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(54) **Titre : CELLULES SOUCHES MESENCHYMATEUSES ET LEURS UTILISATIONS**
(54) **Title: MESENCHYMAL STEM CELLS AND USES THEREFOR**

(57) **Abrégé/Abstract:**
Methods of treating autoimmune diseases, allergic responses, cancer, or inflammatory diseases in an animal, promoting would healing, and promoting angiogenesis in an organ or tissue of an animal by administering to the animal mesenchymal stem cells in an effective amount.

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(54) Title: MESENCHYMAL STEM CELLS AND USES THEREFOR

(57) Abstract: Methods of treating autoimmune diseases, allergic responses, cancer, or inflammatory diseases in an animal, promoting would healing, and promoting angiogenesis in an organ or tissue of an animal by administering to the animal mesenchymal stem cells in an effective amount.

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MESENCHYMAL STEM CELLS AND USES THEREFOR

This invention relates to mesenchymal stem cells. More particularly, this invention relates to novel uses for mesenchymal stem cells, including promoting angiogenesis in various tissues and organs, treating autoimmune diseases, treating allergic responses, treating cancer, treating inflammatory diseases and disorders, and promoting would healing.

Mesenchymal stem cells (MSCs) are multipotent stem cells 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 may be useful in the repair 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)).

MSCs express major histocompatibility complex (MHC) class I antigen on their surface but limited 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 no 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 may enhance their transplant engraftment and limit the ability of the recipient 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).

Applicants presently have examined the interactions of mesenchymal 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 mesenchymal stem cells may regulate the production of various factors that may regulate several steps in the immune response process. Thus, the mesenchymal stem cells may be employed in the treatment of disease conditions and disorders involving the immune system, or diseases, conditions, or disorders involving inflammation 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.

In addition, it is believed that mesenchymal stem cells stimulate peripheral blood mononuclear cells (PBMCs) to produce vascular endothelial growth factor, or VEGF, which promotes angiogenesis by stimulating the formation of new blood vessels.

Furthermore, it is believed that mesenchymal stem cells stimulate dendritic cells (DCs) to produce Interferon-Beta (IFN-ß), which promotes tumor suppression and immunity against viral infection.

In accordance with an aspect of the present invention, there is provided a method of treating an autoimmune disease in an animal. The method comprises administering to the animal mesenchymal stem cells in an amount effective to treat the autoimmune disease in the animal.

Although the scope of this aspect of the present invention 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 is by causing the release of Interleukin-10 (IL-10) from regulatory T-cells (T_{reg} cells) and/or dendritic cells (DC),

Autoimmune diseases which may be treated in accordance with the present invention include, but are not limited to, multiple sclerosis, Type 1 diabetes, rheumatoid arthritis, uveitis, autoimmune thyroid disease, inflammatory bowel disease, autoimmune lymphoproliferative disease (ALPS), demyelinating disease, autoimmune encephalomyelitis, autoimmune gastritis (AIG), and autoimmune glomerular diseases. It is to be understood, however, that the scope of the present invention is not to be limited to the treatment of the specific autoimmune diseases mentioned herein.

In one embodiment, the animal to which the mesenchymal stem cells are administered is a mammal. The mammal may be a primate, including human and non-human primates.

In general, the mesenchymal stem cell (MSC) therapy is based, for example, on the following sequence: harvest of MSC-containing tissue, isolation and expansion of MSCs, and administration of the MSCs to the animal, with or without biochemical or genetic manipulation.

The mesenchymal stem cells that are administered may be a homogeneous composition or may be a mixed cell population enriched in MSCs. Homogeneous mesenchymal stem cell compositions may be obtained by culturing adherent marrow or periosteal cells, and the mesenchymal stem cell compositions may be obtained by culturing adherent marrow or periosteal cells, and the mesenchymal stem cells may be identified by specific cell surface markers which are identified with unique monoclonal antibodies. A method for obtaining a cell population enriched in mesenchymal stem cells is described, for example, in U.S. Patent No. 5,486,359. Alternative sources for mesenchymal stem cells include, but are not limited to, blood, skin, cord blood, muscle, fat, bone, and perichondrium.

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

The mesenchymal stem cells may be from a spectrum of sources including autologous, allogeneic, or xenogeneic.

The mesenchymal stem cells are administered in an amount effective to treat an autoimmune disease in an animal. The mesenchymal stem cells may be administered in an amount of from about $1x10^5$ cells/kg to about $1x10^7$ cells/kg, preferably from about $1x10^6$ cells/kg to about $5x10^6$ 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 patient, the autoimmune disease to be treated, and the extent and severity thereof.

The mesenchymal stem cells may be administered in conjunction with an acceptable pharmaceutical carrier. For example, the mesenchymal stem cells may be administered as a cell suspension in a pharmaceutically acceptable liquid medium for injection.

In accordance with another aspect of the present invention, 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.

Although the scope of this aspect of the present invention 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 Interferon-γ (IFN-γ) in certain inflammatory reactions, such as those associated with psoriasis, for example.

In one embodiment, the inflammatory responses which may be treated are those associated with psoriasis.

In another embodiment, the mesenchymal stem cells may be administered to an animal such that the mesenchymal stem cells contact microglia and/or astrocytes in the brain to reduce inflammation, whereby 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.

In yet another embodiment, the mesenchymal stem cells may be administered to an animal such that the mesenchymal stem cells contact keratinocytes and Langerhans cells in the epidermis of the skin to reduce inflammation as may occur in psoriasis, chronic dermatitis, and contact dermatitis. Although this embodiment is not to be limited to any theoretical reasoning, it is believed that the mesenchymal stem cells may 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 tumor necrosis factor-alpha (TNF-α) and increased regulatory T-cell (Treg cell) population.

In a further embodiment, the mesenchymal stem cells may 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 listed in the website www.arthritis.org/conditions/diseases. Although the scope of this embodiment is not intended to be limited to any theoretical reasoning, it is believed that the mesenchymal stem cells may inhibit Interleukin-17 secretion by memory T-cells in the synovial fluid.

In another embodiment, the mesenchymal stem cells may 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 invention is not intended to be limited to any theoretical reasoning, it is believed that the mesenchymal stem cells promote increased secretion of Interleukin-10 (IL-10) and the generation of regulatory T-cells (Treg cells).

In another embodiment, the mesenchymal stem cells may 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 beleived the mesenchymal stem cells promote secretion of suppressive cytokines such as IL-10, and inhibit macrophage migration inhibitory factor.

In another embodiment, the mesenchymal stem cells may 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 Treg cells.

In yet another embodiment, the mesenchymal stem cells may be used to control 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 induce peripheral blood mononuclear cells to express vascular endothelial growth factor, or VEGF, which stimulates glomerular structuring.

In a further embodiment, the mesenchymal stem cells may 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).

In yet another embodiment, the mesenchymal stem cells may 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 β-cells.

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

The mesenchymal stem cells may be administered to a mammal, including human and non-human primates, as hereinabove described.

The mesenchymal stem cells also may be administered systemically, as hereinabove described. Alternatively, in the case of osteoarthritis or rheumatoid arthritis, the mesenchymal stem cells may be administered directly to an arthritic joint.

The mesenchymal stem cells are administered in an amount effective to treat an inflammatory response in an animal. The mesenchymal stem cells may be administered in an amount of from about $1x10^5$ cells/kg to about $1x10^7$ cells/kg, preferably from about $1x10^6$ cells/kg to about $5x10^6$ 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 patient, the inflammatory response being treated, and the extent and severity thereof.

The mesenchymal stem cells may be administered in conjunction with an acceptable pharmaceutical carrier, as hereinabove described.

In accordance with yet another aspect of the present invention, 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.

Although the scope of this aspect of the present invention 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 may 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 invention is not to be limited to any specific type of cancer.

The animal may be a mammal, including human and non-human primates, as hereinabove described.

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 $1x10^5$ cells/kg to about $1x10^7$ cells/kg, preferably from about $1x10^6$ cells/kg to about $5x10^6$ 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 patient, the type of cancer being treated, and the extent and severity thereof.

The mesenchymal stem cells are administered in conjunction with an acceptable pharmaceutical carrier, and may be administered sytemically, as hereinabove described. Alternatively, the mesenchymal stem cells may be administered directly to the cancer being treated.

In accordance with still another aspect of the present invention, 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.

Although the scope of this aspect of the present invention 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.

Allergic diseases or disorders which may 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 invention is not to be limited to any specific allergic disease or disorder

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 may be a mammal. The mammal may be a primate, including human and non-human primates. In general, the mesenchymal stem cells are administered in an amount of from about $1x10^5$ cells/kg to about $1x10^7$ cells/kg, preferably from about $1x10^6$ cells/kg to about $5x10^6$ cells/kg. The exact dosage is dependent upon a variety of factors, including the age, weight, and sex of the patient, the allergic disease or disorder being treated, and the extent and severity thereof.

The mesenchymal stem cells may be administered in conjunction with an acceptable pharmaceutical carrier, as hereinabove described. The mesenchymal stem cells may be administered systemically, such as by intravenous or intraarterial administration, for example.

In accordance with a further aspect of the present invention, 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.

Specific aspects of the invention include:

- use of cultured allogeneic human mesenchymal stem cells for promoting wound healing in a human; wherein the mesenchymal stem cells express CD73, CD105, and CD166 cell surface markers; and wherein the mesenchymal stem cells promote the secretion of anti-inflammatory cytokines.

- use of cultured allogeneic human mesenchymal stem cells in the manufacture of a medicament for promoting wound healing in a human; and

- a pharmaceutical composition for promoting wound healing in a human, comprising cultured allogenic human mesenchymal stem cells and a pharmaceutically acceptable carrier.

Although the scope of the present invention 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 Interleukin-10 (IL-10). The IL-10 limits or controls inflammation in a wound, thereby promoting healing of a wound.

Furthermore, the mesenchymal stem cells may promote wound healing and fracture healing by inducing secretion factors by other cell types. For example, the

mesenchymal stem cells may induce prostaglandin E2 (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.

The mesenchymal stem cells are administered to the animal in an amount effective to promote wound healing in the animal. The animal may be a mammal, and the mammal may be a primate, including human and non-human primates. In general, the mesenchymal stem cells are administered in an amount of from about $1x10^5$ cells/kg to about $1x10^7$ cells/kg, preferably from about $1x10^6$ cells/kg to about $5x10^6$ 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 patient, and the extent and severity of the wound being treated.

The mesenchymal stem cells may be administered in conjunction with an acceptable pharmaceutical carrier, as hereinabove described. The mesenchymal stem cells may be administered systemically, as hereinabove described. Alternataively, the mesenchymal stem cells may be administered directly to a wound, such as in a fluid on a dressing or reservoir containing the mesenchymal stem cells.

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

The mesenchymal stem cells may be administered to the animal in order to treat or prevent any type of fibrosis in the animal, including, but not limited to, cirrhosis of the liver, fibrosis of the kidneys associated with end-stage renal disease, and fibrosis of the lungs. It is to be understood that the scope of the present invention is not to be limited to any specific type of fibrosis.

The mesenchymal stem cells are administered to the animal in an amount effective to treat or prevent fibrosis in the animal. The animal may be a mammal, and the mammal may be a primate, including human and non-human primates. In general, the mesenchymal stem cells are administered in an amount of from about $1x10^5$ cells/kg to about $1x10^7$ cells/kg, preferably from about $1x10^6$ cells/kg to about $5x10^6$ 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 patient, and the extent and severity of the fibrosis being treated or prevented.

The mesenchymal stem cells may be administered in conjunction with an acceptable pharmaceutical carrier, as hereinabove described. The mesenchymal stem cells may be administered systemically, also as hereinabove described.

It is another object of the present invention to promote angiogenesis 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 invention, 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.

Angiogenesis is the formation of new blood vessels from a pre-existing microvascular bed.

The induction of angiogenesis may be used to treat coronary and peripheral artery insufficiency, and thus may be a noninvasive and curative approach to the treatment of coronary artery disease, ischemic heart disease, and peripheral artery disease. Angiogenesis may 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 may 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.

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.

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 may be administered systemically, such as by intravenous, intraarterial, or intraperitoneal administration, or the mesenchymal stem cells may 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.

The mesenchymal stem cells may be from a spectrum of sources including autologous, allogeneic, or xenogeneic.

Although the scope of the present invention is not to be limited to any theroretical 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.

In one embodiment, the animal is a mammal. The mammal may be a primate, including human and non-human primates.

The mesenchymal stem cells, in accordance with the present invention, may 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 may be administered to an animal to treat blocked arteries, including those in the extremities, i.e., arms, legs, hands, and feet, as well as the neck or in various organs. For example, the mesenchymal stem cells may be used to treat

blocked arteries which supply the brain, thereby treating or preventing stroke. Also, the mesenchymal stem cells may be used to treat blood vessels in embryonic and post-natal corneas and may be used to provide glomerular structuring. In another embodiment, the mesenchymal stem cells may 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.

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

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

In another embodiment, the mesenchymal stem cells may 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.

The mesenchymal stem cells are administered in an amount effective in promoting angiogenesis in an animal. The mesenchymal stem cells may be administered in an amount of from about $1x10^5$ cells/kg to about $1x10^7$ cells/kg, preferably from about $1x10^6$ cells/kg to about $5x10^6$ 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 patient, the disease or disorder to be treated, alleviated, or prevented, and the extent and severity thereof.

The mesenchymal stem cells may be administered in conjunction with an acceptable pharmaceutical carrier. For example, the mesenchymal stem cells may be administered as a cell suspension in a pharmaceutically acceptable liquid medium for

injection. Injection can be local, i.e., directly into the tissue or organ in need of angiogenesis, or systemic.

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

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. Patent No. 6,355,239.

It is to be understood that the mesenchymal stem cells, when employed in the above-mentioned therapies and treatments, may 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 may be administered with soluble carriers for cells such as hyalurionic acid, or in combination with solid matrices, such collagen, gelatin, or other biocompatible polymers, as appropriate.

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

The invention now will be described with respect to the drawings, wherein:

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.

Fig. 2 MSCs inhibit pro-inflammatory effector T cell function. (A) Flow cytometric analysis of T_{Reg} cell numbers (in %) by staining PBMCs or 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)** T_H1 cells generated in presence of MSCs secreted reduced levels of IFN-γ (primary y-axis) and T_H2 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.

Fig. 3 MSCs lead to increased numbers of T_{reg} cell population and increased GITR expression. (A) A $CD4^+$ $CD25^+$ 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, following which ^3H thymidime 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.

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 is 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 (□) and IFN-γ secretion from T_H1 cells (▥) as compared to controls. Data were calculated as % change from cultures generated in absence of MSCs and PGE_2 inhibitors **(C)** 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.

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 may play a role in modulating immune functions in an inflammatory setting.

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 ^{3}H 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).

Fig. 7 Schematic diagram of proposed MSC mechanism of action

MSCs mediate their immuno-modulatory effects by affecting cells from both the innate (DCs- 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 T_H1 or humoral T_H2 immunity). During MSC-DC interaction, MSCs, by means of direct cell-cell contact or via secreted factor, may 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 may interact with them to skew the balance of T_H1 (pathway 1) responses towards T_H2 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) may result in a tolerant phenotype and may aid a recipient host by dampening bystander inflammation in their local micro-environment. Dashed line (----) represents proposed mechanism.

The invention now will be described with respect to the following example; it is to be understood, however, that the scope of the present invention is not to be limited thereby.

Example 1

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, California) containing 1% antibiotic-antimyotic solution (Invitrogen, Carlsbad, California) and 10% fetal bovine serum (FBS, JRH BioSciences, Lenexa, Kansas). 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)).

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, California). Precursors of DCs of plasmacytoid lineage were isolated from PBMCs by immuno-magnetic sorting of positively labeled antibody coated cells ($BDCA2^+$) (Miltenyi Biotech, Auburn, California).

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.

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, California) columns in a magnetic field, while NK cells passed through and were collected.

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, California). 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.

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 ^3H thymidine was added. Incorporated radioactivity was counted after 24 hours.

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.

Generation of T_H1/T_H2 cells

Peripheral blood mononuclear cells (PBMCs) were plated at $2x10^6$ 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 T_H1 (IL-2 (4 ng/ml) + IL-12 (5 ng/ml) + anti-IL-4 (1 µg/ml)) or T_H2 (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, Minnesota).

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 prostaglandin E_2 (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).

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Proliferation of PBMCs

Purified PBMCs were prepared by centrifuging Leukopack™ (Cambrex, Walkersville, Maryland) 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, Missouri). In selected experiments, PBMCs were resuspended in medium containing PGE_2 inhibitors Indomethacin (Sigma Chemicals, St. Louis, Missouri) or NS-938 (Cayman Chemicals, Ann Arbor, Michigan). (^{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.

Quantitative RT-PCR

Total RNA from cell pellets were prepared using a commercially available kit (Qiagen, Valencia, California) and according to the manufacturer's instructions. Contaminating genomic DNA was removed using the DNA-free kit (Ambion, Austin, Texas). Quantitative RT-PCR was performed on a MJ Research Opticon detection system (South San Francisco, California) using QuantiTect SYBR Green™ RT-PCR kit (Qiagen, Valencia, California) 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 sequence for COX-1 and COX-2 specific primers were: COX-1: 5'-CCG GAT GCC AGT CAG GAT GAT G-3'(forward), 5'-CTA GAC AGC CAG ATG CTG ACA G-3' (reverse); COX-2: 5'-ATC TAC CCT CCT CAA GTC CC-3'(forward), 5'-TAC CAG AAG GGC AGG ATA CAG-3' (reverse).

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 ^{3}H 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.

Results

In the present studies, the interaction of human MSCs with isolated immune cell populations, including dendritic cells (DC1 and DC2), effector T cells (T_H1 and T_H2) and NK cells was examined. The interaction of MSCs with each immune cell type had specific consequences, suggesting that MSCs may modulate several steps in the immune response process. The production of secreted factor(s) that modulate and may be responsible for MSC immuno-modulatory effects was evaluated and prostaglandin synthesis was implicated.

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 ($1x10^3$ IU/ml and $1x10^3$ 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. 1A). 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. 1B). 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. 1B). 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. 1C) suggesting a MSC-mediated shift from pro-inflammatory to anti-inflammatory T cell phenotype.

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. 2A), 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. 3A,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 T_H1 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 T_H1 cells and increased IL-4 secretion by T_H2 cells (Fig. 2B). 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 T_H1 or T_H2 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.

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. 2C), thereby suggesting that MSCs can modulate NK cell functions also.

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-1constitutively, 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 $x10^6$ cells/ml) contained approx. 1000 pg/ml of PGE_2 (Fig. 4A). There was no detectable presence of known inducers of PGE_2 secretion e.g. TNF-α, IFN-γ or IL-1β

(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. 4A). 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. 4B). Modest changes in COX-1 levels were seen suggesting that the increase in PGE_2 secretion upon MSC-PBMC co-culture (Fig. 5) is mediated by COX-2 up-regulation. 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 T_H1 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 T_H1 cells (Fig. 4C), respectively, suggesting that MSC effects on immune cell types may 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 (^3H) thymidine incorporation by PHA-treated PBMCs in MSC containing cultures as compared to controls without inhibitors (Fig. 4D).

In summary, a model of MSC interaction with other immune cell types (Fig. 7) is proposed. When mature T cells are present, MSCs may 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.

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

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CLAIMS:

1. Use of cultured allogeneic human mesenchymal stem cells for promoting wound healing in a human;

wherein the mesenchymal stem cells express CD73, CD105, and CD166 cell surface markers; and

wherein the mesenchymal stem cells promote the secretion of anti-inflammatory cytokines.

2. Use of cultured allogeneic human mesenchymal stem cells in the manufacture of a medicament for promoting wound healing in a human.

3. The use of claim 1, wherein the mesenchymal stem cells are for direct application to a wound.

4. The use of claim 3, wherein the mesenchymal stem cells are present in a fluid on a dressing or in a reservoir.

5. The use of any one of claims 1 to 4, wherein the wound is an external wound.

6. The use of any one of claims 1 to 4, wherein the wound is an internal wound.

7. The use of any one of claims 1 to 4, wherein the wound is a dermal ulcer.

8. The use of claim 7, wherein the dermal ulcer is a dermal ulcer found in the feet, hands, legs or arms.

9. The use of any one of claims 1 to 8, wherein the mesenchymal stem cells are not genetically manipulated.

10. The use of any one of claims 1 to 9, wherein the mesenchymal stem cells induce the secretion of a factor selected from the group consisting of prostaglandin E2 (PGE2), vascular endothelial growth factor (VEGF), growth hormone, insulin, insulinlike growth factor 1 (IGF-1), insulin-like growth factor binding protein-3 (IGFBP-3), and endothelin-1.

11. The use of any one of claims 1 to 10, wherein the mesenchymal stem cells bind to an antibody selected from the group consisting of 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, and an antibody produced from hybridoma cell line SH4, ATCC accession number HB 10745.

12. The use of any one of claims 1 to 11, wherein the anti-inflammatory cytokines comprise IL4 or IL10.

13. A pharmaceutical composition for promoting wound healing in a human, comprising cultured allogeneic human mesenchymal stem cells and a pharmaceutically acceptable carrier.

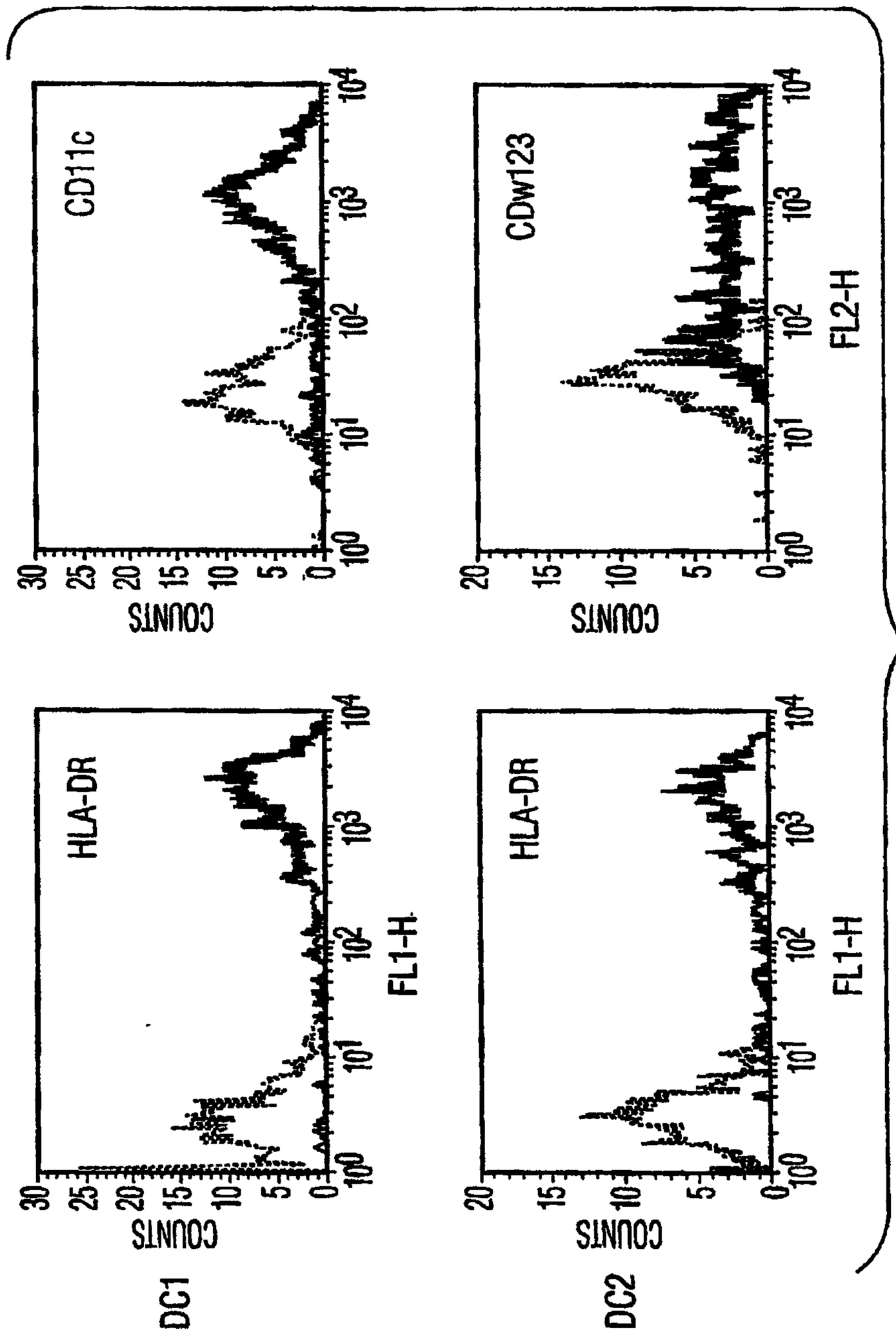

FIG. 1A

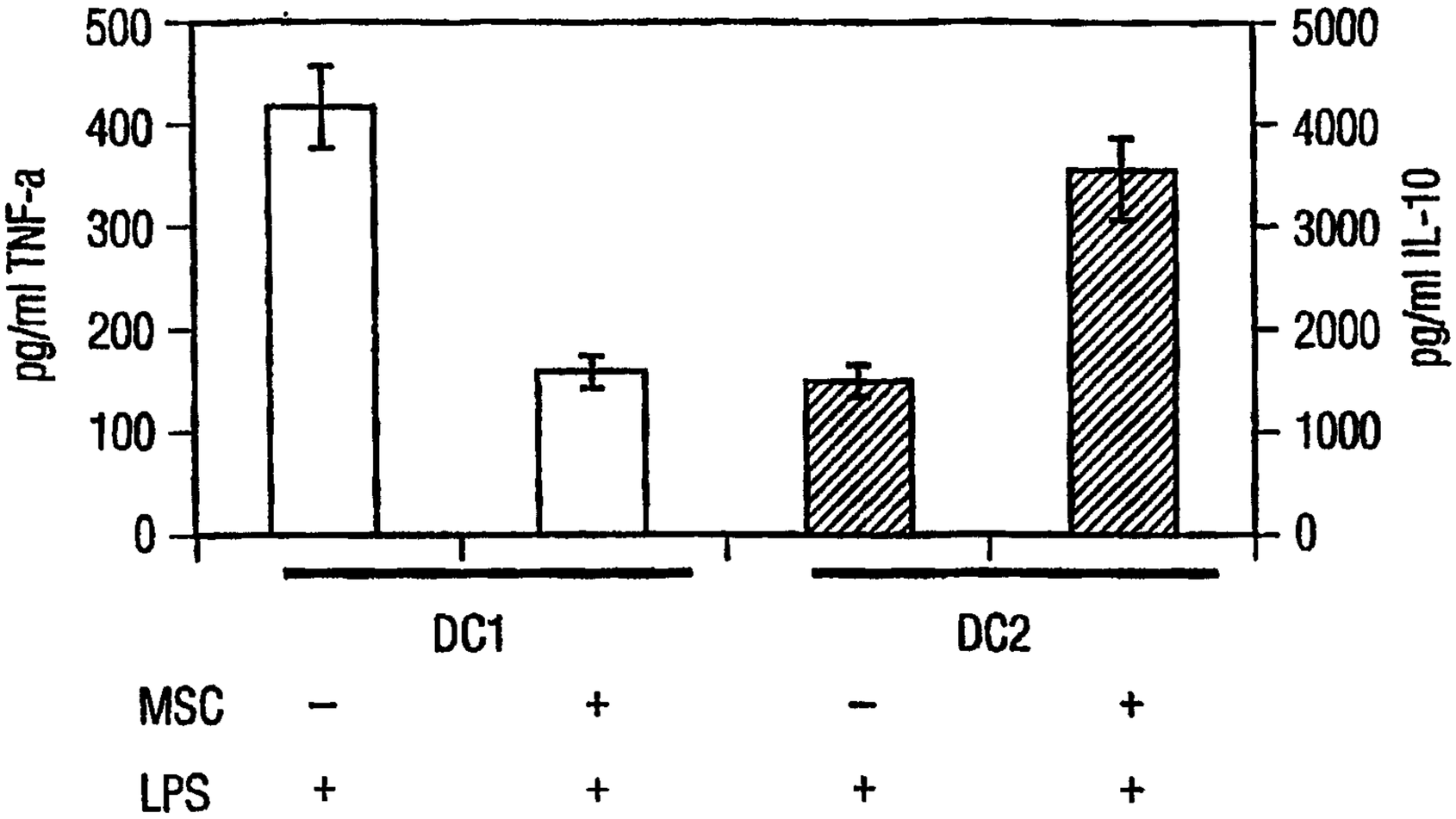
500
400
300
200
100
0
pg/ml TNF-a
5000
4000
3000
2000
1000
0
pg/ml IL-10
DC1
DC2
MSC – + – +
LPS + + + +

FIG. 1B

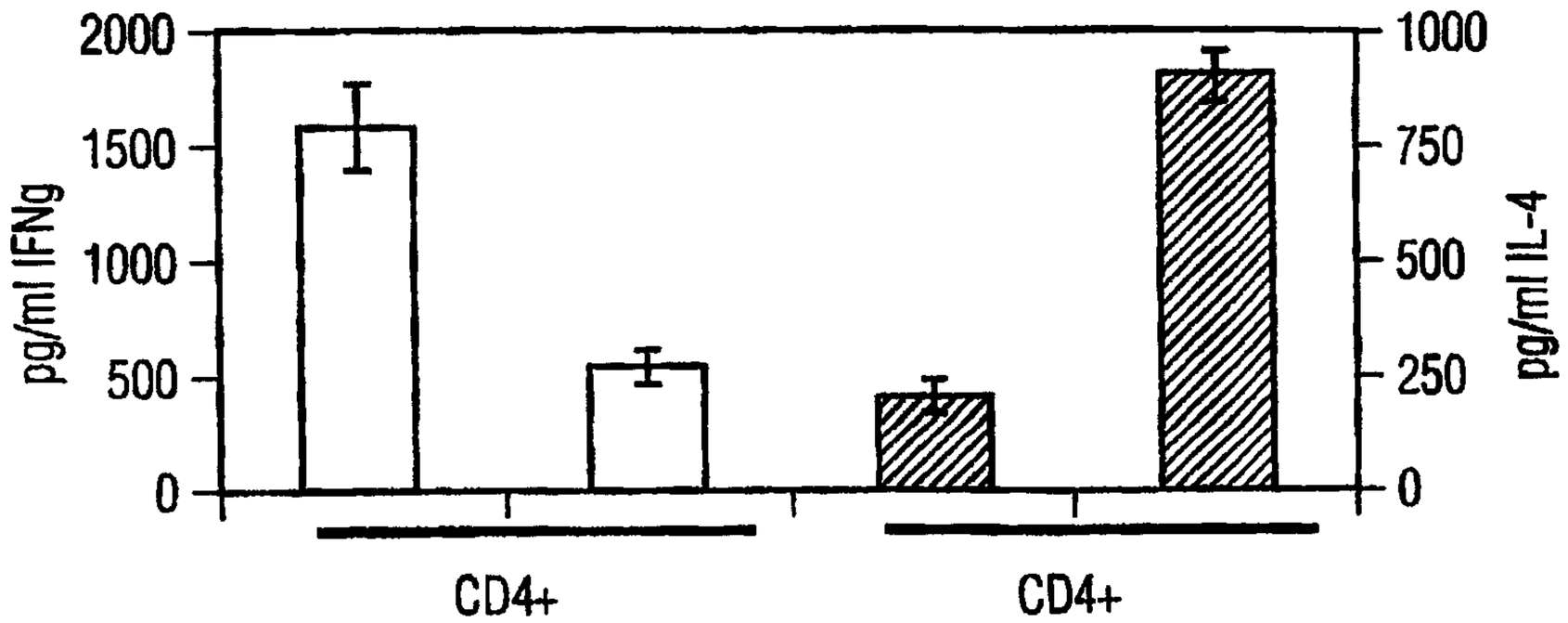
2000
1500
1000
500
0
pg/ml IFNg
1000
750
500
250
0
pg/ml IL-4
CD4+
CD4+
MSC – + – +
DC1 + + – –
DC2 – – + +
PHA + + + +

FIG. 1C

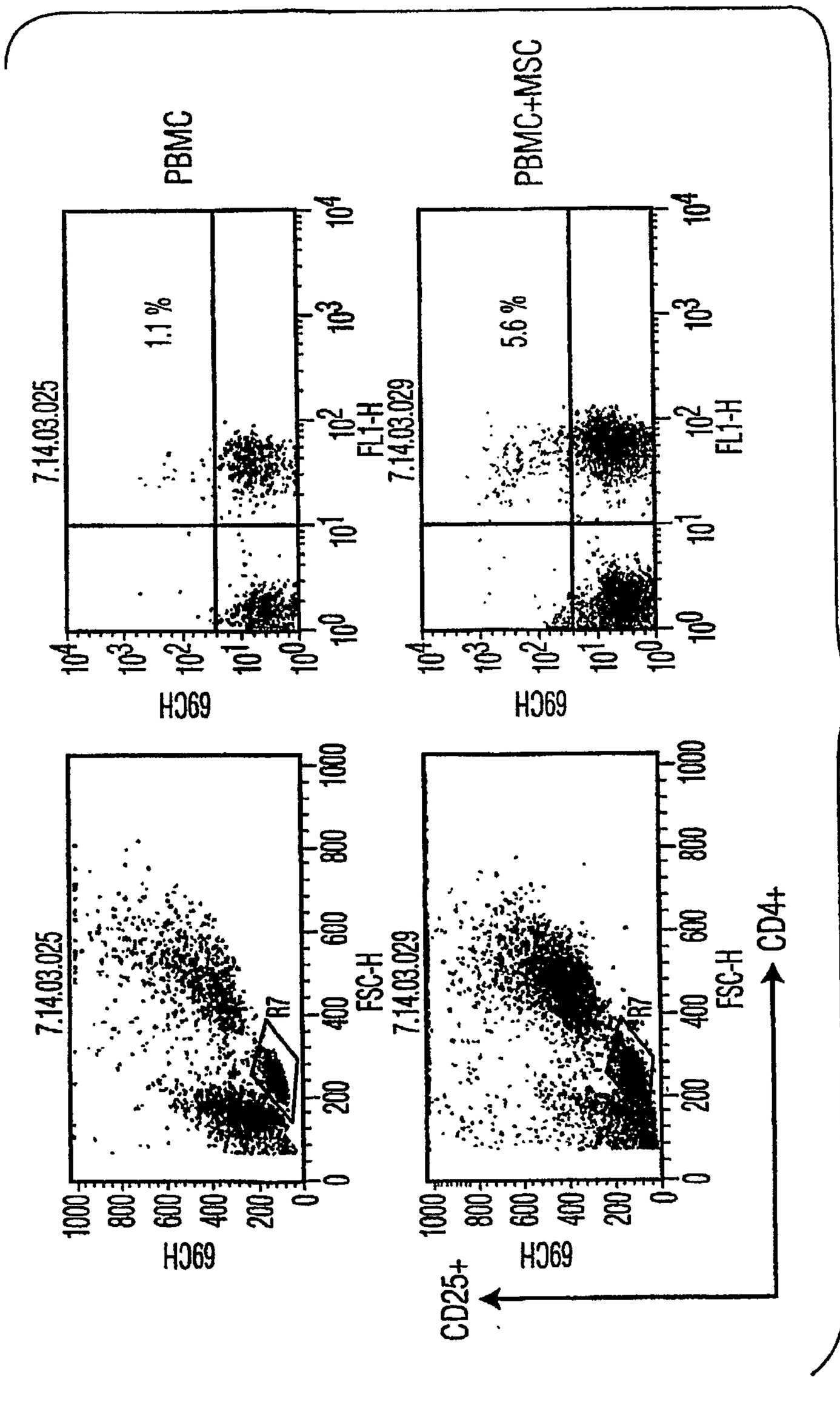
PBMC
PBMC+MSC
7.14.03.025
7.14.03.029
1.1 %
5.6 %
FL1-H
69CH
FSC-H
CD4+
CD25+
R7

FIG. 2A

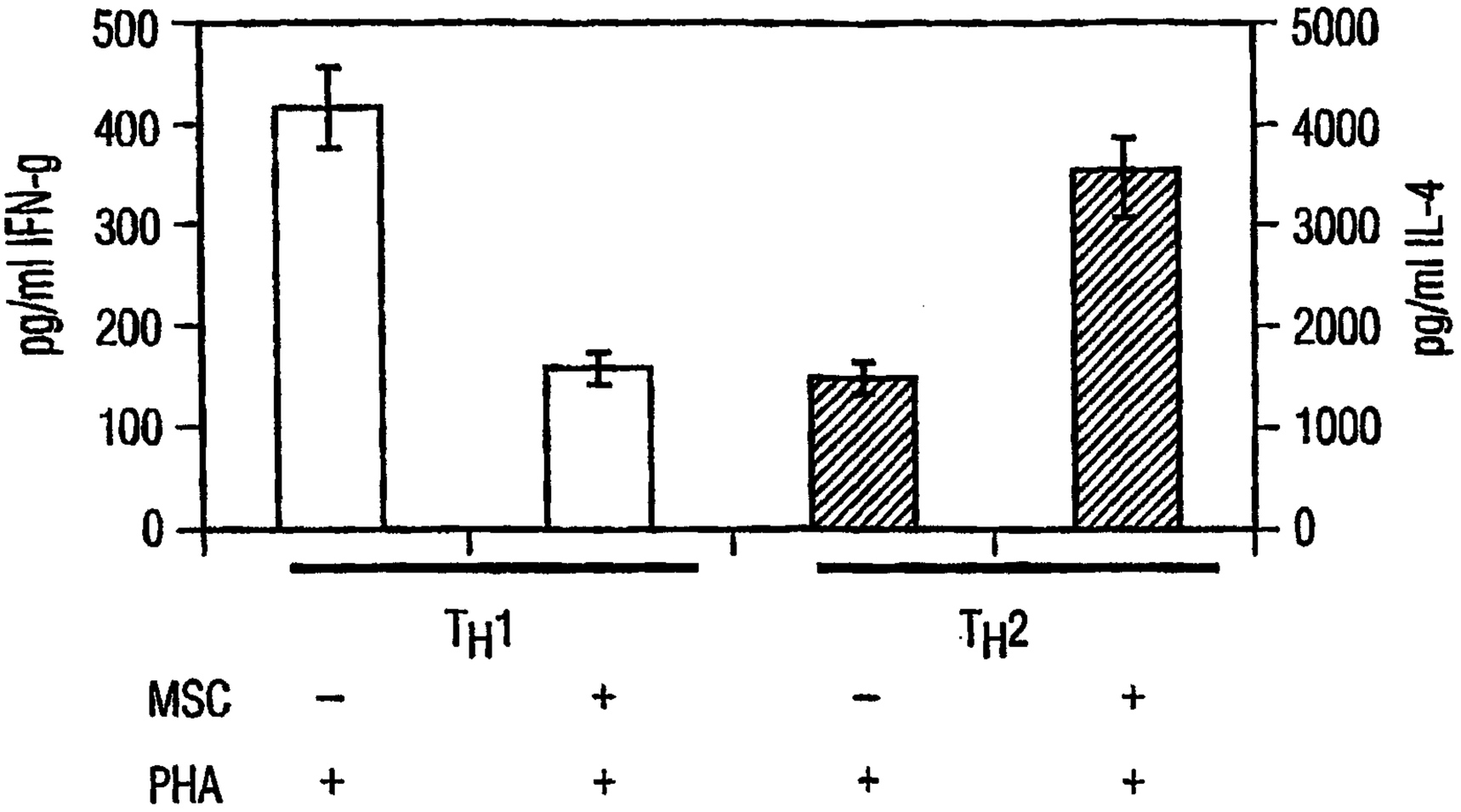
500
400
300
200
100
0
pg/ml IFN-g
5000
4000
3000
2000
1000
0
pg/ml IL-4
TH1
TH2
MSC – + – +
PHA + + + +

FIG. 2B

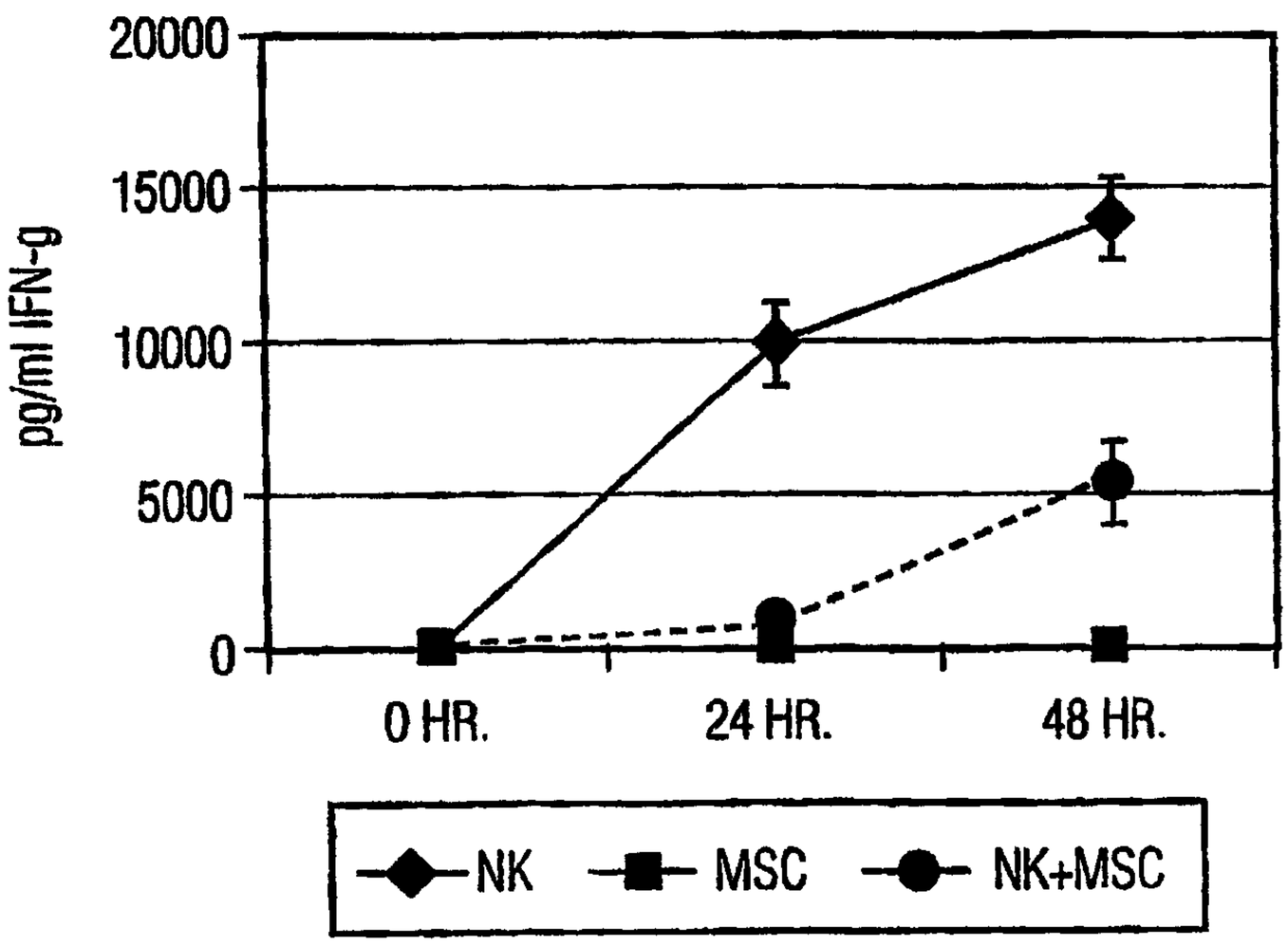
20000
15000
10000
5000
0
pg/ml IFN-g
0 HR.
24 HR.
48 HR.
NK
MSC
NK+MSC

FIG. 2C

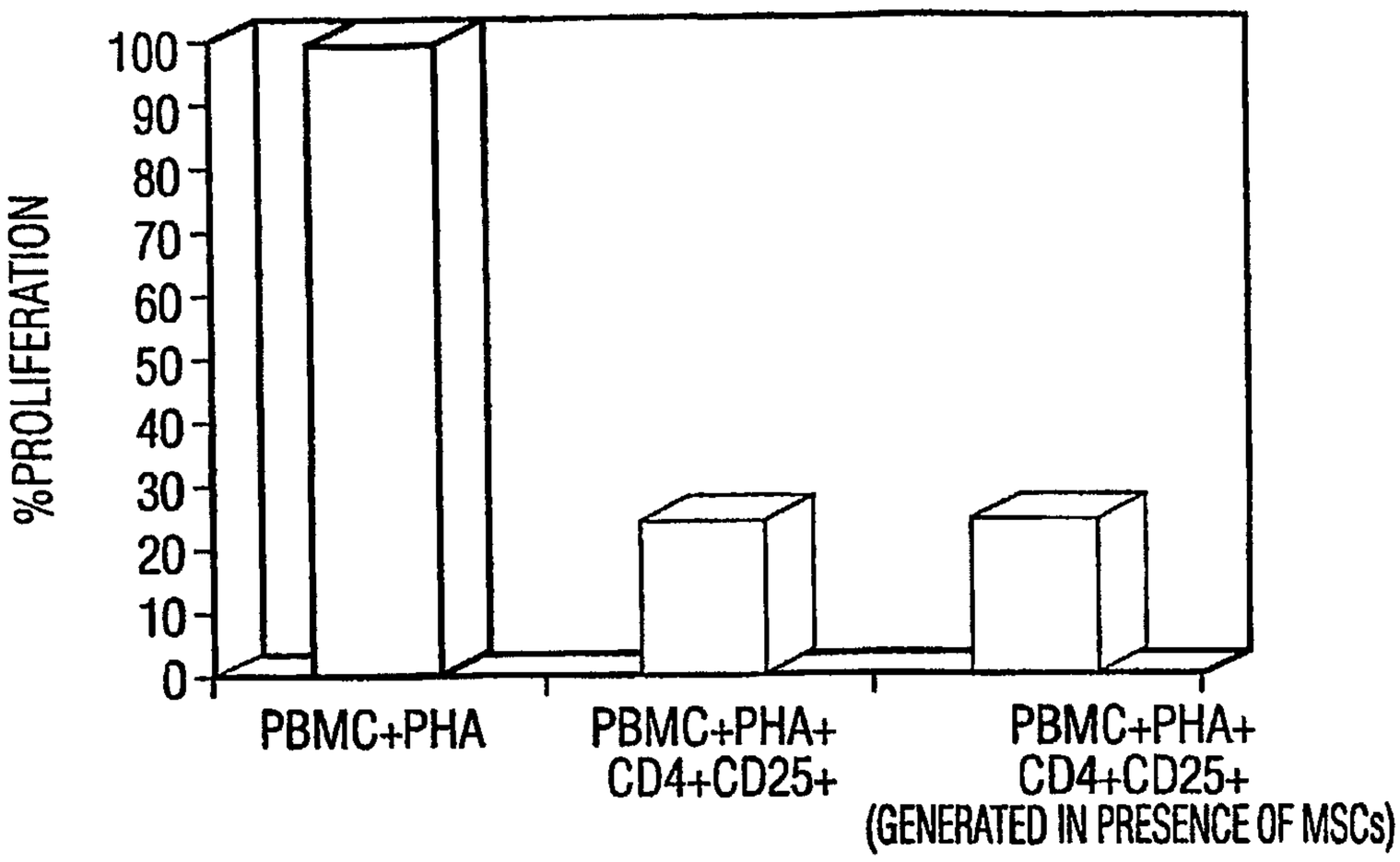
%PROLIFERATION
100
90
80
70
60
50
40
30
20
10
0
PBMC+PHA
PBMC+PHA+
CD4+CD25+
PBMC+PHA+
CD4+CD25+
(GENERATED IN PRESENCE OF MSCs)

FIG. 3A

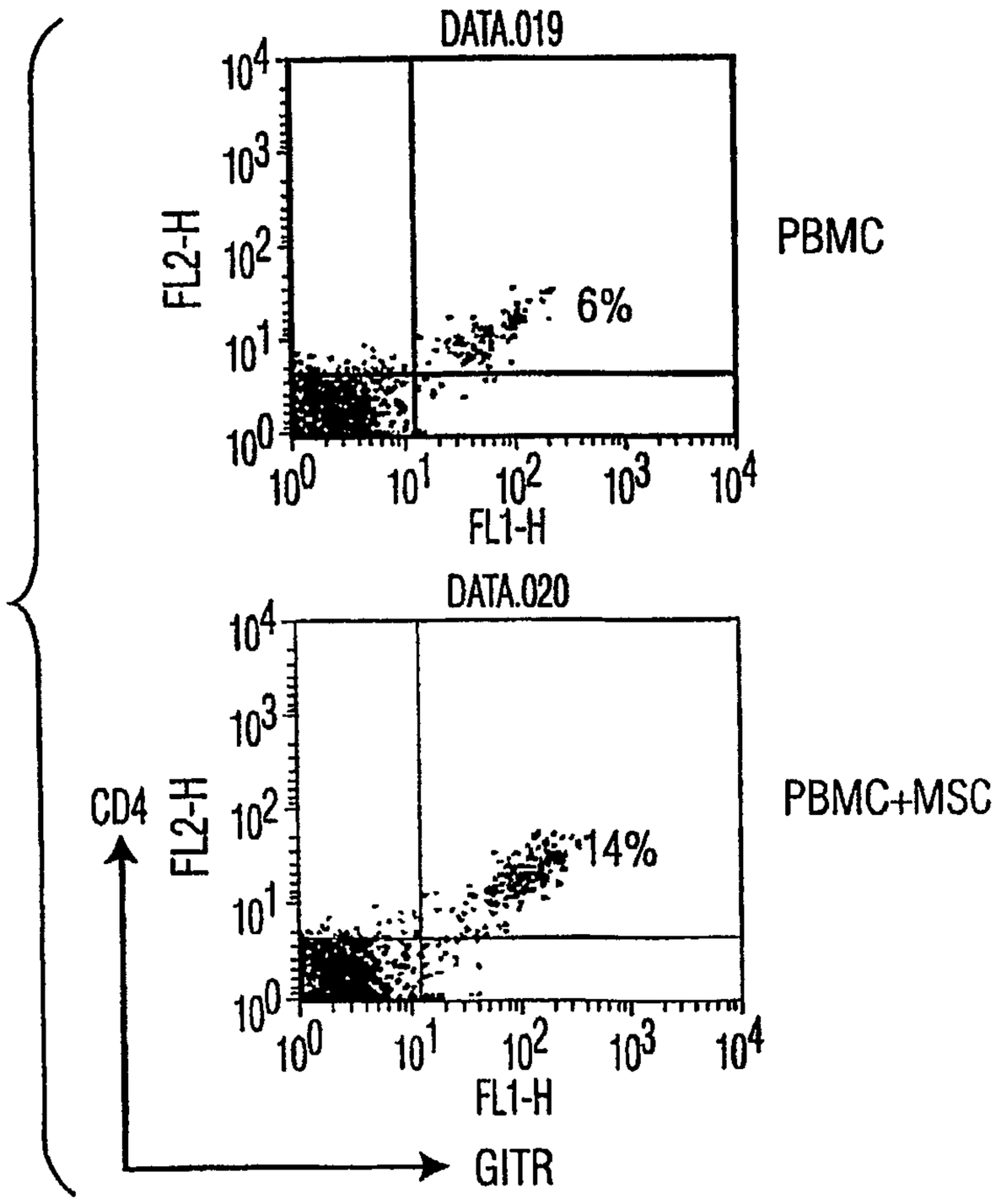
DATA.019
FL2-H
FL1-H
6%
PBMC
DATA.020
CD4
GITR
14%
PBMC+MSC

FIG. 3B

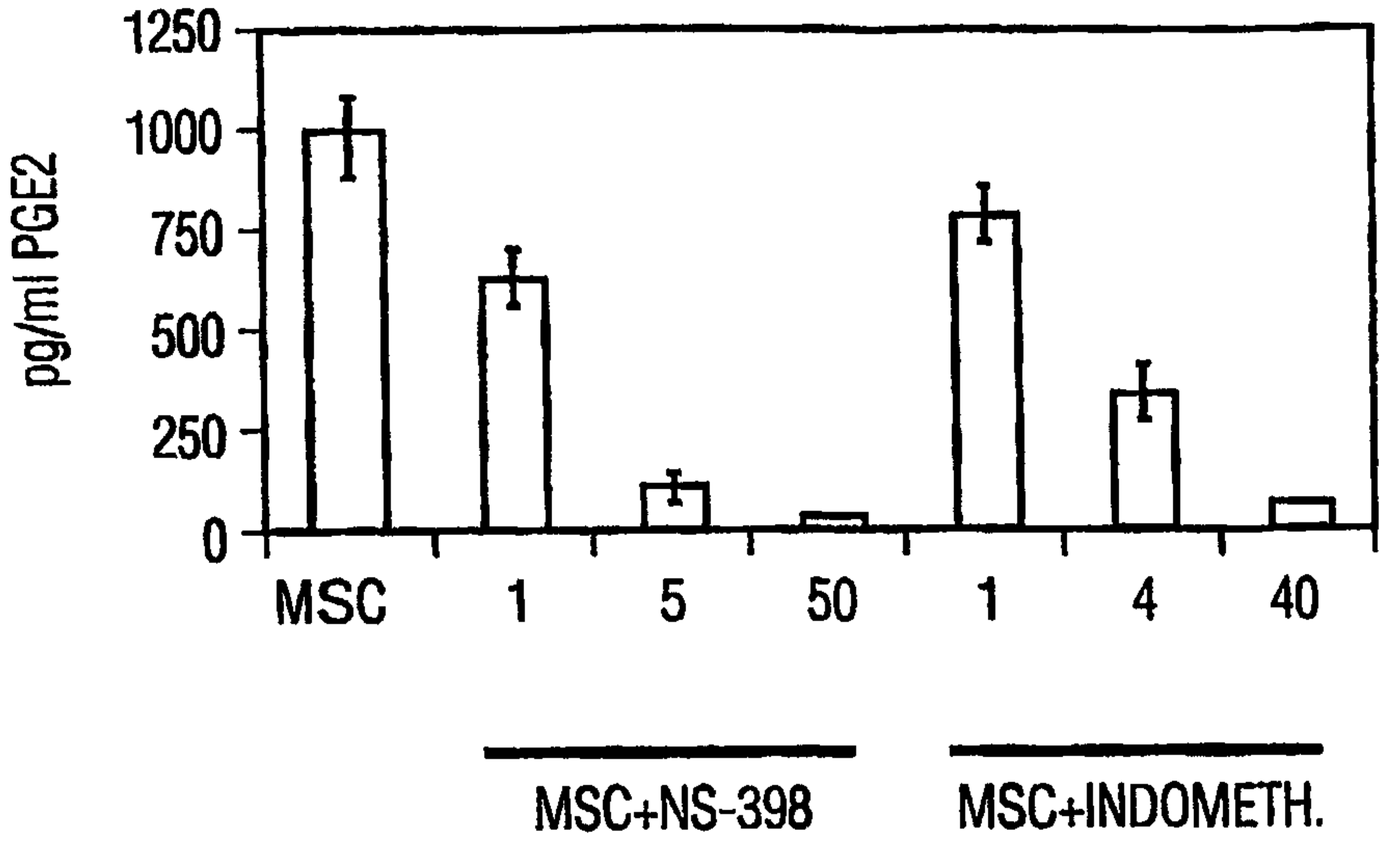
pg/ml PGE2
1250
1000
750
500
250
0
MSC
1
5
50
1
4
40
MSC+NS-398
MSC+INDOMETH.

FIG. 4A

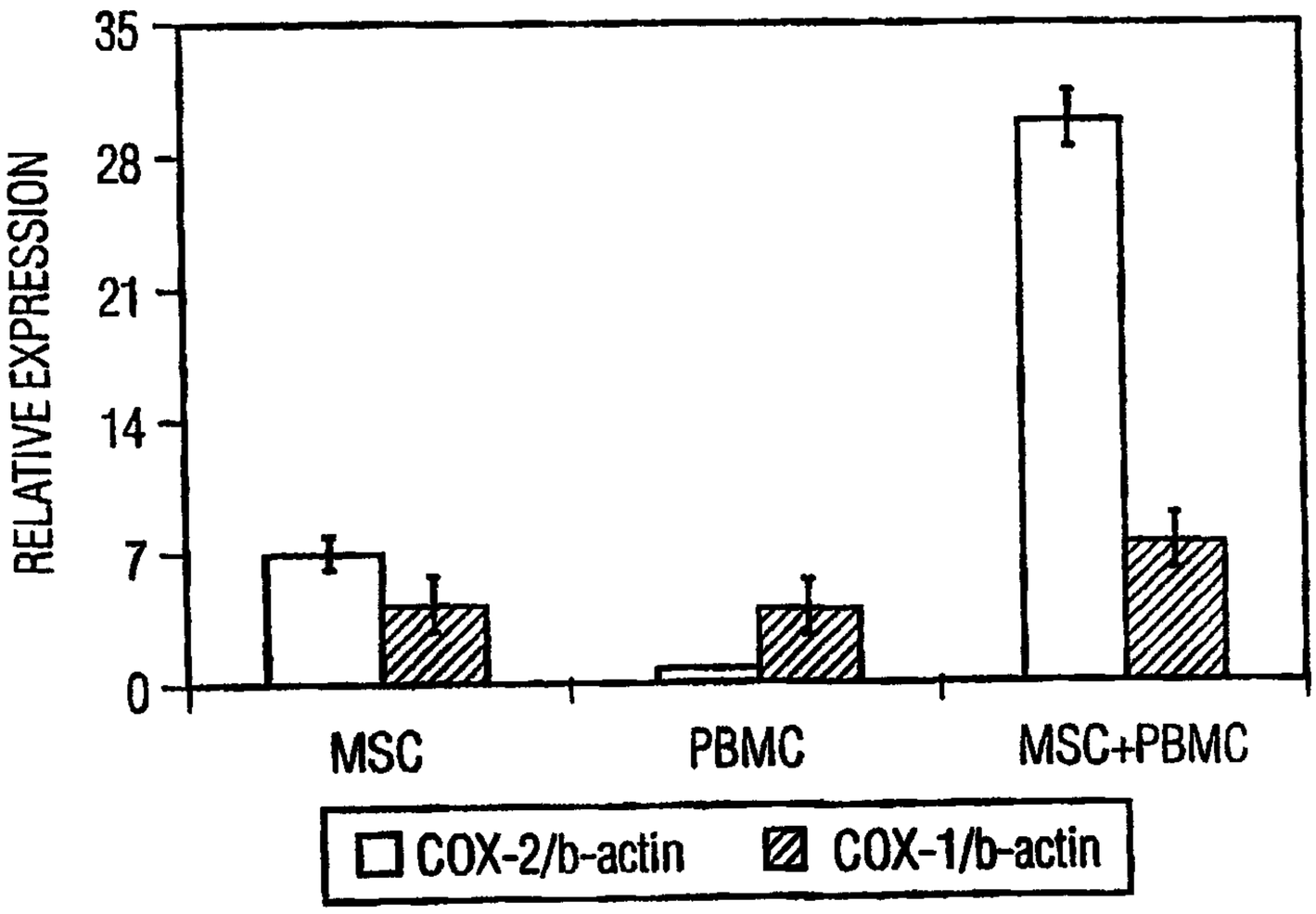
RELATIVE EXPRESSION
35
28
21
14
7
0
MSC
PBMC
MSC+PBMC
COX-2/b-actin
COX-1/b-actin

FIG. 4B

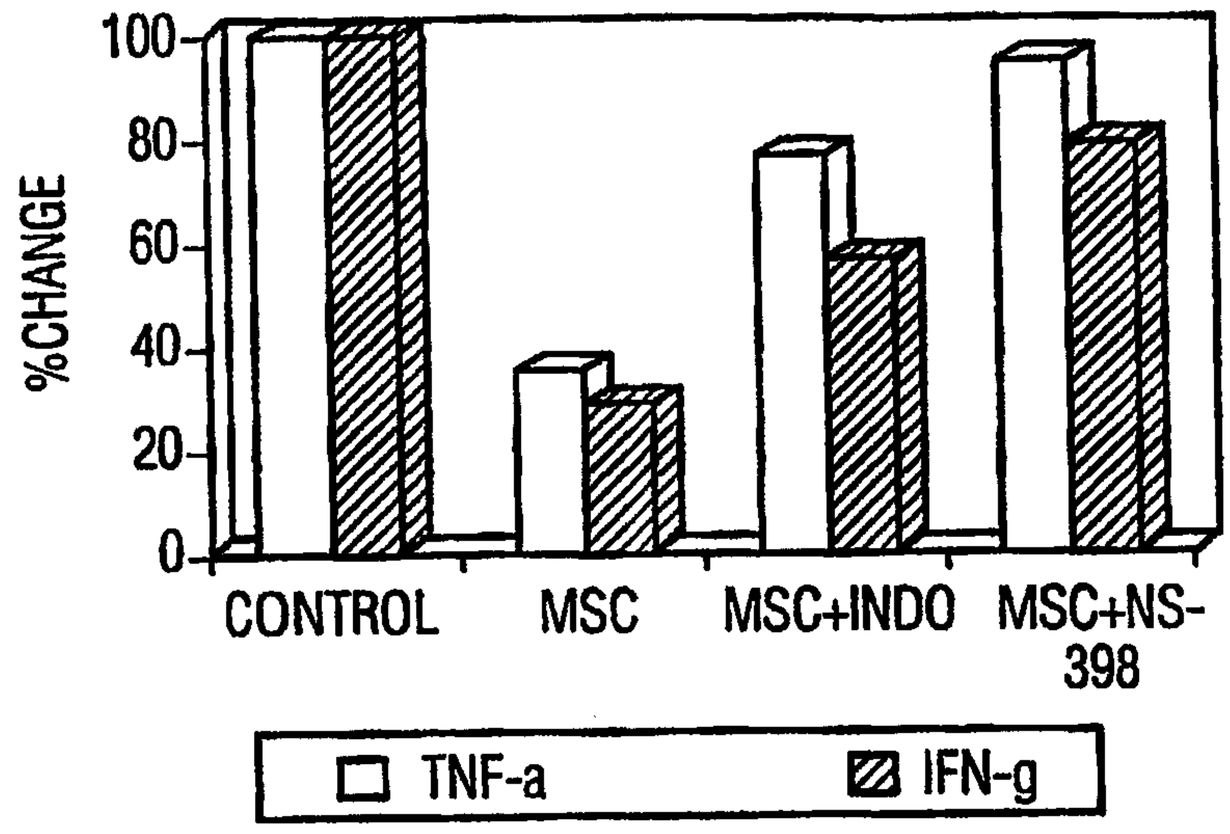
%CHANGE
100
80
60
40
20
0
CONTROL
MSC
MSC+INDO
MSC+NS-398
TNF-a
IFN-g

FIG. 4C

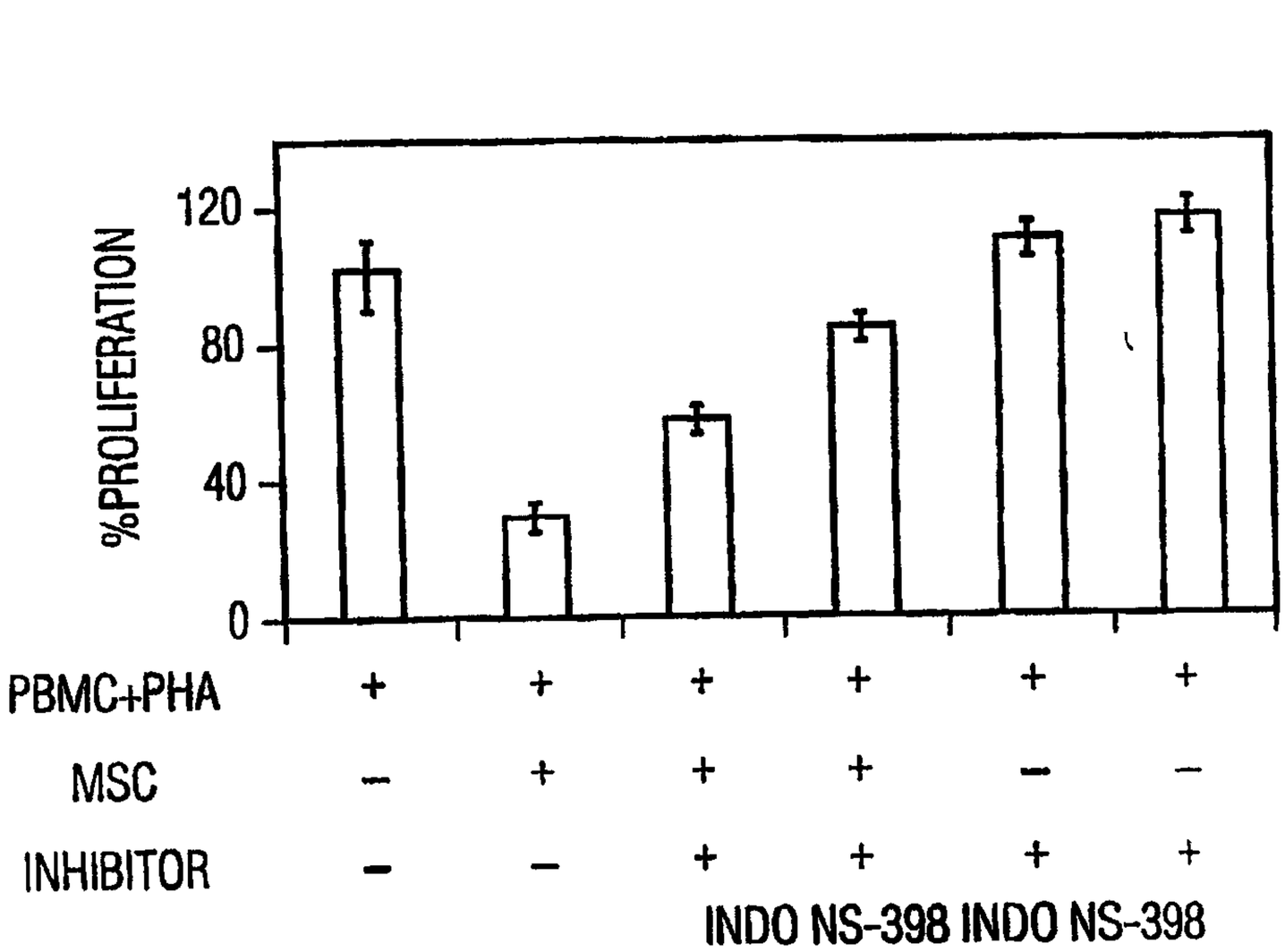
%PROLIFERATION
120
80
40
0
PBMC+PHA + + + + + +
MSC - + + + - -
INHIBITOR - - + + + +
INDO NS-398 INDO NS-398

FIG. 4D

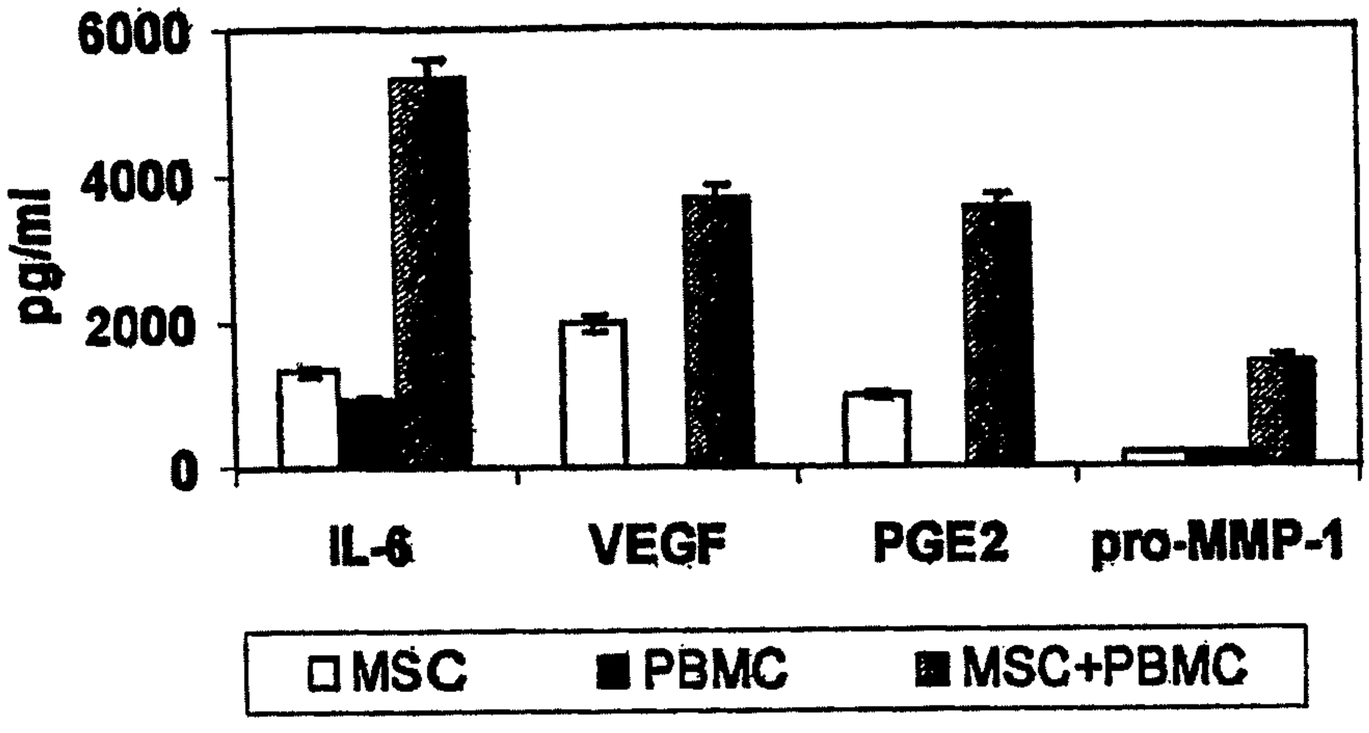

FIG. 5

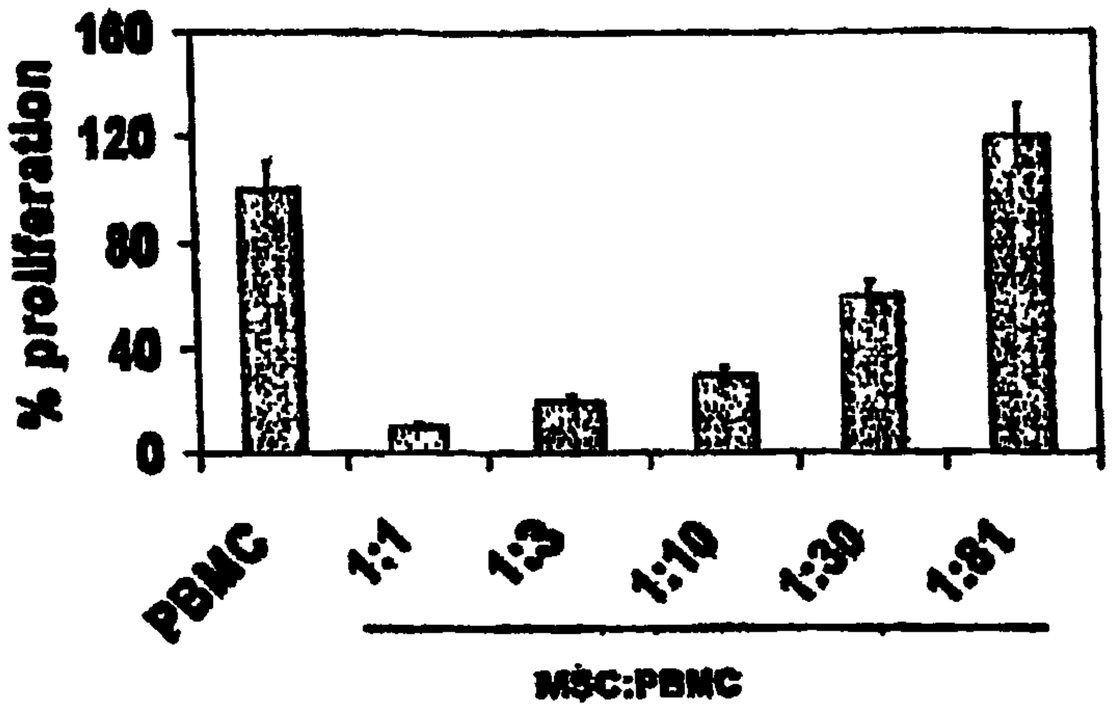

FIG. 6

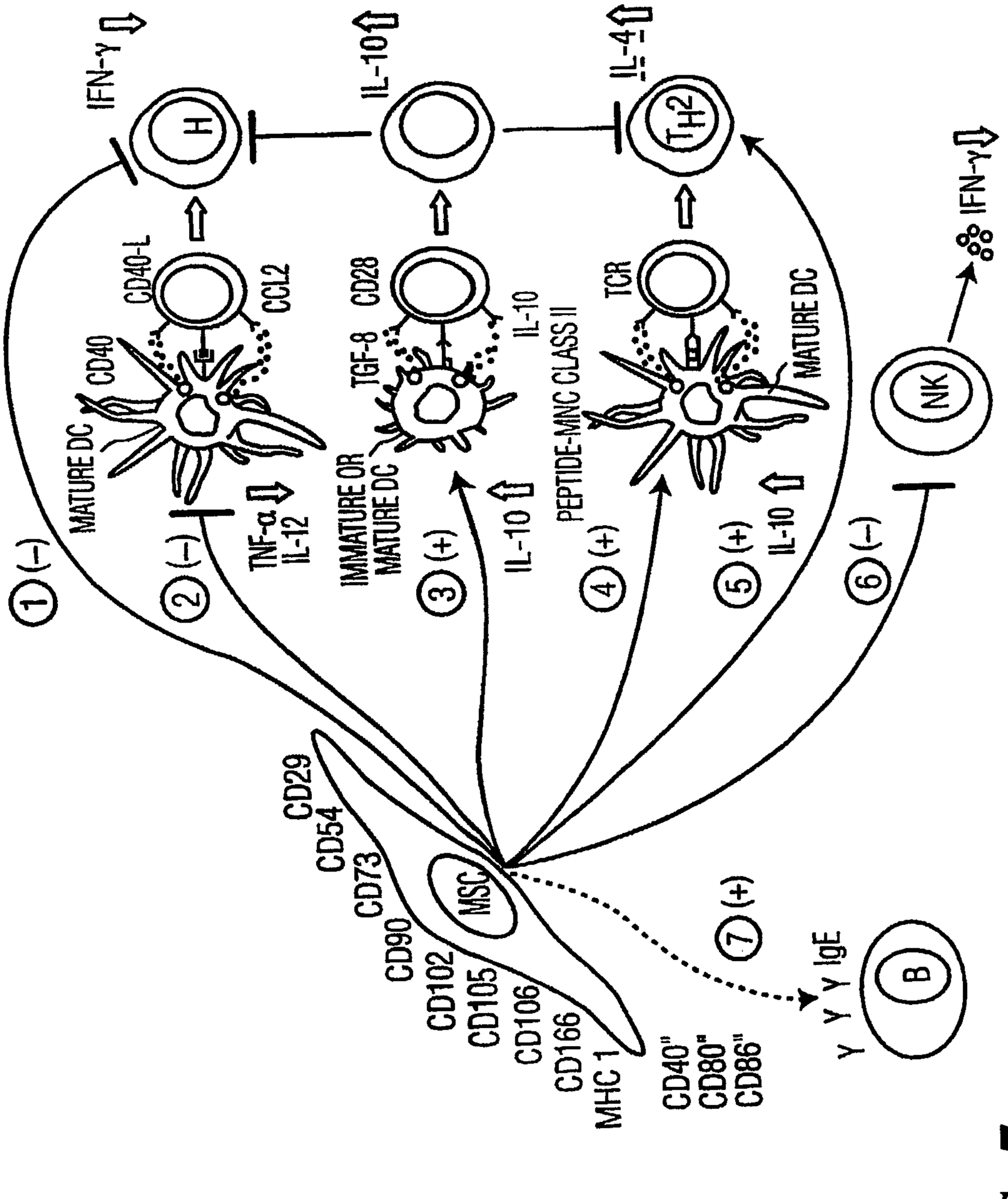
IFN-γ
IL-10
IL-4
H
$T_{H}2$
CD40-L
CCL2
CD40
MATURE DC
TNF-α
IL-12
TGF-8
CD28
IL-10
IMMATURE OR MATURE DC
IL-10
PEPTIDE-MNC CLASS II
TCR
MATURE DC
NK
IFN-γ
1 (−)
2 (−)
3 (+)
4 (+)
5 (+)
IL-10
6 (−)
CD29
CD54
CD73
CD90
CD102
CD105
CD106
CD166
MHC 1
MSC
CD40"
CD80"
CD86"
7 (+)
γ γ γ IgE
B

FIG. 7