.

[0050] 1-3: Confirmation of pain relief and cartilage regeneration effects by treatment of mixed cell

[0051] After 2 weeks of MIA injection, mixed cells {(1.2×10^6 cells: hChonJ (9×10^5 cells) + hChonJb# 7 (3.0×10^5 cells)}, hChonJ (9×10^5 cells), hChonJb#7 (3.0×10^5 cells) or a control {CryoStor-10 (CS-10)} were treated to the articular cavity of the left knee. General symptoms were observed, and body weights were measured once a week.

[0052] The pain relief was observed by performing a von Frey filament test, and as a result, as illustrated in FIG. 2, pain levels decreased from 15 days after cell treatment, and such effects lasted until the end of observation (56th day after treatment). In the case of a group treated with hChonJb#7 (3.0×10^5 cells) expressing TGF-β, the pain relief was observed from 15 days, but after 6 weeks, it was confirmed that the effect disappeared. In contrast, in a group treated with hChonJ (9×10^5 cells) which was a normal human chondrocyte, the pain relief effect was not observed.

Claims:

1. A composition for treating rheumatoid arthritis, which comprises (i) a chondrocyte; and (ii) a cell into which a gene encoding TGF-β (transforming growth factor-beta) is introduced, wherein the cell in (ii) is hChonJb#7.

2. The composition according to claim 1, wherein a ratio of (i) a chondrocyte; and (ii) the cell into which the gene encoding TGF-β (transforming growth factor-beta) is introduced ranges from 1:1 to 100:1.

3. The composition according to claim 2, wherein the ratio of (i) the chondrocyte; and (ii) the cell into which the gene encoding TGF-β (transforming growth factor-beta) is introducedranges from 1:1 to 3:1.

4. A use of a composition comprising (i) a chondrocyte; and (ii) a cell into which a gene encoding TGF-β is introduced in the preparation of a medicament for treating an inflammatory disease induced by a hyperimmune response, wherein the cell in (ii) is hChonJb#7.

5. Use of a composition comprising (i) a chondrocyte; and (ii) a cell into which a gene encoding TGF-β is introduced in the preparation of a medicament for inhibiting the expression or activity of a cytokine selected from the group consisting of IFN-γ,

IL-6, IL-17A, IL-1β, and TNF-α, wherein the cell in (ii) is hChonJb#7.