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Pittenger et al.

(54) **MULTIPOTENT ADULT STEM CELLS AND USES OF MULTIPOTENT ADULT STEM CELLS TO TREAT INFLAMMATION**

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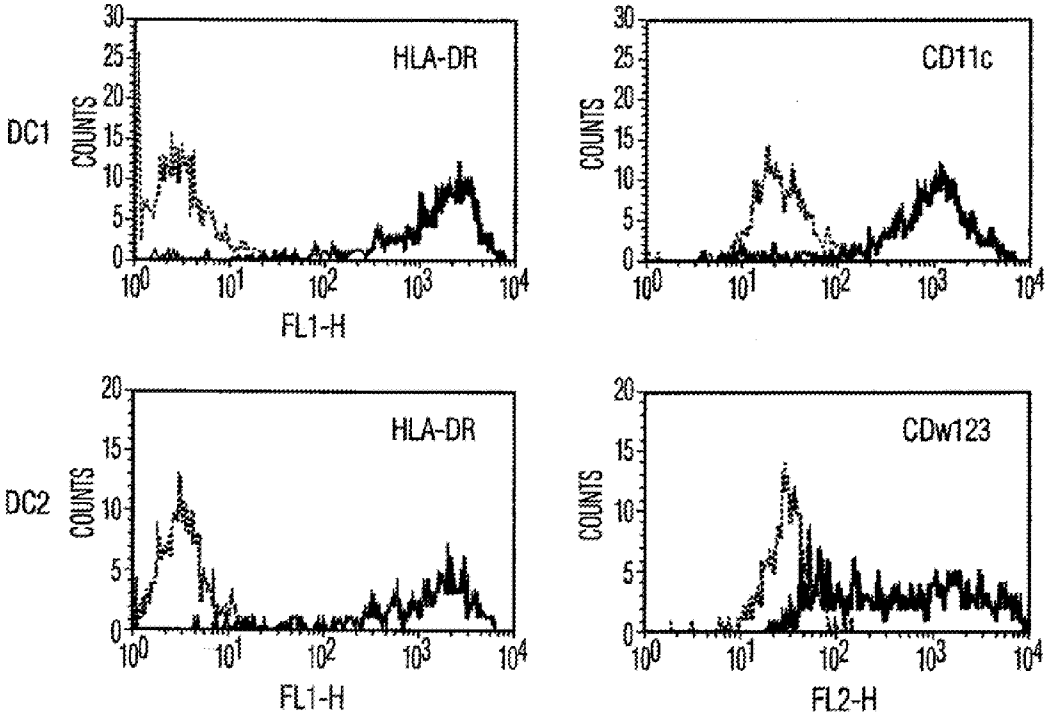
(57) **ABSTRACT**

Disclosed are cell preparations comprising multipotent adult stem cells and methods for using multipotent adult stem cells to treat autoimmune diseases, treat allergic responses, treat cancer, treat inflammatory diseases, treat fibrotic disorders, reduce inflammation and/or fibrosis, promote would healing, repair epithelial damage, and/or promote angiogenesis.



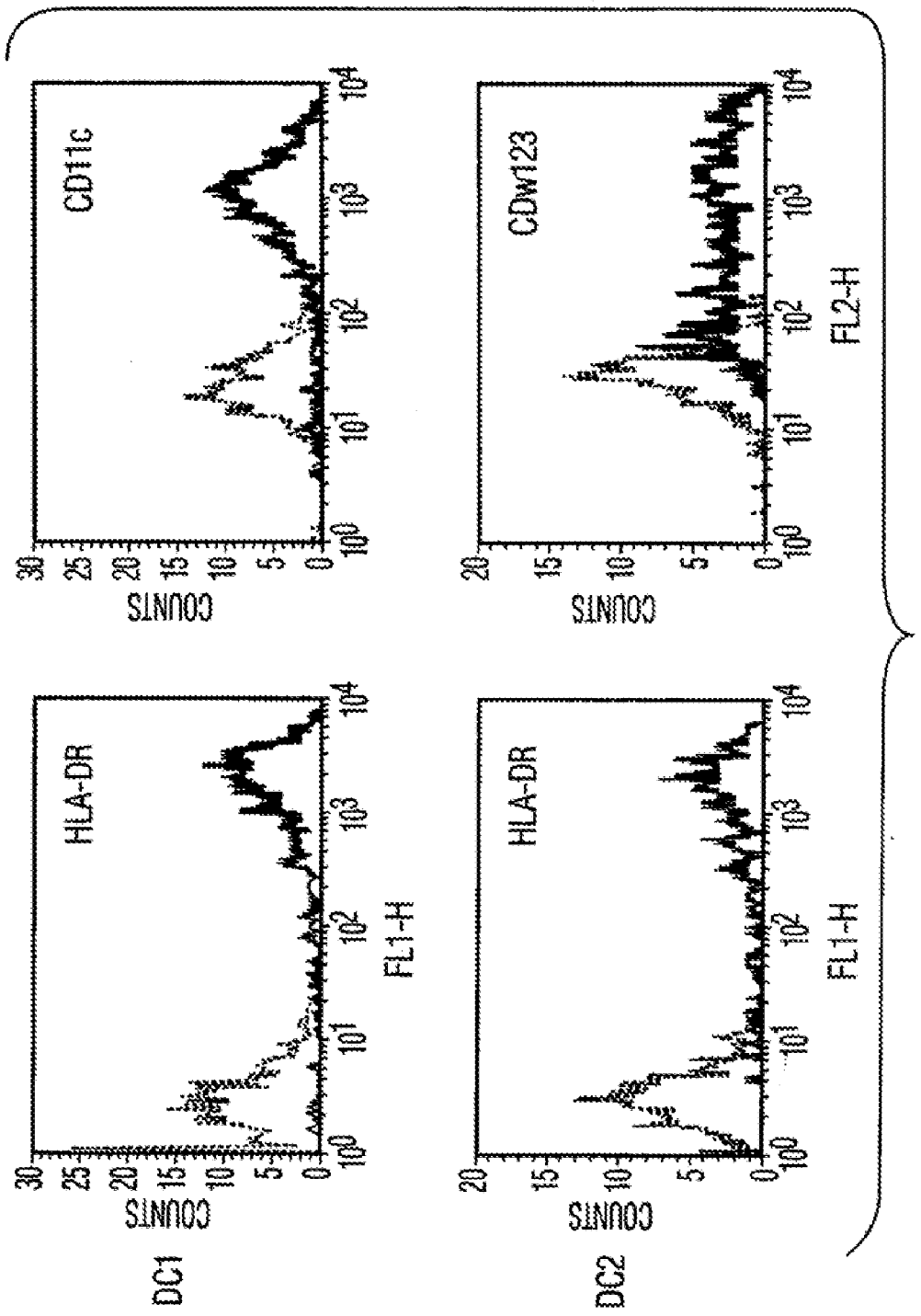


FIG. 1A

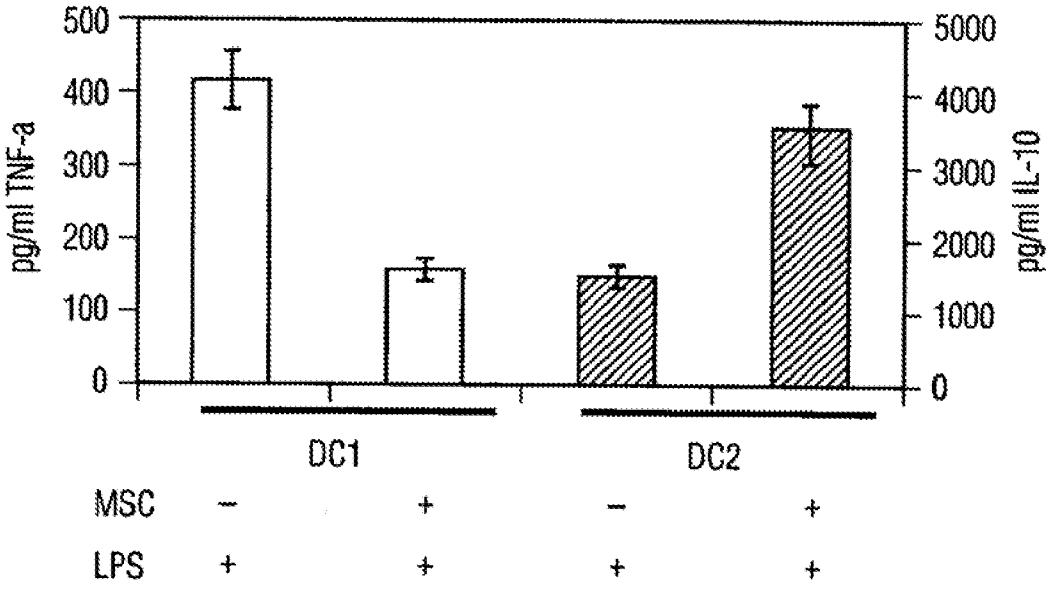

500
400
300
200
100
0
pg/ml TNF-a
5000
4000
3000
2000
1000
0
pg/ml IL-10
DC1
DC2
MSC – + – +
LPS + + + +

FIG. 1B

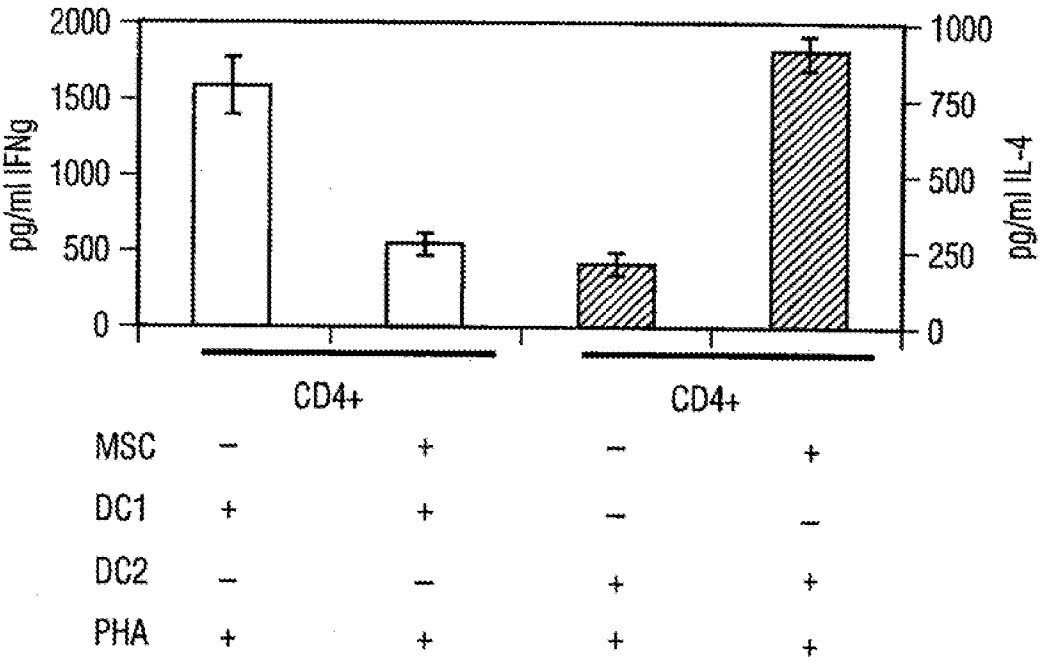

2000
1500
1000
500
0
pg/ml IFNg
1000
750
500
250
0
pg/ml IL-4
CD4+
CD4+
MSC – + – +
DC1 + + – –
DC2 – – + +
PHA + + + +

FIG. 1C

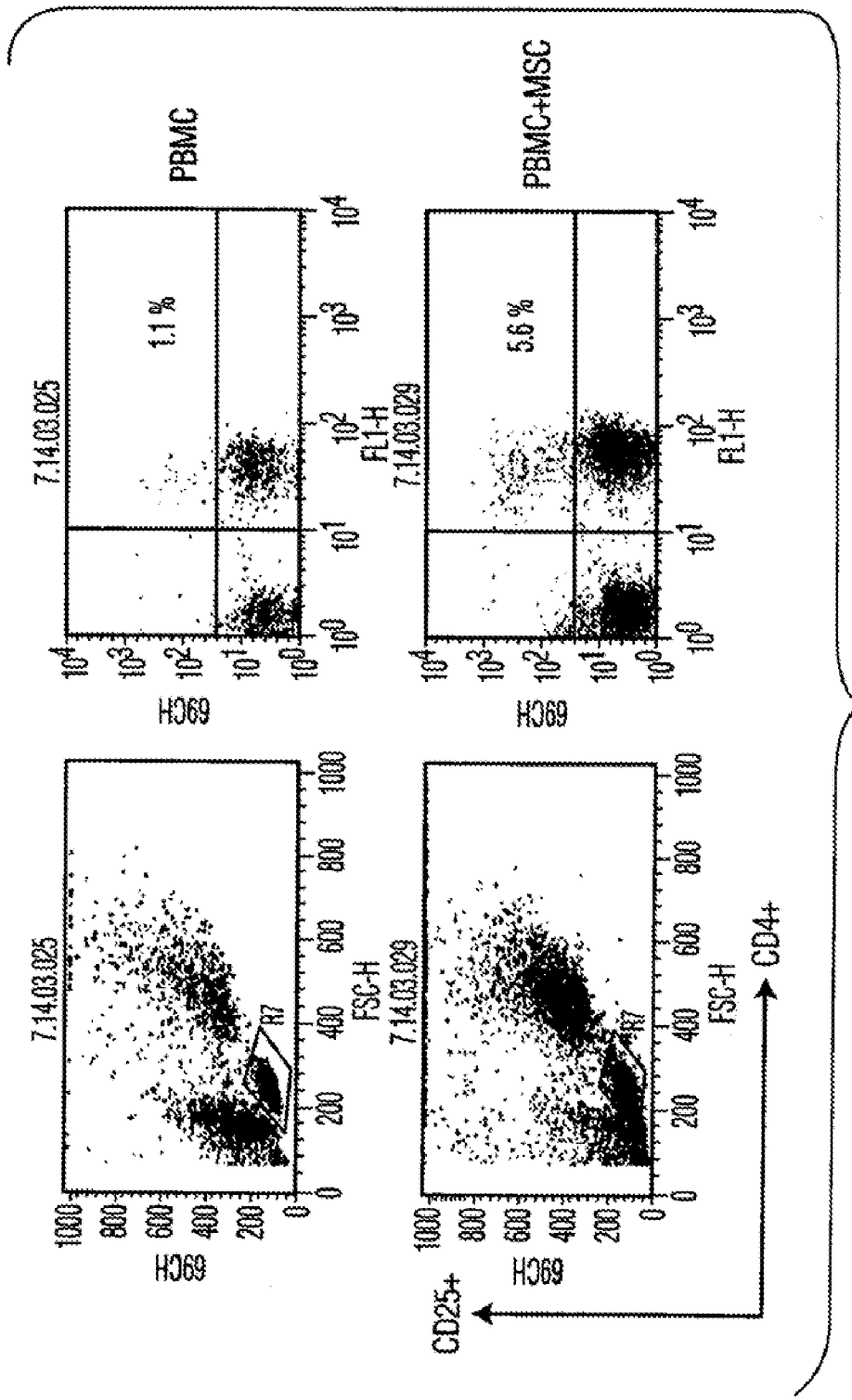

PBMC
PBMC+MSC
1.1 %
5.6 %
7.14.03.025
7.14.03.029
FL1-H
HC69
FSC-H
R7
CD25+
CD4+

FIG. 2A

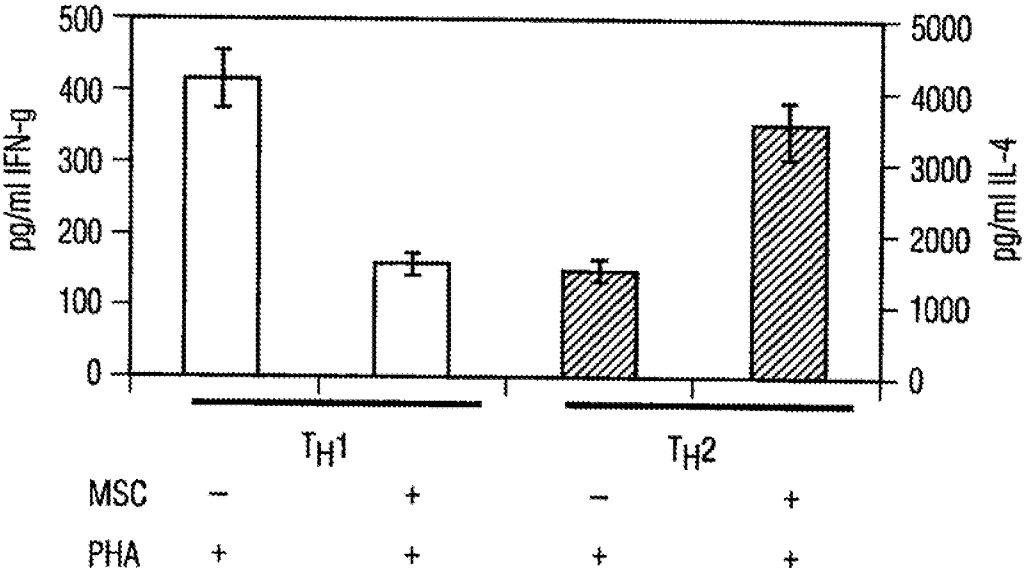

500
400
300
200
100
0
pg/ml IFN-g
5000
4000
3000
2000
1000
0
pg/ml IL-4
TH1
TH2
MSC – + – +
PHA + + + +

FIG. 2B

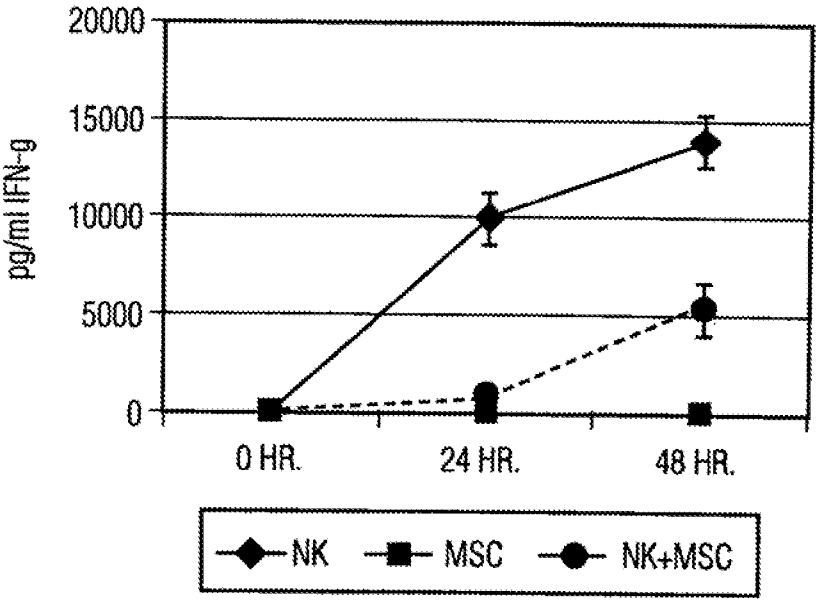

20000
15000
10000
5000
0
pg/ml IFN-g
0 HR.
24 HR.
48 HR.
NK
MSC
NK+MSC

FIG. 2C

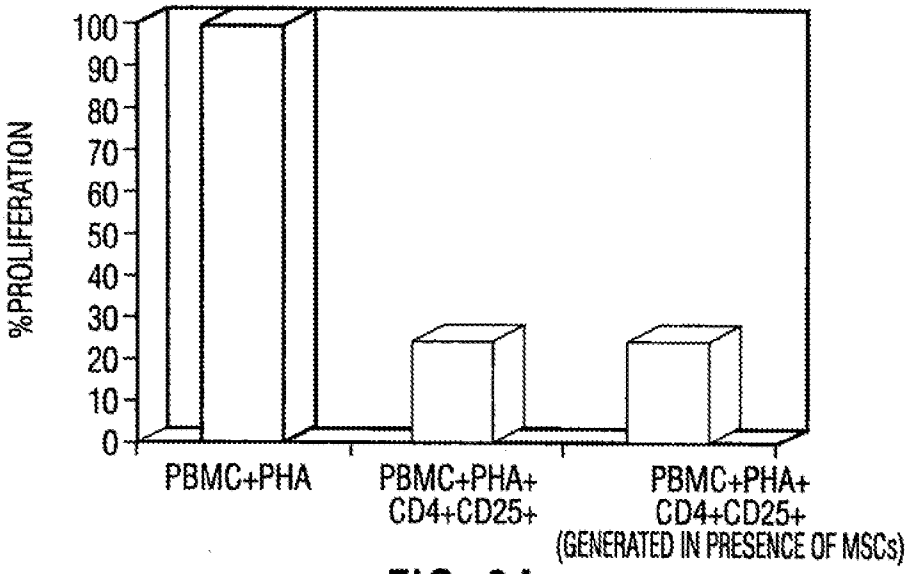

%PROLIFERATION
100
90
80
70
60
50
40
30
20
10
0
PBMC+PHA
PBMC+PHA+ CD4+CD25+
PBMC+PHA+ CD4+CD25+ (GENERATED IN PRESENCE OF MSCs)

FIG. 3A

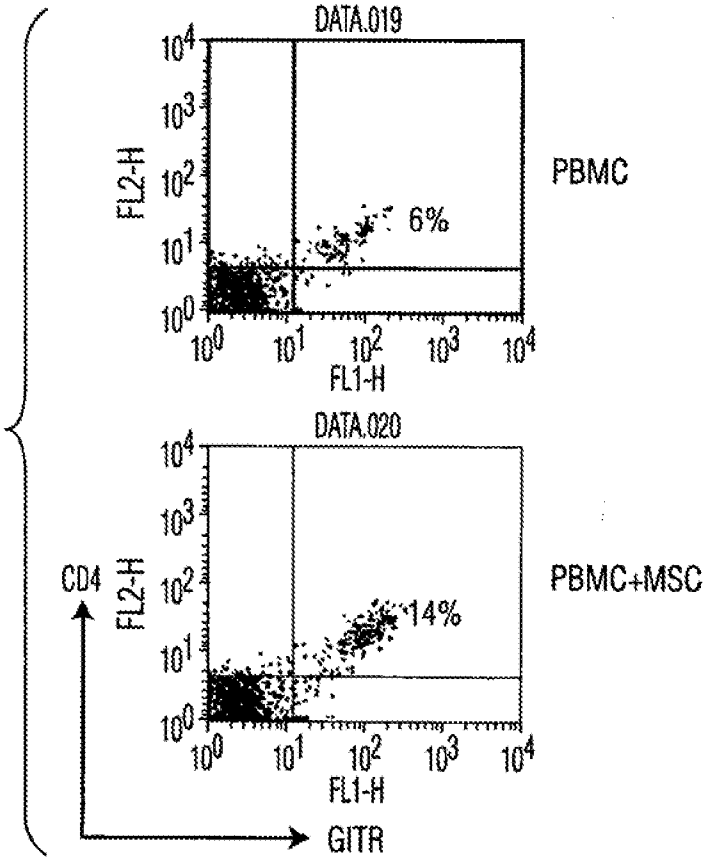

DATA.019
FL2-H
FL1-H
6%
PBMC
DATA.020
CD4
GITR
14%
PBMC+MSC

FIG. 3B

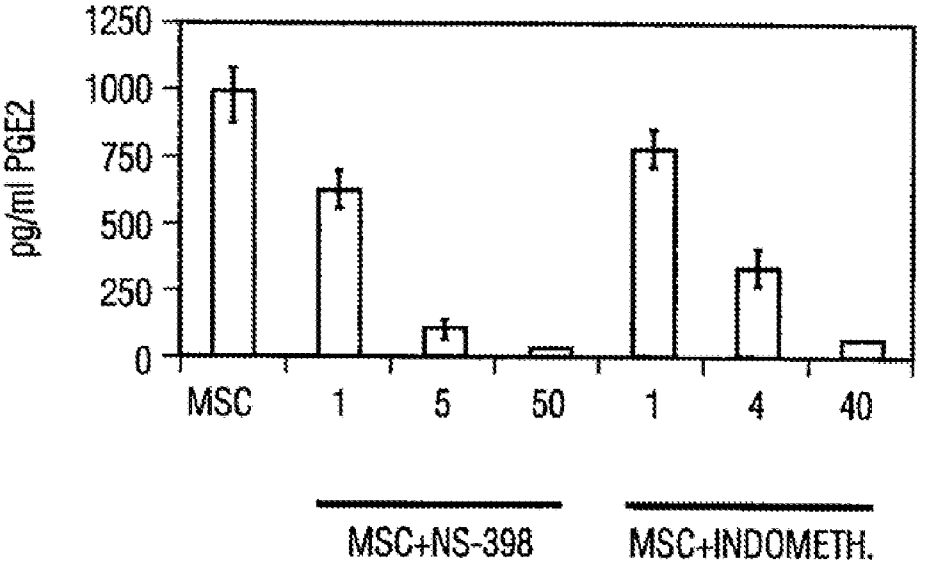

1250
1000
750
500
250
0
pg/ml PGE2
MSC
1
5
50
1
4
40
MSC+NS-398
MSC+INDOMETH.

FIG. 4A

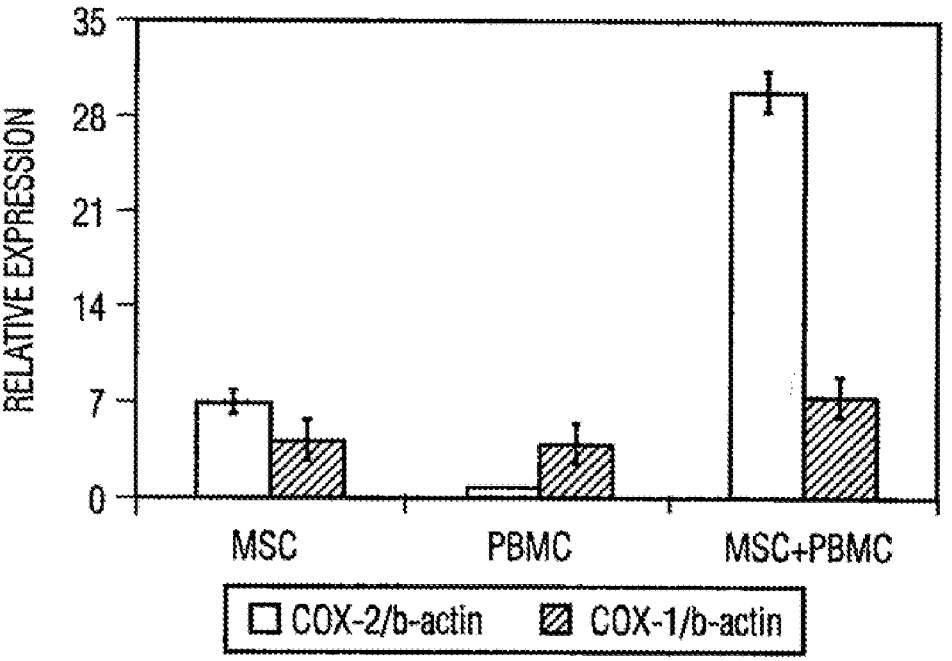

35
28
21
14
7
0
RELATIVE EXPRESSION
MSC
PBMC
MSC+PBMC
COX-2/b-actin
COX-1/b-actin

FIG. 4B

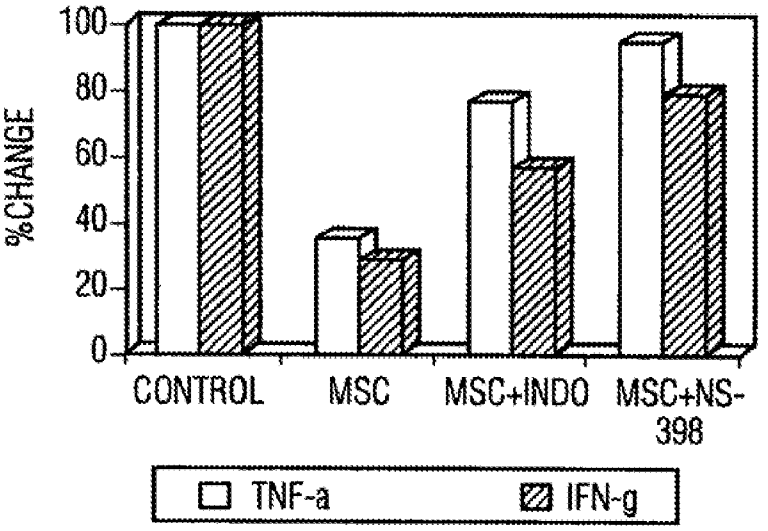

100
80
60
40
20
0
%CHANGE
CONTROL
MSC
MSC+INDO
MSC+NS-398
TNF-a
IFN-g

FIG. 4C

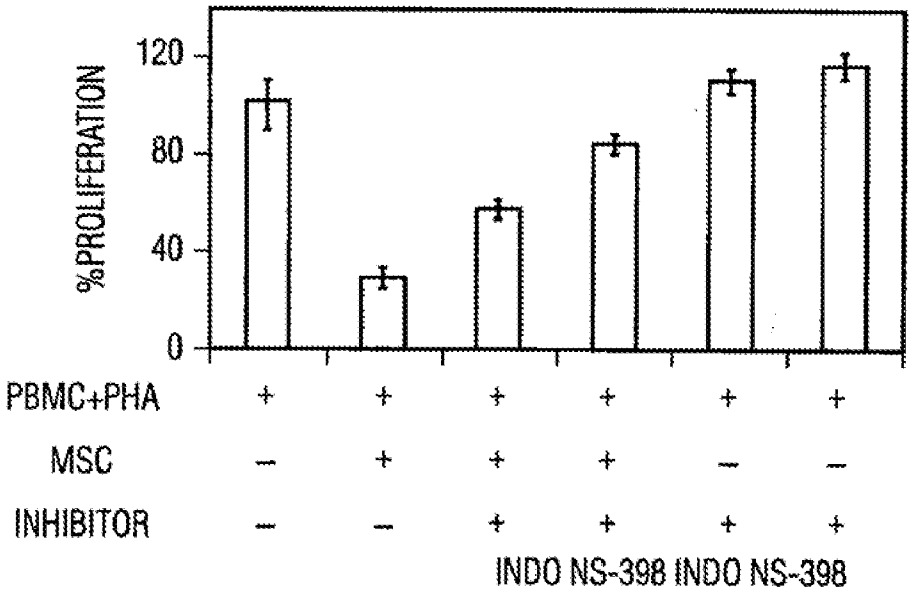

120
80
40
0
%PROLIFERATION
PBMC+PHA + + + + + +
MSC – + + + – –
INHIBITOR – – + + + +
INDO NS-398 INDO NS-398

FIG. 4D

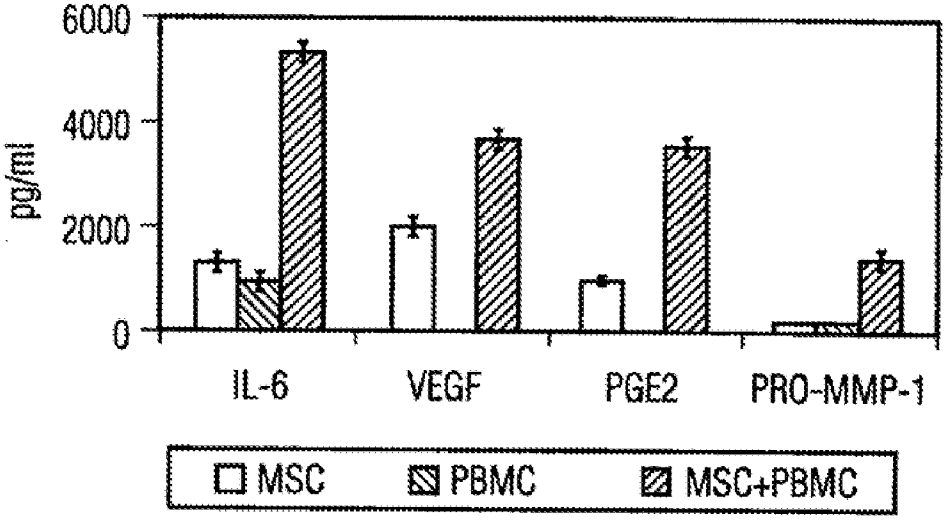

6000
4000
2000
0
pg/ml
IL-6
VEGF
PGE2
PRO-MMP-1
MSC
PBMC
MSC+PBMC

FIG. 5

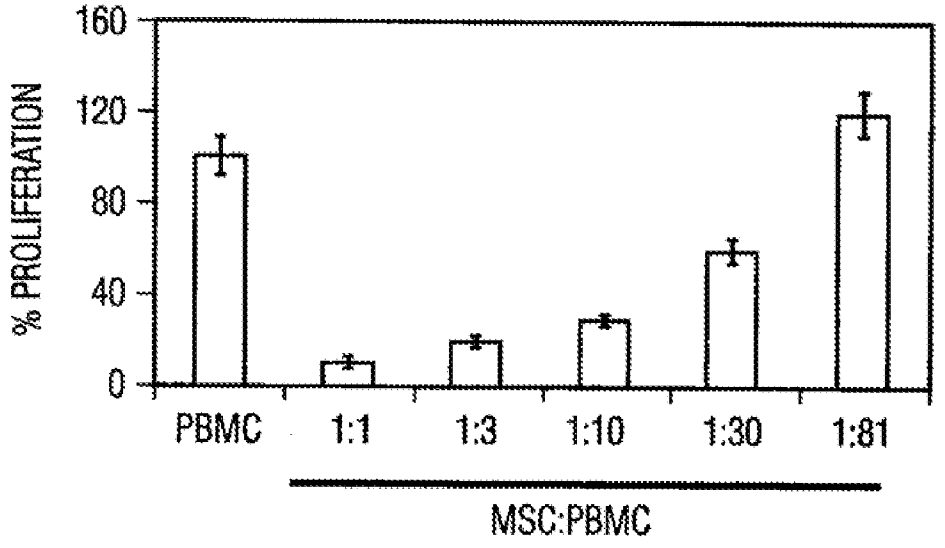

160
120
80
40
0
% PROLIFERATION
PBMC
1:1
1:3
1:10
1:30
1:81
MSC:PBMC

FIG. 6

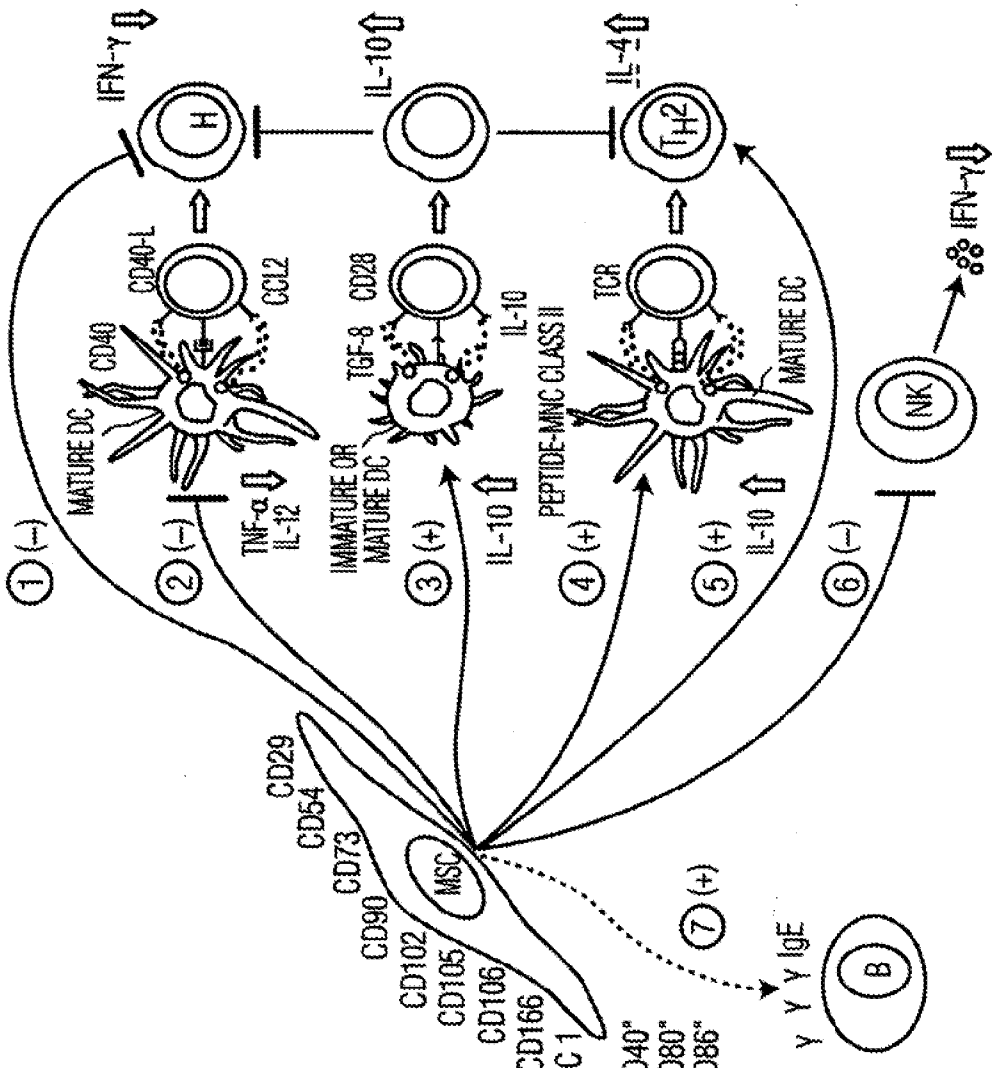

IFN-γ
IL-10
IL-4
H
TH2
NK
B
MSC
MATURE DC
IMMATURE OR MATURE DC
MATURE DC
CD40-L
CCL2
CD40
CD28
TGF-β
IL-10
TCR
PEPTIDE-MHC CLASS II
TNF-α
IL-12
IL-10
IL-10
IFN-γ
IgE
CD29
CD54
CD73
CD90
CD102
CD105
CD106
CD166
MHC 1
CD40*
CD80*
CD86*
① (–)
② (–)
③ (+)
④ (+)
⑤ (+)
⑥ (–)
⑦ (+)

FIG. 7

MULTIPOTENT ADULT STEM CELLS AND USES OF MULTIPOTENT ADULT STEM CELLS TO TREAT INFLAMMATION

RELATED APPLICATIONS

[0001] This application is a continuation of application Ser. No. 11/541,853, filed Oct. 2, 2006, which is a continuation-in-part of application Ser. No. 11/080,298, filed Mar. 15, 2005, which claims priority based on provisional application Ser. No. 60/555,118, filed Mar. 22, 2004; the contents of each application are hereby incorporated by reference in their entireties.

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] The present technology was made with Government support under Contract No. N66001-02-C-8068 awarded by the Department of the Navy. The Government has certain rights in this technology

BACKGROUND OF THE INVENTION

[0003] Mesenchymal stem cells (MSCs), which are present in adult bone marrow, are a kind of multipotent stem cell that can differentiate readily into lineages including osteoblasts, myocytes, chondrocytes, and adipocytes. (Pittenger, et al., *Science*, Vol. 284, pg. 143 (1999); Haynesworth, et al., *Bone*, Vol. 13, pg. 69 (1992); Prockop, *Science*, Vol. 276, pg. 71 (1997)). In vitro studies have demonstrated the capability of MSCs to differentiate into muscle (Wakitani, et al., *Muscle Nerve*, Vol. 18, pg. 1417 (1995)), neuronal-like precursors (Woodbury, et al., *J. Neurosci. Res.*, Vol. 69, pg. 908 (2002); Sanchez-Ramos, et al., *Exp. Neurol.*, Vol. 171, pg. 109 (2001)), cardiomyocytes (Toma, et al., *Circulation*, Vol. 105, pg. 93 (2002); Fakuda, *Artif. Organs*, Vol. 25, pg. 187 (2001)) and possibly other cell types. In addition, MSCs have been shown to provide effective feeder layers for expansion of hematopoietic and embryonic stem cells (Eaves, et al., *Ann. N.Y. Acad. Sci.*, Vol. 938, pg. 63 (2001); Wagers, et al., *Gene Therapy*, Vol. 9, pg. 606 (2002)). Recent studies with a variety of animal models have shown that MSCs can be useful in the repair and/or regeneration of damaged bone, cartilage, meniscus or myocardial tissues (DeKok, et al., *Clin. Oral Implants Res.*, Vol. 14, pg. 481 (2003)); Wu, et al., *Transplantation*, Vol. 75, pg. 679 (2003); Noel, et al., *Curr. Opin. Investig. Drugs*, Vol. 3, pg. 1000 (2002); Ballas, et al., *J. Cell. Biochem. Suppl.*, Vol. 38, pg. 20 (2002); Mackenzie, et al., *Blood Cells Mol. Dis.*, Vol. 27 (2002)). Several investigators have used MSCs with encouraging results for transplantation in animal disease models including osteogenesis imperfecta (Pereira, et al., *Proc. Nat. Acad. Sci.*, Vol. 95, pg. 1142 (1998)), parkinsonism (Schwartz, et al., *Hum. Gene Ther.*, Vol. 10, pg. 2539 (1999)), spinal cord injury (Chopp, et al., *Neuroreport*, Vol. 11, pg. 3001 (2000); Wu, et al., *J. Neurosci. Res.*, Vol. 72, pg. 393 (2003)) and cardiac disorders (Tomita, et al., *Circulation*, Vol. 100, pg. 247 (1999). Shake, et al., *Ann. Thorac. Surg.*, Vol. 73, pg. 1919 (2002)). Importantly, promising results also have been reported in clinical trials for osteogenesis imperfecta (Horwitz, et al., *Blood*, Vol. 97, pg. 1227 (2001); Horowitz, et al. *Proc. Nat. Acad. Sci.*, Vol. 99, pg. 8932 (2002)) and enhanced engraftment of heterologous bone marrow transplants (Frassoni, et al., *Int. Society for Cell Therapy*, SA006 (abstract) (2002); Koc, et al., *J. Clin. Oncol.*, Vol. 18, pg. 307 (2000)).

[0004] MSCs express major histocompatibility complex (MHC) class I antigen on their surface, but do not express MHC class II (Le Blanc, et al., *Exp. Hematol.*, Vol. 31, pg. 890 (2003); Potian, et al., *J. Immunol.*, Vol. 171, pg. 3426 (2003)) and do not express B7 or CD40 co-stimulatory molecules (Majumdar, et al., *J. Biomed. Sci.*, Vol. 10, pg. 228 (2003)), suggesting that these cells have a low-immunogenic phenotype (Tse, et al., *Transplantation*, Vol. 75, pg. 389 (2003)). MSCs also inhibit T-cell proliferative responses in an MHC-independent manner (Bartholomew, et al., *Exp. Hematol.*, Vol. 30, pg. 42 (2002); Devine, et al., *Cancer J.*, Vol. 7, pg. 576 (2001); DiNicola, et al., *Blood*, Vol. 99, pg. 3838 (2002)). These immunological properties of MSCs can enhance their transplant engraftment and limit the ability of the recipient's immune system to recognize and reject allogeneic cells following transplantation. The production of factors by MSCs, that modulate the immune response and support hematopoiesis together with their ability to differentiate into appropriate cell types under local stimuli make them desirable stem cells for cellular transplantation studies (Majumdar, et al., *Hematother. Stem Cell Res.*, Vol. 9, pg. 841 (2000); Haynesworth, et al., *J. Cell. Physiol.*, Vol. 166, pg. 585 (1996).

BRIEF SUMMARY OF THE INVENTION

[0005] The present technology generally relates to multipotent adult stem cells, such as adult bone marrow-derived stem cells. More particularly, the present technology relates to new and heretofore unappreciated uses for multipotent adult stem cells, such as mesenchymal stem cells, including, but not limited to promoting angiogenesis in various tissues and organs, treating autoimmune diseases, treating allergic responses, treating cancer, treating inflammatory diseases and disorders, promoting wound healing, treating inflammation, and repairing epithelial damage.

[0006] In accordance with the present technology, multipotent adult stem cells can be used, for example, to treat an autoimmune disease, treat an inflammatory response, treat an allergic disease, treat a pulmonary disease having fibrotic and/or inflammatory components, repair epithelial damage, and promote wound healing in subjects, including a human subject. Autoimmune diseases that can be treated with multipotent adult stem cells include, for example, Type 1 Diabetes, inflammatory bowel disease, Crohn's disease, and uveitis. Pulmonary diseases that can be treated with multipotent adult stem cells include, but are not limited to, Acute Respiratory Distress Syndrome (ARDS), Chronic Obstructive Pulmonary Disease (COPD), and asthma. Inflammatory responses, including those associated with autoimmune diseases or pulmonary diseases, can be reduced with multipotent adult stem cells. An inflammatory response can be reduced by, for example, reducing the production or expression of pro-inflammatory mediators, increasing the production or expression of anti-inflammatory mediators, or a combination thereof.

[0007] Multipotent adult stem cells of the present technology have the capacity to differentiate into at least one cell type of each of the mesodermal, ectodermal, and endodermal lineages. For example, the cells can be induced to differentiate into cells of at least osteoblast, chondrocyte, adipocyte, fibroblast, marrow stroma, skeletal muscle, smooth muscle, cardiac muscle, endothelial, epithelial, hematopoietic, glial, neuronal or oligodendrocyte cell types, among others. Multipotent adult stem cells of the present technology are capable

of differentiating into at least one cell type of at least one of the endodermal, ectodermal, or mesodermal embryonic lineages.

[0008] Adult bone marrow is an accessible and renewable source of adult multipotent stem cells that can be greatly expanded in culture. For example, bone marrow-derived mesenchymal stem cells (MSCs) are multipotent cells that have been identified and cultured from various avian and mammalian species.

[0009] The present technology provides one or more cell preparations comprising adult bone marrow-derived stem cells in one or more doses effective to treat an inflammatory response in a subject. The subject can be a human having, for example, inflammatory bowel disease. The stem cells can be capable of differentiating into at least one cell type of each of the endodermal, ectodermal, and mesodermal embryonic lineages. The effective dose of the cell preparation can contain a sufficient number of stem cells to provide about 1×10^5 to about 1×10^7 cells per kilogram of the subject. In certain embodiments, the subject can be a mammal. The mammal can be, for example, a primate, including a human and a non-human primate. Administration of the cell preparation can, for example, elevate interferon-beta levels in the subject.

[0010] The present technology also provides a method of treating inflammatory bowel disease in a subject, comprising administering to the subject allogeneic multipotent adult stem cells in an amount effective to treat inflammatory bowel disease. In certain embodiments, the subject can be an animal. The animal can be, for example, a primate, including a human and a non-human primate. The multipotent adult stem cells can be administered systemically, such as intravenously, intraarterially, or intraperitoneally. The multipotent adult stem cells can be administered in conjunction with an acceptable pharmaceutical carrier, such as a pharmaceutically acceptable liquid medium. The multipotent adult stem cells can be administered as a suspension of cells. The amount effective to treat inflammatory bowel disease can be about 1×10^5 to about 1×10^7 cells per kilogram of the subject.

[0011] The present technology further provides a method of treating a gastrointestinal autoimmune disease in a subject, comprising administering to the subject allogeneic multipotent adult stem cells in an amount effective to repair or regenerate intestinal tissue. The autoimmune disease can be selected from the group consisting of Crohn's disease, inflammatory bowel disease, and autoimmune gastritis. In certain embodiments, the subject can be an animal. The animal can be, for example, a primate, including a human and a non-human primate. The multipotent adult stem cells can be administered systemically, such as intravenously, intraarterially, or intraperitoneally. The multipotent adult stem cells can be administered in conjunction with an acceptable pharmaceutical carrier, such as a pharmaceutically acceptable liquid medium. The multipotent adult stem cells can be administered as a suspension of cells. The amount effective to repair or regenerate intestinal tissue can be about 1×10^5 to about 1×10^7 cells per kilogram of the subject.

[0012] The multipotent adult stem cells of the present technology can be positive for one or more cell surface markers selected from, for example, integrin al (CD49a); integrin $\alpha2$ (CD49b); integrin $\alpha3$ (CD49c); integrin $\alpha5$ (CD49e); integrin αV (CD51); integrin $\beta1$ (CD29); integrin $\beta3$ (CD61); integrin $\beta4$ (CD104); IL-1R (CD121a); IL-3Rα (CD123); IL-4R (CDw124); IL-6R (CD126); IL-7R (CDw127); IFNγR (CDw119); TNFIR (CD120a); TNFIIR (CD120b); TGFβ1R; TGFβIIR; bFGFR; PDGFR (CD140a); transferrin (CD71); ICAM-1 (CD54); ICAM-2 (CD102); VCAM-1 (CD106); L-Selectin (CD62L); LFA-3 (CD58); ALCAM (CD166); hyaluronate (CD44); endoglin (CD105); Thy-1 (CD90); or CD9. The stem cells can also be positive for each of CD49b, CD49e, and CD140a. Further, the stem cells can be negative for one or more cell surface markers such as integrin $\alpha4$ (CD49d); integrin αL (CD11a); integrin C$\beta2$ (CD18); CD4; CD14; CD34; CD45; IL-2R (CD25); EGFR-3; Fas ligand; ICAM-3 (CD50); E-Selectin (CD62E); P-Selectin (CD62P); vW Factor; cadherin 5; or Lewis x (CD15). In addition, the multipotent adult stem cells of the present technology can be adult bone marrow-derived stem cells such as, for example, mesenchymal stem cells.

[0013] The present technology also provides a method of repairing and/or regenerating intestinal tissue in a subject, comprising treating the subject with allogeneic mesenchymal stem cells. In certain embodiments, the subject can be an animal. The animal can be, for example, a primate, including a human and a non-human primate. The stem cells can be from an adult. The mesenchymal stem cells can be administered to the subject as part of, for example, a pharmaceutical formulation having a pharmaceutically acceptable carrier, such as a liquid injectable carrier, a liquid topical carrier, a gel injectable carrier, a gel topical carrier, a solid matrix, and combinations thereof. The mesenchymal stem cell pharmaceutical formulation can be administered via intravenous, intraarterial, or intraperitoneal injection. Mesenchymal stem cells can be administered in an amount effective to repair or regenerate intestinal tissue in a subject. The amount effective to repair or regenerate intestinal tissue can be about 1×10^5 to about 1×10^7 cells per kilogram of the subject.

[0014] Applicants presently have examined the interactions of multipotent adult stem cells with isolated immune cell populations, including dendritic cells (DC1 and DC2), effector T-cells (Th1 and Th2), and NK cells. Based on such interactions, Applicants discovered that multipotent adult stem cells regulate the production of various factors that affect several steps in the inflammatory and/or immune response process. Thus, the multipotent adult stem cells of the present technology can be employed in the treatment of disease conditions and disorders involving the immune system, or diseases, conditions, or disorders involving inflammation, epithelial damage, or allergic responses. Such diseases, conditions, and disorders include, but are not limited to, autoimmune diseases, allergies, arthritis, inflamed wounds, alopecia araeta (baldness), periodontal diseases including gingivitis and periodontitis, and other diseases, conditions or disorders involving an immune response.

[0015] In addition, it is believed that multipotent adult stem cells, including mesenchymal stem cells, express and secrete vascular endothelial growth factor, or VEGF, which promotes angiogenesis by stimulating the formation of new blood vessels. Mesenchymal stem cells also stimulate peripheral blood mononuclear cells (PBMCs) to produce VEGF.

[0016] Furthermore, it is believed that multipotent adult stem cells stimulate dendritic cells (DCs) to produce Interferon-Beta (IFN-β), which promotes tumor suppression and immunity against viral infection.

BRIEF DESCRIPTION OF SEVERAL VIEWS OF THE DRAWINGS

[0017] The present technology now will be described with respect to the drawings.

[0018] FIG. **1**. MSCs modulate dendritic cell functions. (A) Flow cytometric analysis of mature monocytic DC1 cells using antibodies against HLA-DR and CD11c and of plasmacytoid DC2 cells using antibodies against HLA-DR and CD123 (IL-3 receptor). (---): isotype control; (-): FITC/PE conjugated antibodies. (B) MSCs inhibit TNF-α secretion (primary y-axis) and increase IL-10 secretion (secondary y-axis) from activated DC1 and DC2 respectively. (C) MSCs cultured with mature DC1 cells inhibit IFN-γ secretion (primary y-axis) by T cells and increase IL-4 levels (secondary y-axis) as compared to MSC or DC alone. The decreased production of pro-inflammatory IFN-γ and increased production of anti-inflammatory IL-4 in the presence of MSCs indicated a shift in the T cell population towards an anti-inflammatory phenotype.

[0019] FIG. **2**. MSCs inhibit pro-inflammatory effector T cell function. (A) Flow cytometric analysis of T_{reg} cell numbers (in %) by staining PBMCs or the non-adherent fraction in MSC+PBMC culture (MSC+PBMC) with FITC-conjugated CD4 (x-axis) and PE conjugated CD25 (y-axis) antibodies. Gates were set based on isotype control antibodies as background. Graphs are representative of 5 independent experiments. (B) Th1 cells generated in presence of MSCs secreted reduced levels of IFN-γ (primary Y-axis) and Th2 cells generated in presence of MSCs secreted increased amounts of IL-4 (secondary y-axis) in cell culture supernatants. (C) MSCs inhibit IFN-γ secretion from purified NK cells cultured for 0, 24, or 48 hours in a 24-well plate. Data shown are mean±SD cytokine secretion in one experiment and are representative of 3 independent experiments.

[0020] FIG. **3**. MSCs lead to increased numbers of T_{reg} cell population and increased GITR expression. (A) A CD44+ CD25+T_{reg} cell population from PBMC or MSC+PBMC (MSC to PBMC ratio 1:10) cultures (cultured without any further stimulation for 3 days) was isolated using a 2-step magnetic isolation procedure. These cells were irradiated (to block any further proliferation) and used as stimulators in a mixed lymphocyte reaction (MLR), where responders were allogeneic PBMCs (stimulator to responder ratio 1:100) in the presence of phytohemagglutinin (PHA) (2.5 mg/ml). The cells were cultured for 48 hours; 3H thymidine was added; and incorporated radioactivity was counted after 24 hours. The results showed that the T_{reg} population, generated in the presence of MSCs (lane 3) was similar functionally to the T_{reg} cells generated in the absence of MSCs (lane 2). (B) PBMCs were cultured for 3 days in the absence (top plot) or presence (bottom plot) of MSCs (MSC to PBMC ratio 1:10), following which the non-adherent fraction was harvested and immunostained with FITC-labeled GITR and PE-labeled CD4. Results show a greater than twofold increase in GITR expression in cells cultured in the presence of MSCs.

[0021] FIG. **4**. MSCs produce PGE_2 and blocking PGE_2 reverses MSC-mediated immuno-modulatory effects. (A) PGE_2 secretion (mean±SD) in culture supernatants obtained from MSCs cultured in the presence or absence of PGE_2 blockers NS-398 or indomethacin (Indometh.) at various concentrations. Inhibitor concentrations are in μM and data presented are values obtained after 24 hour culture (B) COX-1 and COX-2 expression in MSCs and PBMCs using real-time RT-PCR. MSCs expressed significantly higher levels of COX-2 as compared to PBMCs, and when MSCs were cultured in presence of PBMCs, there was a >3-fold increase in COX-2 expression in MSCs. Representative data from 1 of 3 independent experiments are shown. The MSC+PBMC cultures were setup in a trans-well chamber plate where MSCs were plated onto the bottom chamber and PBMCs onto the top chamber. (C) Presence of PGE_2 blockers indomethacin (Ind.) or NS-398 increases TNF-α secretion from activated DCs (open bars) and IFN-γ secretion from Th1 cells (hatched bars) as compared to controls. Data were calculated as % change from cultures generated in absence of MSCs and PGE_2 inhibitors (D) Presence of PGE_2 blockers indomethacin (Indo) and NS-398 during MSC¬PBMC co-culture (1:10) reverses MSC-mediated anti-proliferative effects on PHA-treated PBMCs. Data shown are from one experiment and are representative of 3 independent experiments.

[0022] FIG. **5**. Constituitive MSC cytokine secretion is elevated in the presence of allogeneic PBMCs. Using previously characterized human MSCs, the levels of the cytokines IL-6 and VEGF, lipid mediator PGE_2, and matrix metalloproteinase 1 (pro MMP-1) in culture supernatant of MSCs cultured for 24 hours in the presence (hatched bars) or absence (open bars) of PBMCs (MSC to PBMC ratio 1:10) were analyzed. The MSCs produced IL-6, VEGF, and PGE_2 constituitively, and the levels of these factors increased upon co-culture with PBMCs, thereby suggesting that MSCs can play a role in modulating immune functions in an inflammatory setting.

[0023] FIG. **6**. MSCs inhibit mitogen-induced T-cell proliferation in a dose-dependent manner. Increasing numbers of allogeneic PBMCs were incubated with constant numbers of MSCs (2,000 cells/well) plated on a 96-well plate in the presence or absence of PHA (2.5 mg/ml) for 72 hours, and 3H thymidine incorporation determined (in counts per minute, or cpm). There was a dose-dependent inhibition of the proliferation of PHA-treated PBMCs in the presence of MSCs. Representative results from 1 of 3 independent experiments are shown. Similar results were reported by LeBlanc, et al., Scand J. Immunol., Vol. 57, pg. 11 (2003).

[0024] FIG. **7**. Schematic diagram of proposed MSC mechanism of action. MSCs mediate their immuno-modulatory effects by affecting cells from both the innate (DC-pathways 2-4; and NK-pathway 6) and adaptive (T-pathways 1 and 5 and B-pathway 7) immune systems. In response to an invading pathogen, immature DCs migrate to the site of potential entry, mature and acquire an ability to prime naïve T cells (by means of antigen specific and co-stimulatory signals) to become protective effector T cells (cell-mediated Th1 or humoral Th2 immunity). During MSC-DC interaction, MSCs, by, for example, means of direct cell-cell contact or via secreted factor, can alter the outcome of immune response by limiting the ability of DCs to mount a cell-mediated response (pathway 2) or by promoting the ability to mount a humoral response (pathway 4). Also, when mature effector T cells are present, MSCs can interact with them to skew the balance of Th1 (pathway 1) responses towards TH2 responses (pathway 5), and probably towards an increased IgE producing B cell activity (pathway 7), desirable outcomes for suppression of GvHD and autoimmune disease symptoms. MSCs in their ability to result in an increased generation of T_{reg} population (pathway 3) can result in a tolerant phenotype and can aid a recipient host by dampening bystander inflammation in their local micro-environment. Dashed line (----) represents proposed mechanism.

DETAILED DESCRIPTION OF THE INVENTION

[0025] In accordance with an aspect of the present technology, there is provided a method of treating a disease selected

from the group consisting of autoimmune diseases and graft-versus-host disease in an animal, including, for example, a human. The method comprises at least the step of administering to the animal mesenchymal stem cells in an amount effective to treat the disease in the animal.

[0026] Although the scope of this aspect of the present technology is not to be limited to any theoretical reasoning, it is believed that at least one mechanism by which the mesenchymal stem cells suppress autoimmune disease and graft-versus-host disease is by causing the release of Interleukin-10 (IL-10) from regulatory T-cells (T_{reg} cells) and/or dendritic cells (DC).

[0027] Autoimmune diseases which can be treated in accordance with the present technology include, but are not limited to, multiple sclerosis, Type 1 diabetes, rheumatoid arthritis, uveitis, autoimmune thyroid disease, inflammatory bowel disease, scleroderma, Graves' Disease, lupus, Crohn's disease, autoimmune lymphoproliferative disease (ALPS), demyelinating disease, autoimmune encephalomyelitis, autoimmune gastritis (AIG), and autoimmune glomerular diseases. Also, as noted hereinabove, graft-versus-host disease can be treated. It is to be understood, however, that the scope of the present technology is not to be limited to the treatment of the specific diseases mentioned herein.

[0028] In certain embodiments, the mesenchymal stem cells are administered to a mammal. The mammal can be a primate, including human and non-human primates.

[0029] In general, the multipotent adult stem cell therapy is based, for example, on the following sequence: harvest of stem cell-containing tissue such as, for example, bone marrow; isolation and/or expansion of stem cells; and administration of the stem cells, with or without biochemical or genetic manipulation, to the animal.

[0030] The mesenchymal stem cells that are administered can be, for example, a homogeneous composition or a mixed cell population enriched in mesenchymal stem cells. The mesenchymal stem cell compositions can be obtained, for example, by culturing adherent bone marrow or periosteal cells. Adult bone marrow-derived stem cells, such as bone marrow-derived mesenchymal stem cells, can be identified by specific cell surface markers, which are capable of being bound by unique monoclonal antibodies.

[0031] A method for obtaining a cell population enriched in mesenchymal stem cells is described in, for example, U.S. Pat. No. 5,486,359, the contents of which are hereby incorporated by reference in its entirety. Adult bone marrow contains multipotent stem cells, including mesenchymal stem cells. Alternative sources for multipotent adult stem cells such as mesenchymal stem cells include, but are not limited to, blood, skin, cord blood, muscle, fat, bone, and perichondrium.

[0032] Multipotent adult stem cells can be obtained from a variety of sources, including bone marrow. For example, multipotent adult stem cells such as human mesenchymal stem cells can be obtained from bone marrow from a number of different sources, including plugs of femoral head cancellous bone pieces, patients with degenerative joint disease during hip or knee replacement surgery, and aspirated marrow from normal donors or oncology patients who have marrow harvested for future bone marrow transplantation. Harvested marrow can be prepared for cell culture by a number of different mechanical isolation processes depending upon the source of the harvested marrow (i.e., the presence of bone chips, peripheral blood, etc.) that are well known in the art. Exemplary culture media and culture conditions are identified in, for example, U.S. Pat. No. 5,486,359 and include media and conditions that allow for expansion, growth, and isolation of mesenchymal stem cells, without differentiation.

[0033] Multipotent stem cells isolated from human adult bone marrow can be surface antigen positive for integrin al (CD49a); integrin α2 (CD49b); integrin α3 (CD49c); integrin α5 (CD49e); integrin αV (CD51); integrin β1 (CD29); integrin β3 (CD61); integrin β4 (CD104); IL-1R (CD121a); IL-3Rα (CD123); IL-4R (CDw124); IL-6R (CD126); IL-7R (CDw127); IFNγR (CDw119); TNFIR (CD120a); TNFIIR (CD120b); TGFβ1R; TGFβIIR; bFGFR; PDGFR (CD140a); transferrin (CD71); ICAM-1 (CD54); ICAM-2 (CD102); VCAM-1 (CD106); L-Selectin (CD62L); LFA-3 (CD58); ALCAM (CD166); hyaluronate (CD44); endoglin (CD105); Thy-1 (CD90); CD9; and combinations thereof. Cell isolated from human adult bone marrow can be surface antigen negative for integrin α4 (CD49d); integrin αL (CD11a); integrin Cβ2 (CD18); CD4; CD14; CD34; CD45; IL-2R (CD25); EGFR-3; Fas ligand, ICAM-3 (CD50); E-Selectin (CD62E); P-Selectin (CD62P); vW Factor; cadherin 5; Lewis x (CD15); and combinations thereof.

[0034] Cell preparations having greater than about 95%, usually greater than about 98%, of multipotent adult human stem cells can be achieved using techniques for isolation, purification, and culture expansion of stem cells. For example, isolated, cultured adult bone marrow-derived stem cells such as mesenchymal stem cells can comprise a single phenotypic population (about 95% or about 98% homogeneous) by flow cytometric analysis of expressed surface antigens. The desired cells in such composition can be identified, for example, by expression of a cell surface marker (e.g., CD73 or CD105) specifically bound by an antibody produced from hybridoma cell line SH2, ATCC accession number HB 10743, an antibody produced from hybridoma cell line SH3, ATCC accession number HB 10744, or an antibody produced from hybridoma cell line SH4, ATCC accession number HB 10745. Such antibodies selectively bind bone marrow-derived mesenchymal stem cells and, therefore, can be used to identify, quantify, isolate, or purify mesenchymal stem cells from bone marrow samples.

[0035] The mesenchymal stem cells can be administered by a variety of procedures. The mesenchymal stem cells can be administered systemically, such as by intravenous, intraarterial, or intraperitoneal administration.

[0036] The mesenchymal stem cells can be from a spectrum of sources including autologous, allogeneic, or xenogeneic.

[0037] The mesenchymal stem cells are administered in an amount effective to treat an autoimmune disease or graft-versus-host disease in an animal. The mesenchymal stem cells can be administered in an amount of from about 1×10^5 cells/kg to about 1×10^7 cells/kg. In other embodiments, the mesenchymal stem cells are administered in an amount of from about 1×10^6 cells/kg to about 5×10^6 cells/kg. In other embodiments, the mesenchymal stem cells are administered in an amount of from about 2×10^6 cells/kg to about 4×10^6 cells/kg. In still other embodiments, the mesenchymal stem cells are administered in an amount of about 3×10^6 cells/kg. The amount of mesenchymal stem cells to be administered is dependent upon a variety of factors, including the age, weight, and sex of the subject and/or patient, the autoimmune disease to be treated, and the extent and severity thereof.

[0038] The mesenchymal stem cells can be administered in conjunction with an acceptable pharmaceutical carrier. For example, the mesenchymal stem cells can be administered as a cell suspension in a pharmaceutically acceptable liquid medium or gel for injection or topical application.

[0039] In accordance with another aspect of the present technology, there is provided a method of treating an inflammatory response in an animal. The method comprises administering to the animal mesenchymal stem cells in an amount effective to treat the inflammatory response in the animal. Without wishing to be bound by any particular theory, the mesenchymal stem cells prevent or reverse an inflammatory response by, for example, increasing expression, production, and/or secretion of pro-inflammatory cytokines such as, for example, tumor necrosis factor-alpha (TNF-α) and Interferon-γ (IFN-γ); decreasing expression, production, and/or secretion of anti-inflammatory cytokines such as, for example, IL-10 and IL-4; and/or combinations thereof.

[0040] Although the scope of this aspect of the present technology is not to be limited to any theoretical reasoning, it is believed that the mesenchymal stem cells promote T-cell maturation to regulatory T-cells (T_{reg}), thereby controlling inflammatory responses. It is also believed that the mesenchymal stem cells inhibit T helper 1 cells (Th1 cells), thereby decreasing the expression of the IFN-γ in certain inflammatory reactions, such as those associated with psoriasis, for example.

[0041] In certain embodiments, the inflammatory responses which can be treated are those associated with psoriasis.

[0042] In other embodiments, the mesenchymal stem cells can be administered to an animal such that the mesenchymal stem cells prevent or reduce inflammation in the brain by, for example, contacting or secreting factors that affect, microglia and/or astrocytes in the brain. The mesenchymal stem cells limit neurodegeneration caused by activated glial cells in diseases or disorders such as Alzheimer's disease, Parkinson's disease, stroke, or brain cell injuries.

[0043] In yet other embodiments, the mesenchymal stem cells can be administered to an animal such that the mesenchymal stem cells reduce skin inflammation as can occur in psoriasis, chronic dermatitis, and contact dermatitis by, for example, contacting or secreting factors that affect, keratinocytes and Langerhans cells in the epidermis of the skin. Although this embodiment is not to be limited to any theoretical reasoning, it is believed that the mesenchymal stem cells can contact the keratinocytes and Langerhans cells in the epidermis, and alter the expression of T-cell receptors and cytokine secretion profiles, leading to decreased expression of TNF-α and increased regulatory T-cell (T_{reg} cell) population.

[0044] In further embodiments, the mesenchymal stem cells can be used to reduce inflammation in the bone, as occurs in arthritis and arthritis-like conditions, including but not limited to, osteoarthritis and rheumatoid arthritis, and other arthritic diseases such as ankylosing spondylitis, avascular necrosis (osteonecrosis), fibromyalgia, juvenile dermatomyositis, juvenile rheumatoid arthritis, juvenile spondyloarthopathy, lyme disease, marfan syndrome, myositis, osteogenesis imperfecta, osteoporosis, Paget's disease, Raynaud's Phenomenon, scleroderma, Sjorgren's Syndrome, and systemic lupus erythematosus. Although the scope of this embodiment is not intended to be limited to any theoretical reasoning, it is believed that the mesenchymal stem cells can inhibit Interleukin-17 secretion by memory T-cells in the synovial fluid.

[0045] In other embodiments, the mesenchymal stem cells can be used to limit inflammation in the gut and liver during inflammatory bowel disease and chronic hepatitis, respectively. Although the scope of this aspect of the present technology is not intended to be limited to any theoretical reasoning, it is believed that the mesenchymal stem cells promote increased secretion of IL-10 and the generation of T_{reg} cells.

[0046] In other embodiments, the mesenchymal stem cells can be used to inhibit excessive neutrophil and macrophage activation in pathological conditions such as sepsis and trauma, including burn injury, surgery, and transplants. Although the scope of this embodiment is not to be limited to any theoretical reasoning, it is believed the mesenchymal stem cells promote secretion of suppressive cytokines such as IL-10, and inhibit macrophage migration inhibitory factor (MIF).

[0047] In other embodiments, the mesenchymal stem cells can be used to control inflammation in immune privileged sites such as the eye, including the cornea, lens, pigment epithelium, and retina, brain, spinal cord, pregnant uterus and placenta, ovary, testes, adrenal cortex, liver, and hair follicles. Although the scope of this embodiment is not to be limited to any theoretical reasoning, it is believed that the mesenchymal stem cells promote the secretion of suppressive cytokines such as IL-10 and the generation of T_{reg} cells.

[0048] In yet other embodiments, the mesenchymal stem cells can be used to treat tissue damage associated with end-stage renal disease (ESRD) infections during dialysis and/or glomerulonephritis. Although the scope of this embodiment is not to be limited to any theoretical reasoning, it is believed that mesenchymal stem cells can promote renal repair. Mesenchymal stem cells also express and secrete vascular endothelial growth factor, or VEGF, which stimulates new blood vessel formation, which should aid in the repair of damaged kidney tissue.

[0049] In a further embodiment, the mesenchymal stem cells can be used to control viral infections such as influenza, hepatitis C, Herpes Simplex Virus, vaccinia virus infections, and Epstein-Barr virus. Although the scope of this embodiment is not to be limited to any theoretical reasoning, it is believed that the mesenchymal stem cells promote the secretion of Interferon-Beta (IFN-β).

[0050] In yet other embodiments, the mesenchymal stem cells can be used to control parasitic infections such as *Leishmania* infections and *Helicobacter* infections. Although the scope of this embodiment is not to be limited to any theoretical reasoning, it is believed that the mesenchymal stem cells mediate responses by T helper 2 (Th2) cells, and thereby promote increased production of Immunoglobulin E (IgE) by B-cells.

[0051] It is to be understood, however, that the scope of this aspect of the present technology is not to be limited to the treatment of any particular inflammatory response.

[0052] The mesenchymal stem cells can be administered to a mammal, including human and non-human primates, as described herein.

[0053] The mesenchymal stem cells also can be administered systemically, as described herein. Alternatively, in the case of osteoarthritis or rheumatoid arthritis, the mesenchymal stem cells can be administered directly to an arthritic joint.

[0054] The mesenchymal stem cells are administered in an amount effective to treat an inflammatory response in an animal. The mesenchymal stem cells can be administered in an amount of from about 1×10^5 cells/kg to about 1×10^7 cells/kg. In other embodiments, the mesenchymal stem cells are administered in an amount of from about 1×10^6 cells/kg to about 5×10^6 cells/kg. In other embodiments, the mesenchymal stem cells are administered in an amount of from about 2×10^6 cells/kg to about 4×10^6 cells/kg. In still other embodiments, the mesenchymal stem cells are administered in an amount of about 3×10^6 cells/kg. The exact dosage of mesenchymal stem cells to be administered is dependent upon a variety of factors, including the age, weight, and sex of the subject and/or patient, the inflammatory response being treated, and the extent and severity thereof.

[0055] The mesenchymal stem cells can be administered in conjunction with an acceptable pharmaceutical carrier, as described herein.

[0056] In accordance with another aspect of the present technology, there is provided a method of treating inflammation and/or repairing epithelial damage in an animal. The method comprises administering to the animal mesenchymal stem cells in an amount effective to treat the inflammation and/or epithelial damage in the animal.

[0057] Although the scope of this aspect of the present technology is not to be limited to any theoretical reasoning, it is believed that the mesenchymal stem cells cause a decrease in the secretion of the pro-inflammatory cytokines TNF-α and IFN-γ by T-cells, and an increase in the secretion of the anti-inflammatory cytokines IL-10 and Interleukin-4 (IL-4) by T-cells. It is also believed that the mesenchymal stem cells cause a decrease in IFN-γ secretion by natural killer (NK) cells.

[0058] The inflammation and/or epithelial damage which can be treated in accordance with at least this aspect of the present technology includes, but is not limited to, inflammation and/or epithelial damage caused by a variety of diseases and disorders, including, but not limited to, autoimmune disease, rejection of transplanted organs, burns, cuts, lacerations, and ulcerations, including skin ulcerations and diabetic ulcerations.

[0059] In certain embodiments, the mesenchymal stem cells are administered to an animal at least in order to repair epithelial damage resulting from autoimmune diseases, including, but not limited to, rheumatoid arthritis, Crohn's Disease, Type 1 diabetes, multiple sclerosis, scleroderma, Graves' Disease, lupus, inflammatory bowel disease, autoimmune gastritis (AIG), and autoimmune glomerular disease. The mesenchymal stem cells also can repair epithelial damage resulting from graft-versus-host disease (GvHD).

[0060] This aspect of the present technology is applicable particularly to the repair of epithelial damage resulting from graft-versus-host disease, and more particularly, to the repair of epithelial damage resulting from severe graft-versus-host disease, including Grades III and IV GvHD affecting, for example, the skin and/or the gastrointestinal system. Applicants have discovered, in particular, that mesenchymal stem cells, when administered to a patient suffering from severe graft-versus-host disease, and in particular, Grades III and IV GvHD, the administration of the mesenchymal stem cells resulted in repair of skin and/or ulcerated intestinal epithelial tissue in the subject and/or patient.

[0061] In other embodiments, the mesenchymal stem cells are administered to an animal in order to repair epithelial damage to a transplanted organ or tissue including, but not limited to, kidney, heart, and lung, caused by rejection of the transplanted organ or tissue.

[0062] In yet other embodiments, the mesenchymal stem cells are administered to an animal to repair epithelial damage caused by burns, cuts, lacerations, and ulcerations, including, but not limited to, skin ulcerations and diabetic ulcerations.

[0063] The mesenchymal stem cells can be administered to an animal such as a mammal, including human and non-human primates, as described herein.

[0064] The mesenchymal stem cells also can be administered systemically, as described herein.

[0065] The mesenchymal stem cells are administered in an amount effective to repair epithelial damage in an animal. The mesenchymal stem cells can be administered in an amount of from about 1×10^5 cells/kg to about 1×10^7 cells/kg. In other embodiments, the mesenchymal stem cells are administered in an amount of from about 1×10^6 cells/kg to about 5×10^6 cells/kg. In other embodiments, the mesenchymal stem cells are administered in an amount of from about 2×10^6 cells/kg to about 4×10^6 cells/kg. In still other embodiments, the mesenchymal stem cells are administered in an amount of about 3×10^6 cells/kg. The exact dosage of mesenchymal stem cells to be administered is dependent upon a variety of factors, including the age, weight, and sex of the subject and/or patient, the type of epithelial damage being repaired, and the extent and severity thereof.

[0066] In accordance with yet another aspect of the present technology, there is provided a method of treating cancer in an animal. The method comprises administering to the animal mesenchymal stem cells in an amount effective to treat cancer in the animal.

[0067] Although the scope of this aspect of the present technology is not to be limited to any theoretical reasoning, it is believed that the mesenchymal stem cells interact with dendritic cells, which leads to IFN-β secretion, which in turn acts as a tumor suppressor. Cancers which can be treated include, but are not limited to, hepatocellular carcinoma, cervical cancer, pancreatic cancer, prostate cancer, fibrosarcoma, medullablastoma, and astrocytoma. It is to be understood, however, that the scope of the present technology is not to be limited to any specific type of cancer.

[0068] As noted herein, it shall be appreciated by those of skill in the art that the term "animal" includes a mammal, such as a human or a non-human primate.

[0069] The mesenchymal stem cells are administered to the animal in an amount effective to treat cancer in the animal. In general, the mesenchymal stem cells are administered in an amount of from about 1×10^5 cells/kg to about 1×10^7 cells/kg. In other embodiments, the mesenchymal stem cells are administered in an amount of from about 1×10^6 cells/kg to about 5×10^6 cells/kg. The exact amount of mesenchymal stem cells to be administered is dependent upon a variety of factors, including the age, weight, and sex of the subject and/or patient, the type of cancer being treated, and the extent and severity thereof.

[0070] The mesenchymal stem cells are administered in conjunction with an acceptable pharmaceutical carrier, and can be administered systemically, as described herein. Alternatively, the mesenchymal stem cells can be administered directly to the cancer being treated.

[0071] In accordance with still another aspect of the present technology, there is provided a method of treating an allergic disease or disorder in an animal. The method comprises

administering to the animal mesenchymal stem cells in an amount effective to treat the allergic disease or disorder in the animal.

[0072] Although the scope of this aspect of the present technology is not to be limited to any theoretical reasoning, it is believed that mesenchymal stem cells, when administered after an acute allergic response, provide for inhibition of mast cell activation and degranulation. Also, it is believed that the mesenchymal stem cells downregulate basophil activation and inhibit cytokines such as TNF-α, chemokines such as Interleukin-8 and monocyte chemoattractant protein, or MCP-1, lipid mediators such as leukotrienes, and inhibit main mediators such as histamine, heparin, chondroitin sulfates, and cathepsin.

[0073] Allergic diseases or disorders which can be treated include, but are not limited to, asthma, allergic rhinitis, atopic dermatitis, and contact dermatitis. It is to be understood, however, that the scope of the present technology is not to be limited to any specific allergic disease or disorder.

[0074] The mesenchymal stem cells are administered to the animal in an amount effective to treat the allergic disease or disorder in the animal. The animal can be a mammal. The mammal can be a primate, including human and non-human primates. In general, the mesenchymal stem cells are administered in an amount of from about 1×10^5 cells/kg to about 1×10^7 cells/kg. In other embodiments, the mesenchymal stem cells are administered in an amount of from about 1×10^6 cells/kg to about 5×10^6 cells/kg. The exact dosage is dependent upon a variety of factors, including the age, weight, and sex of the subject and/or patient, the allergic disease or disorder being treated, and the extent and severity thereof.

[0075] The mesenchymal stem cells can be administered in conjunction with an acceptable pharmaceutical carrier, as described herein. The mesenchymal stem cells can be administered systemically, such as by intravenous or intraarterial administration, for example.

[0076] In accordance with a further aspect of the present technology, there is provided a method of promoting wound healing in an animal. The method comprises administering to the animal mesenchymal stem cells in an amount effective to promote wound healing in the animal.

[0077] Although the scope of the present technology is not to be limited to any theoretical reasoning, it is believed that, as mentioned hereinabove, the mesenchymal stem cells cause T_{reg} cells and dendritic cells to release IL-10, which limits or controls inflammation in a wound, thereby promoting healing of a wound.

[0078] Furthermore, the mesenchymal stem cells can promote wound healing and fracture healing by inducing secretion factors by other cell types. For example, the mesenchymal stem cells can induce prostaglandin E_2 (PGE_2)-mediated release of vascular endothelial growth factor (VEGF) by peripheral blood mononuclear cells (PBMCs), as well as PGE_2-mediated release of growth hormone, insulin, insulin-like growth factor 1 (IGF-1) insulin-like growth factor binding protein-3 (IGFBP-3), and endothelin-1.

[0079] Wounds which can be healed include, but are not limited to, those resulting from cuts, lacerations, burns, and skin ulcerations.

[0080] The mesenchymal stem cells are administered to the animal in an amount effective to promote wound healing in the animal. The animal can be a mammal, and the mammal can be a primate, including human and non-human primates. In general, the mesenchymal stem cells are administered in an amount of from about 1×10^5 cells/kg to about 1×10^7 cells/kg. In other embodiments, the mesenchymal stem cells are administered in an amount of from about 1×10^6 cells/kg to about 5×10^6 cells/kg. The exact amount of mesenchymal stem cells to be administered is dependent upon a variety of factors, including the age, weight, and sex of the subject and/or patient, and the extent and severity of the wound being treated.

[0081] The mesenchymal stem cells can be administered in conjunction with an acceptable pharmaceutical carrier, as described herein. The mesenchymal stem cells can be administered systemically, as described herein. Alternatively, the mesenchymal stem cells can be administered directly to a wound, such as in a fluid on a dressing or reservoir containing the mesenchymal stem cells.

[0082] In accordance with yet another aspect of the present technology, there is provided a method of treating or preventing fibrosis or fibrotic disorder in an animal. The method comprises administering to the animal mesenchymal stem cells in an amount effective to treat or prevent fibrosis or a fibrotic disorder in an animal.

[0083] The mesenchymal stem cells can be administered to the animal in order to treat or prevent any type of fibrosis or fibrotic disorder and in the animal, including, but not limited to, cirrhosis of the liver, fibrosis of the kidneys associated with end-stage renal disease, and lung disorders or diseases having fibrotic and can include in addition, inflammatory components, including, but not limited to, Acute Respiratory Distress Syndrome (ARDS), Chronic Obstructive Pulmonary Disease (COPD). It is to be understood that the scope of the present technology is not to be limited to any specific type of fibrosis or fibrotic disorder.

[0084] The mesenchymal stem cells are administered to the animal in an amount effective to treat or prevent fibrosis or a fibrotic disorder in the animal. The animal can be a mammal, and the mammal can be a primate, including human and non-human primates. In general, the mesenchymal stem cells are administered in an amount of from about 1×10^5 cells/kg to about 1×10^7 cells/kg. In other embodiments, the mesenchymal stem cells are administered in an amount of from about 1×10^6 cells/kg to about 5×10^6 cells/kg. The exact amount of mesenchymal stem cells to be administered is dependent upon a variety of factors, including the age, weight, and sex of the subject and/or patient, and the extent and severity of the fibrosis or fibrotic disorder being treated or prevented.

[0085] The mesenchymal stem cells can be administered in conjunction with an acceptable pharmaceutical carrier, as described herein. The mesenchymal stem cells can be administered systemically, also as described herein.

[0086] It is another object of the present technology to promote angiogenesis (i.e., the formation of new blood vessels from a pre-existing microvascular bed) in a tissue or organ of an animal, wherein such tissue or organ is in need of angiogenesis. Thus, in accordance with a further aspect of the present technology, there is provided a method of promoting angiogenesis in an organ or tissue of an animal. The method comprises administering to the animal mesenchymal stem cells in an amount effective to promote angiogenesis in an organ or tissue of the animal.

[0087] The induction of angiogenesis can be used to treat coronary and peripheral artery insufficiency, and thus can be a noninvasive and curative approach to the treatment of coronary artery disease, ischemic heart disease, and peripheral artery disease. Angiogenesis can play a role in the treatment

of diseases and disorders in tissue and organs other than the heart, as well as in the development and/or maintenance of organs other than the heart. Angiogenesis can provide a role in the treatment of internal and external wounds, as well as dermal ulcers. Angiogenesis also plays a role in embryo implantation, and placental growth, as well as the development of the embryonic vasculature. Angiogenesis also is essential for the coupling of cartilage resorption with bone formation, and is essential for correct growth plate morphogenesis.

[0088] Furthermore, angiogenesis is necessary for the successful engineering and maintenance of highly metabolic organs, such as the liver, where a dense vascular network is necessary to provide sufficient nutrient and gas transport.

[0089] The mesenchymal stem cells can be administered to the tissue or organ in need of angiogenesis by a variety of procedures. The mesenchymal stem cells can be administered systemically, such as by intravenous, intraarterial, or intraperitoneal administration, or the mesenchymal stem cells can be administered directly to the tissue or organ in need of angiogenesis, such as by direct injection into the tissue or organ in need of angiogenesis.

[0090] The mesenchymal stem cells can be from a spectrum of sources including autologous, allogeneic, or xenogeneic.

[0091] Although the scope of the present technology is not to be limited to any theoretical reasoning, it is believed that the mesenchymal stem cells, when administered to an animal, stimulate peripheral blood mononuclear cells (PBMCs) to produce vascular endothelial growth factor, or VEGF, which stimulates the formation of new blood vessels.

[0092] In certain embodiments, the animal is a mammal. The mammal can be a primate, including human and non-human primates.

[0093] The mesenchymal stem cells, in accordance with the present technology, can be employed in the treatment, alleviation, or prevention of any disease or disorder which can be alleviated, treated, or prevented through angiogenesis. Thus, for example, the mesenchymal stem cells can be administered to an animal to treat blocked arteries, including those in the extremities, such as arms, legs, hands, and feet, as well as the neck or in various organs. For example, the mesenchymal stem cells can be used to treat blocked arteries which supply the brain, thereby treating or preventing stroke. Also, the mesenchymal stem cells can be used to treat blood vessels in embryonic and post-natal corneas and can be used to provide glomerular structuring. In other embodiments, the mesenchymal stem cells can be employed in the treatment of wounds, both internal and external, as well as the treatment of dermal ulcers found in the feet, hands, legs or arms, including, but not limited to, dermal ulcers caused by diseases such as diabetes and sickle cell anemia.

[0094] Furthermore, because angiogenesis is involved in embryo implantation and placenta formation, the mesenchymal stem sells can be employed to promote embryo implantation and prevent miscarriage.

[0095] In addition, the mesenchymal stem cells can be administered to an unborn animal, including humans, to promote the development of the vasculature in the unborn animal.

[0096] In other embodiments, the mesenchymal stem cells can be administered to an animal, born or unborn, in order to promote cartilage resorption and bone formation, as well as promote correct growth plate morphogenesis.

[0097] The mesenchymal stem cells are administered in an amount effective in promoting angiogenesis in an animal. The mesenchymal stem cells can be administered in an amount of from about 1×10^5 cells/kg to about 1×10^7 cells/kg. In other embodiments, the mesenchymal stem cells are administered in an amount of from about 1×10^6 cells/kg to about 5×10^6 cells/kg. The amount of mesenchymal stem cells to be administered is dependent upon a variety of factors, including the age, weight, and sex of the subject and/or patient, the disease or disorder to be treated, alleviated, or prevented, and the extent and severity thereof.

[0098] The mesenchymal stem cells can be administered in conjunction with an acceptable pharmaceutical carrier. For example, the mesenchymal stem cells can be administered as a cell suspension in a pharmaceutically acceptable liquid medium for injection. Injection can be local, such as by administration directly into the tissue or organ in need of angiogenesis, or systemic, such as intravenously or intraarterially.

[0099] The mesenchymal stem cells can be genetically engineered with one or more polynucleotides encoding a therapeutic agent. The polynucleotides can be delivered to the mesenchymal stem cells via an appropriate expression vehicle. Expression vehicles which can be employed to genetically engineer the mesenchymal stem cells include, but are not limited to, retroviral vectors, adenoviral vectors, and adeno-associated virus vectors.

[0100] The selection of an appropriate polynucleotide encoding a therapeutic agent is dependent upon various factors, including the disease or disorder being treated, and the extent and severity thereof. Polynucleotides encoding therapeutic agents, and appropriate expression vehicles are described further in U.S. Pat. No. 6,355,239, the contents of which are hereby incorporated by reference in its entirety.

[0101] It is to be understood that the mesenchymal stem cells, when employed in the above-mentioned therapies and treatments, can be employed in combination with other therapeutic agents known to those skilled in the art, including, but not limited to, growth factors, cytokines, drugs such as anti-inflammatory drugs, and cells other than mesenchymal stem cells, such as dendritic cells, and can be administered with soluble carriers for cells such as hyaluronic acid, or in combination with solid matrices, such collagen, gelatin, or other biocompatible polymers, as appropriate.

[0102] It is to be understood that the methods described herein can be carried out in a number of ways and with various modifications and permutations thereof that are well known in the art. It also can be appreciated that any theories set forth as to modes of action or interactions between cell types should not be construed as limiting this technology in any manner, but are presented such that the methods of the present technology can be understood more fully.

[0103] It is to be understood that the scope of the present technology is not to be limited to the specific embodiments described above. The present technology can be practiced other than as particularly described and still be within the scope of the accompanying claims.

[0104] Likewise, the following examples are presented in order to more fully illustrate the present technology. They should in no way be construed, however, as limiting the broad scope of the technology disclosed herein.

EXAMPLES

[0105] The present technology now will be described with respect to the following examples; it is to be understood, however, that the scope of the present technology is not to be limited thereby.

Example 1

[0106] Materials and Methods Culture of human MSCs. Human MSCs were cultured as described by Pittenger et al., *Science*, Vol. 284, pg. 143 (1999). Briefly, marrow samples were collected from the iliac crest of anonymous donors following informed consent by Poietics Technologies, Div of Cambrex Biosciences. MSCs were cultured in complete Dulbecco's Modified Eagle's Medium-Low Glucose (Life Technologies, Carlsbad, Calif.) containing 1% antibiotic-antimyotic solution (Invitrogen, Carlsbad, Calif.) and 10% fetal bovine serum (FBS, JRH BioSciences, Lenexa, Kans.). MSCs grew as an adherent monolayer and were detached with trypsin/EDTA (0.05% trypsin at 37° C. for 3 minutes). All MSCs used were previously characterized for multilineage potential and retained the capacity to differentiate into mesenchymal lineages (chondrocytic, adipogenic, and osteogenic) (Pittenger, et al., *Science*, Vol. 284, pg. 143 (1999)).

[0107] Isolation of Dendritic cells. Peripheral blood mononuclear cells (PBMCs) were obtained from Poietics Technologies, Div of Cambrex Biosciences (Walkersville, Md.). Precursors of dendritic cells (DCs) of monocytic lineage (CD1c+) were positively selected from PBMCs using a 2-step magnetic separation method according to Dzionek, et. al., *J. Immunol*., Vol. 165, pg. 6037 (2000). Briefly, CD1c expressing B cells were magnetically depleted of CD19+ cells using magnetic beads, followed by labeling the B-cell depleted fraction with biotin-labeled CD1c (BDCA1+) and anti-biotin antibodies and separating them from the unlabeled cell fraction utilizing magnetic columns according to the manufacturer's instructions (Miltenyi Biotech, Auburn, Calif.). Precursors of DCs of plasmacytoid lineage were isolated from PBMCs by immuno-magnetic sorting of positively labeled antibody coated cells (BDCA2+) (Miltenyi Biotech, Auburn, Calif.).

[0108] MSC-DC culture. In most experiments, human MSCs and DCs were cultured in equal numbers for various time periods and cell culture supernatant collected and stored at −80° C. until further evaluation. In selected experiments, MSCs were cultured with mature DC1 or DC2 cells (1:1 MSC:DC ratio) for 3 days, and then the combined cultures (MSCs and DCs) were irradiated to prevent any proliferation. Next, antibody purified, naïve, allogeneic T cells (CD4+, CD45RA+) were added to the irradiated MSCs/DCs and cultured for an additional 6 days. The non-adherent cell fraction (purified T cells) was then collected from the cultures, washed twice and re-stimulated with PHA for another 24 hours, following which cell culture supernatants were harvested and analyzed for secreted IFN-γ and IL-4 by ELISA.

[0109] Isolation of NK cells. Purified populations of NK cells were obtained by depleting non-NK cells that are magnetically labeled with a cocktail of biotin-conjugated monoclonal antibodies (anti-CD3, -CD14, -CD19, -CD36 and anti-IgE antibodies) as a primary reagent and anti-biotin monoclonal antibodies conjugated to Microbeads as secondary labeling reagent. The magnetically labeled non-NK cells were retained in MACS (Miltenyi Biotech, Auburn, Calif.) columns in a magnetic field, while NK cells passed through and were collected.

[0110] Isolation of T_{Reg} cell population. The T_{Reg} cell population was isolated using a 2-step isolation procedure. First non-$CD4^+$T cells were indirectly magnetically labeled with a cocktail of biotin labeled antibodies and anti-biotin microbeads. The labeled cells were then depleted by separation over a MACS column (Miltenyi Biotech, Auburn, Calif.). Next, $CD4^+CD25^+$ cells were directly labeled with CD25 microbeads and isolated by positive selection from the pre-enriched $CD4^+$. T cell fraction. The magnetically labeled $CD4^+CD25^+$T cells were retained on the column and eluted after removal of the column from the magnetic field.

[0111] In order to determine whether the increased CD4+ CD25+ population generated in the presence of MSCs were suppressive in nature, CD4+CD25+T_{reg} cell populations were isolated from PBMC or MSC+PBMC (MSC to PBMC ratio 1:10) cultures (cultured without any further stimulation for 3 days) using a 2-step magnetic isolation procedure. These cells were irradiated to block any further proliferation and used as stimulators in a mixed lymphocyte reaction (MLR), where responders were allogeneic PBMCs (stimulator to responder ratio 1:100) in the presence of PHA (2.5 μg/ml). The culture was carried out for 48 hours, following which ^{3}H thymidine was added. Incorporated radioactivity was counted after 24 hours.

[0112] PBMCs were cultured in the absence or presence of MSCs (MSC to PBMC ratio 1:10), following which the non-adherent fraction was harvested and immunostained with FITC-labeled glucocorticoid-induced TNF receptor, or GITR, and PE-labeled CD4.

[0113] Generation of Th1/Th2 cells. Peripheral blood mononuclear cells (PBMCs) were plated at 2×10^6 cells/ml for 45 min. at 37° C. in order to remove monocytes. Non-adherent fraction was incubated in the presence of plate-bound anti-CD3 (5 μg/ml) and anti-CD28 (1 μg/ml) antibodies under Th1 (IL-2 (4 ng/ml) IL-12 (5 ng/ml)+anti-IL-4 (1 μg/ml)) or Th2 (IL-2 (4 ng/ml)+IL-4 (4 ng/ml)+anti-IFN-γ (1 μg/ml)) conditions for 3 days in the presence or absence of MSCs. The cells were washed and then re-stimulated with PHA (2.5 μg/ml) for another 24 or 48 hours, following which levels of IFN-γ and IL-4 were measured in culture supernatants by ELISA (R&D Systems, Minneapolis, Minn.).

[0114] Analysis of levels of VEGF, PGE_2, and pro-MMP-1 in culture supernatant of MSCs. Using previously characterized human MSCs, the levels of Interleukin-6 (IL-6), VEGF, lipid mediator PGE_2, and matrix metalloproteinase 1 (pro-MMP-1) were analyzed in culture supernatant of MSCs cultured for 24 hours in the presence or absence of PBMCs (MSC to PBMC ratio 1:10).

[0115] Proliferation of PBMCs. Purified PBMCs were prepared by centrifuging leukopack (Cambrex, Walkersville, Md.) on Ficoll-Hypaque (Lymphoprep, Oslo, Norway). Separated cells were cultured (in triplicates) in the presence or absence of MSCs (plated 3-4 hours prior to PBMC addition to allow them to settle) for 48 hours in presence of the mitogen PHA (Sigma Chemicals, St. Louis, Mo.). In selected experiments, PBMCs were resuspended in medium containing PGE_2 inhibitors Indomethacin (Sigma Chemicals, St. Louis, Mo.) or NS-938 (Cayman Chemicals, Ann Arbor, Mich.). (^{3}H)-thymidine was added (20 μl in a 200 μl culture) and the cells harvested after an additional 24 hour culture using an

automatic harvester. The effects of MSCs or PGE_2 blockers were calculated as the percentage of the control response (100%) in presence of PHA.

[0116] Quantitative RT-PCR. Total RNA from cell pellets were prepared using a commercially available kit (Qiagen, Valencia, Calif.) and according to the manufacturer's instructions. Contaminating genomic DNA was removed using the DNA-free kit (Ambion, Austin, Tex.). Quantitative RT-PCR was performed on a MJ Research Opticon detection system (South San Francisco, Calif.) using QuantiTect SYBR Green RT-PCR kit (Qiagen, Valencia, Calif.) with primers at concentration of 0.5 μM. Relative changes in expression levels in cells cultured under different conditions were calculated by the difference in Ct values (crossing point) using β-actin as internal control. The sequences for COX-1 and COX-2 specific primers were: COX-1: 5'-CCG GAT GCC AGT CAG GAT GAT G-3' (forward) (SEQ ID NO:1), 5'-CTA GAC AGC CAG ATG CTG ACA G-3' (reverse) (SEQ ID NO:2); COX-2: 5'-ATC TAC CCT CCT CAA GTC CC-3' (forward) (SEQ ID NO:3), 5'-TAC CAG AAG GGC AGG ATA CAG-3' (reverse) (SEQ ID NO:4).

[0117] Increasing numbers of allogeneic PBMCs were incubated with constant numbers of MSCs (2,000 cells/well) plated on a 96-well plate in the presence of PHA (2.5 μg/ml) for 72 hours, and ^3H thymidine incorporation (counts per minute, cpm) was determined. The PBMCs and MSCs were cultured at ratios of MSC:PBMC of 1:1, 1:3, 1:10, 1:30, and 1:81.

[0118] Results. In the present studies, the interaction of human MSCs with isolated immune cell populations, including dendritic cells (DC1 and DC2), effector T cells (Th1 and Th2) and NK cells was examined. The interaction of MSCs with each immune cell type had specific consequences, suggesting that MSCs can modulate several steps in the immune response process. The production of secreted factor(s) that modulate and can be responsible for MSC immuno-modulatory effects was evaluated and prostaglandin synthesis was implicated.

[0119] Myeloid (DC1) and plasmacytoid (DC2) precursor dendritic cells were isolated by immuno-magnetic sorting of $BDCA1^+$ and $BDCA2^+$ cells respectively and matured by incubation with GM-CSF and IL-4 (1×10^3 IU/ml and 1×10^3 IU/ml, respectively) for DC1 cells, or IL-3 (10 ng/ml) for DC2 cells. Using flow cytometry, DC1 cells were $HLA\text{-}DR^+$ and $CD11c^+$, whereas DC2 cells were $HLA\text{-}DR^+$ and $CD123^+$ (FIG. **1**A). In the presence of the inflammatory agent bacterial lipopolysaccharide (LPS, 1 ng/ml), DC1 cells produced moderate levels of TNF-α but when MSCs were present (ratios examined 1:1 and 1:10), there was >50% reduction in TNF-α secretion (FIG. **1**B). On the other hand, DC2 cells produced IL-10 in the presence of LPS and its levels were increased greater than 2-fold upon MSC:DC2 co-culture (1:1) (FIG. **1**B). Therefore, the MSCs modified the cytokine profile of activated DCs in culture towards a more tolerogenic phenotype. Additionally, activated DCs, when cultured with MSCs, were able to reduce IFN-γ and increase IL-4 levels secreted by naïve $CD4^+$T cells (FIG. **1**C) suggesting a MSC-mediated shift from pro-inflammatory to anti-inflammatory T cell phenotype.

[0120] As increased IL-10 secretion plays a role in generation of regulatory cells (Kingsley, et al., *J. Immunol.*, Vol. 168, pg. 1080 (2002)), T-regulatory cells (T_{Reg}) were quantified by flow cytometry in co-cultures of PBMCs and MSCs. Upon culture of PBMCs with MSCs for 3-5 days, there was an increase in T_{Reg} cell numbers as determined by staining of PBMCs with anti-CD4 and anti-CD25 antibodies (FIG. **2**A), further supporting a MSC-induced tolerogenic response. The $CD4^+CD25^+$ T_{Reg} cell population, generated in presence of MSCs expressed increased levels of gluocorticoid-induced TNF receptor (GITR), a cell surface receptor expressed on T_{Reg} cell populations, and was suppressive in nature as it suppressed allogeneic T cell proliferation (FIG. **3**A,B). Next, MSCs were investigated as to their direct ability to affect T cell differentiation. Using antibody selected purified T cells ($CD4^+$Th cells), IFN-γ producing Th1 and IL-4 producing T_H2 cells were generated in presence or absence of MSCs. When MSCs were present during differentiation, there was reduced IFN-γ secretion by Th1 cells and increased IL-4 secretion by Th2 cells (FIG. **2**B). No significant change in IFN-γ or IL-4 levels were seen when MSCs were added to the culture after Th cells had differentiated (at 3 days) into effector Th1 or Th2 types (data not shown). These experiments suggest that MSCs can affect effector T cell differentiation directly and alter the T cell cytokine secretion towards a humoral phenotype.

[0121] Similarly, when MSCs were cultured with purified NK cells (CD3-, CD14-, CD19-, CD36") at a ratio 1:1 for different time periods (0-48 hrs), there was decreased IFN-γ secretion in the culture supernatant (FIG. **2**C), thereby suggesting that MSCs can modulate NK cell functions also.

[0122] Previous work has indicated that MSCs modify T-cell functions by soluble factor(s) (LeBlanc, et al., *Exp. Hematol.*, Vol. 31, pg. 890 (2003); Tse, et al., *Transplantation*, Vol. 75, pg. 389 (2003). It was observed that the MSCs secreted several factors, including IL-6, prostaglandin E_2, VEGF and proMMP-lconstitutively, and the levels of each increased upon culture with PBMCs (FIG. **5**). In order to investigate MSC-derived factors leading to inhibition of TNF-α and increase of IL-10 production by DCs, the potential role of prostaglandin E_2 was investigated, as it has been shown to inhibit TNF-α production by activated DCs (Vassiliou, et al., *Cell. Immunol.*, Vol. 223, pg. 120 (2003)). Conditioned media from MSC culture (24 hour culture of 0.5×10^6 cells/ml) contained approximately 1000 pg/ml of PGE_2 (FIG. **4**A). There was no detectable presence of known inducers of PGE_2 secretion, such as TNF-α, IFN-γ or IL-10 (data not shown), in the culture supernatant indicating a constitutive secretion of PGE_2 by MSCs. The PGE_2 secretion by hMSCs was inhibited 60-90% in the presence of known inhibitors of PGE_2 production, NS-398 (5 μM) and indomethacin (4 μM) (FIG. **4**A). As the release of PGE_2 secretion occurs as a result of enzymatic activity of constitutively active cycloxygenase enzyme 1 (COX-1) and inducible cycloxygenase enzyme 2 (COX-2) (Harris, et al., *Trends Immunol.*, Vol. 23, pg. 144 (2002)) the mRNA expression for COX-1 and COX-2 in MSCs and PBMCs using trans-well culture system was analyzed. MSCs expressed significantly higher levels of COX-2 as compared to PBMCs and the expression levels increase >3-fold upon co-culture of MSCs and PBMCs (MSC to PBMC ratio 1:10) for 24 hours (FIG. **4**B). Modest changes in COX-1 levels were seen suggesting that the increase in PGE2 secretion upon MSC-PBMC co-culture (FIG. **5**) is mediated by COX-2 up-regulation.

[0123] To investigate whether the immunomodulatory effects of MSC on DCs and T-cells were mediated by PGE_2, MSCs were cultured with activated dendritic cells (DC1) or Th1 cells in the presence of PGE_2 inhibitors NS-398 or indomethacin. The presence of NS-398 or indomethacin

increased TNF-α secretion by DC1s, and IFN-γ secretion from Th1 cells (FIG. **4**C), respectively, suggesting that MSC effects on immune cell types can be mediated by secreted PGE_2. Recent studies have shown that MSCs inhibit T-cell proliferation induced by various stimuli (DeNicola, et al., *Blood*, Vol. 99, pg. 3838 (2002); LeBlanc, et al., *Scand. J. Immunol.*, Vol. 57, pg. 11 (2003)). It was observed that MSCs inhibit mitogen-induced T cell proliferation in a dose-dependent manner (FIG. **6**) and when PGE_2 inhibitors NS-398 (5 μM) or indomethacin (4 μM) were present, there was a >70% increase in (^{3}H) thymidine incorporation by PHA-treated PBMCs in MSC containing cultures as compared to controls without inhibitors (FIG. **4**D).

[0124] In summary, a model of MSC interaction with other immune cell types (FIG. **7**) is proposed. When mature T cells are present, MSCs can interact with them directly and inhibit the pro-inflammatory IFN-γ production (pathway 1) and promote regulatory T cell phenotype (pathway 3) and anti-inflammatory T_H2 cells (pathway 5). Further, MSCs can alter the outcome of the T cell immune response through DCs by secreting PGE_2, inhibiting pro-inflammatory DC1 cells (pathway 2) and promoting anti-inflammatory DC2 cells (pathway 4) or regulatory DCs (pathway 3). A shift towards T_H2 immunity in turn, suggests a change in B cell activity towards increased generation of IgE/IgG1 subtype antibodies (pathway 7). MSCs, by their ability to inhibit IFN-γ secretion from NK cells likely modify NK cell function (pathway 6). This model of MSC:Immune cell interactions is consistent with the experimentation performed in several other laboratories (LeBlanc, et al., *Exp. Hematol.*, Vol. 31, pg. 890 (2003); Tse, et al., *Transplantation*, Vol. 75, pg. 389 (2003); DiNicola, et al., *Blood*, Vol. 99, pg. 3838 (2002)). Further examination of the proposed mechanisms is underway and animal studies are now necessary to examine the in vivo effects of MSC administration.

Example 2

[0125] Mesenchymal stem cells were given to a 33-year-old female patient suffering from severe Grade IV gastrointestinal graft-versus-host disease (GVHD). The patient was refractory to all other GVHD treatments. Endoscopic views of the patient's colon showed areas of ulceration and inflammation prior to treatment. Histology of the patient's colon showed that the graft-versus-host disease had destroyed the vast majority of the patient's intestinal crypts, prior to treatment.

[0126] The patient was given an intravenous infusion of allogeneic mesenchymal stem cells in 50 ml of Plasma Lyte A (Baxter) in an amount of 3×10^6 cells per kilogram of body weight.

[0127] The patient was evaluated at two weeks post-infusion. At two weeks post-infusion, an endoscopic view of the patient's colon showed that the areas of inflammation and ulceration visible prior to treatment were resolved. In addition, a biopsy of the patient's colon showed significant regeneration of intestinal crypts. Thus, the administration of the mesenchymal stem cells to the patient resulted in a significant reduction in the inflammatory component of gastrointestinal graft-versus-host disease, and resulted in the regeneration of new functional intestinal tissue.

[0128] The disclosures of all patents, publications, including published patent applications, depository accession numbers, and database accession numbers are hereby incorporated by reference in their entireties to the same extent as if each patent, publication, depository accession number, and database accession number were specifically and individually incorporated by reference.

[0129] The presently described technology is now described in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, to practice the same. It is to be understood that the foregoing describes preferred embodiments of the technology and that modifications can be made therein without departing from the spirit or scope of the invention as set forth in the appended claims.

[0130] It is to be understood, however, that the scope of the present technology is not to be limited to the specific embodiments described above. The present technology can be practiced other than as particularly described and still be within the scope of the accompanying claims.

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taccagaagg gcaggataca g 21

1. A cell preparation comprising adult bone marrow-derived stem cells in a dose effective to treat an inflammatory response in a subject.

2. The cell preparation of claim **1**, wherein the stem cells are capable of differentiating into at least one cell type of each of the endodermal, ectodermal, and mesodermal embryonic lineages.

3. The cell preparation of claim **1**, wherein the stem cells are capable of differentiating into at least one cell type of at least one of the endodermal, ectodermal, or mesodermal embryonic lineages.

4. The cell preparation of claim **1**, wherein the dose contains a sufficient number of stem cells to provide about 1×10^5 to about 1×10^7 cells per kilogram of the subject.

5. The cell preparation of claim **1**, wherein the stem cells are positive for one or more cell surface markers selected from the group consisting of integrin al (CD49a); integrin α2 (CD49b); integrin α3 (CD49c); integrin α5 (CD49e); integrin αV (CD51); integrin β1 (CD29); integrin β3 (CD61); integrin β4 (CD104); IL-1R (CD121a); IL-3Rα (CD123); IL-4R (CDw124); IL-6R (CD126); IL-7R (CDw127); IFNγR (CDw119); TNFIR (CD120a); TNFIIR (CD120b); TGFβ1R; TGFβIIR; bFGFR; PDGFR (CD140a); transferrin (CD71); ICAM-1 (CD54); ICAM-2 (CD102); VCAM-1 (CD106); L-Selectin (CD62L); LFA-3 (CD58); ALCAM (CD166); hyaluronate (CD44); endoglin (CD105); Thy-1 (CD90); and CD9.

6. The cell preparation of claim **1**, wherein administration of the cell preparation elevates interferon-beta levels in the subject.

7. The cell preparation of claim **1**, wherein the subject is a subject having inflammatory bowel disease.

8. A method of treating inflammatory bowel disease in a subject, comprising the step of:

administering to the subject allogeneic, multipotent adult bone marrow-derived stem cells in an amount effective to treat the inflammatory bowel disease.

9. The method of claim **8**, wherein the multipotent stem cells are administered intravenously or intraarterially.

10. The method of claim **8**, wherein the multipotent stem cells are positive for one or more cell surface markers selected from the group consisting of integrin al (CD49a); integrin α2 (CD49b); integrin α3 (CD49c); integrin α5 (CD49e); integrin αV (CD51); integrin β1 (CD29); integrin β3 (CD61); integrin β4 (CD104); IL-1R (CD121a); IL-3Rα (CD123); IL-4R (CDw124); IL-6R (CD126); IL-7R (CDw127); IFNγR (CDw119); TNFIR (CD120a); TNFIIR (CD120b); TGFβ1R; TGFβIIR; bFGFR; PDGFR (CD140a); transferrin (CD71); ICAM-1 (CD54); ICAM-2 (CD102); VCAM-1 (CD106); L-Selectin (CD62L); LFA-3 (CD58); ALCAM (CD166); hyaluronate (CD44); endoglin (CD105); Thy-1 (CD90); and CD9.

11. The method of claim **8**, wherein the multipotent stem cells are positive for CD49b, CD49e, and CD140a.

12. The method of claim **8**, wherein the multipotent stem cells are negative for one or more cell surface markers selected from the group consisting of integrin α4 (CD49d); integrin αL (CD11a); integrin Cβ2 (CD18); CD4; CD14; CD34; CD45; IL-2R (CD25); EGFR-3; Fas ligand; ICAM-3 (CD50); E-Selectin (CD62E); P-Selectin (CD62P); vW Factor; cadherin 5; and Lewis x (CD15).

13. The method of claim **8**, wherein the multipotent stem cells are negative for CD34, CD45, CD62E, and CD62P.

14. A method of treating a gastrointestinal autoimmune disease in a subject, comprising the step of:

administering to the subject allogeneic multipotent adult stem cells in an amount effective to repair or regenerate intestinal tissue.

15. The method of claim **14**, wherein the autoimmune disease is selected from the group consisting of Crohn's disease, inflammatory bowel disease, and autoimmune gastritis.

16. The method of claim **14**, wherein the multipotent stem cells are administered intravenously or intraarterially.

17. The method of claim **14**, wherein the multipotent stem cells are positive for one or more cell surface markers selected from the group consisting of integrin al (CD49a); integrin α2 (CD49b); integrin α3 (CD49c); integrin α5 (CD49e); integrin αV (CD51); integrin β1 (CD29); integrin β3 (CD61); integrin β4 (CD104); IL-1R (CD121a); IL-3Rα (CD123); IL-4R (CDw124); IL-6R (CD126); IL-7R (CDw127); IFNγR (CDw119); TNFIR (CD120a); TNFIIR (CD120b); TGFβ1R; TGFβIIR; bFGFR; PDGFR (CD140a); transferrin (CD71); ICAM-1 (CD54); ICAM-2 (CD102); VCAM-1 (CD106); L-Selectin (CD62L); LFA-3 (CD58); ALCAM (CD166); hyaluronate (CD44); endoglin (CD105); Thy-1 (CD90); and CD9.

18. The method of claim **14**, wherein the multipotent stem cells are positive for CD49b, CD49e, and CD140a.

19. The method of claim **14**, wherein the multipotent stem cells are negative for one or more cell surface markers selected from the group consisting of integrin α4 (CD49d); integrin αL (CD11a); integrin Cβ2 (CD18); CD4; CD14; CD34; CD45; IL-2R (CD25); EGFR-3; Fas ligand; ICAM-3 (CD50); E-Selectin (CD62E); P-Selectin (CD62P); vW Factor; cadherin 5; and Lewis x (CD15).

20. The method of claim **14**, wherein the multipotent stem cells are negative for CD34, CD45, CD62E, and CD62P.

21. A method of repairing or regenerating intestinal tissue in a human, comprising the step of:

treating the human with allogeneic adult mesenchymal stem cells.

22. The method of claim **21**, wherein the mesenchymal stem cells are administered to the human.

23. The method of claim **22**, wherein the administration of the mesenchymal stem cell comprises intravenous, intraarterial, or intraperitoneal injection.

* * * * *