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DESCRIPTION

Field of the Invention

[0001] The field of the invention is immunomodulation by multipotent adult progenitor cells ("MAPCs") and their use for modulating immune responses in primary and adjunctive therapies.

Background of the Invention

[0002] The therapeutic use of organ transplants, including bone marrow transplants, has steadily increased since its early beginnings. It has become an important therapeutic option for a number of diseases, including, but not limited to, hematologic, immunologic, and malignant disorders.

[0003] Unfortunately, the therapeutic uses of transplantation often are complicated, rendered ineffectual, or precluded by adverse immune responses engendered by the transplant. Among the most prominent adverse reactions encountered as a result of transplant therapies are (i) the host versus graft response ("HVG") (rejection of the transplant by an immune competent host), and (ii) graft versus host disease ("GVHD") (processes that occur primarily in an immunocompromised host when it is recognized as non-self by immunocompetent cells of a graft).

[0004] Graft rejection in a host can be avoided, of course, by perfectly matching the donor and the host. Except for autologous tissue, however, only identical twins might be expected to be truly syngeneic. Perfect matches between an individual donor and another individual host/recipient are virtually non-existent. Thus, the use of autologous tissue is the only other way to make a perfect match. Unfortunately, the host tissue is typically not suitable or was not isolated in advance of need. Frequently the need for the transplant therapy is, in fact, to replace damaged tissue in the host. The use of syngeneic tissue, therefore, while an effective solution to the problems of adverse host response to graft tissue, is not generally useful in practical applications.

[0005] If syngeneic matching is not possible, the adverse immune effects that arise in transplant therapies can be mitigated by matching an allogeneic donor and host as closely as possible. Blood and/or tissue typing is used to match donors and hosts to provide the highest likelihood of therapeutic success. Even the closest matching of allogeneic tissue, however, does not prevent serious HVG, and, accordingly, transplant therapies involve the use of immunosuppression and immunosuppressive drugs, as discussed below.

[0006] Another approach to avoid the complications of HVG in transplant therapies has been

to disable the immune system of the recipient host. This has been accomplished by using radiation therapy, and/or immunosuppressive chemotherapy, and/or antibody therapy. The resulting suppression of host immune responses often quite effectively aids establishment of the graft (such as bone marrow) in the host. However, immunoablation or suppression compromises the host's immune defenses. This results in the host becoming all too readily susceptible to infections after even minor exposure to infectious agents. The resulting infections are a major cause of morbidity and mortality among transplant patients.

[0007] Compromising the host immune system also engenders or exacerbates another serious problem encountered in transplant therapies - graft versus host disease ("GVHD"). GVHD occurs when donor tissue contains immunocompetent cells that recognize MHC proteins of the recipient as non-self. This activates the T-cells, and they secrete cytokines, such as IL-2 (interleukin 2), IFNγ (interferon gamma), and TNFα (tumor necrosis factor alpha). These signals trigger an immune attack on recipient targets including the skin, GI tract, liver, and lymphoid organs (Ferrara and Deeg, 1991). GVHD is particularly a problem in bone marrow transplants, where it has been shown to be mediated primarily by T lymphocytes (Grebe and Streilein, 1976). In fact, approximately 50% of bone marrow transplant patients develop acute GVHD. Many of these patients die (from 15% to 45%).

[0008] There are also other immune system dysfunctions, disorders, and diseases that arise as primary pathologies and as secondary effects of other pathologies and/or treatments thereof. These include neoplasms, pathologies of the bone marrow, pathologies of the blood, autoimmune disorders, and some inflammatory disorders, as discussed further below. Primary and adjunctive therapy for these disorders and diseases, like primary and adjunctive therapies for HVG and GVHD, often involve the use of immunosuppressive drugs. All of the current therapies have disadvantages and side effects.

Immunosuppressant Drugs

[0009] A good deal of effort has been directed to developing drugs to treat these immune system dysfunctions to ameliorate or eliminate their deleterious effects, without causing additional harmful side effects. There has been some progress toward this goal, and a number of drugs have been developed and are in use to prevent and/or treat these dysfunctions. The introduction of the more effective of these drugs marked a great advance in the medical practice of transplant therapies; but, none are ideal. Indeed, none of the immunosuppressive drugs currently available for clinical use in transplant therapies are entirely effective. All of the drugs have serious drawbacks and deleterious side effects, as summarized briefly below. For review see Farag (2004), "Chronic graft-versus-host disease: where do we go from here?," Bone Marrow Transplantation 33: 569-577.

[0010] Corticosteroids, which are used primarily to treat inflammation and inflammatory diseases, are known to be immunosuppressive and are considered by many to be the best primary treatment for HVG and GVHD. They inhibit T-cell proliferation and T-cell dependent

immune responses, at least in part, by inhibiting the expression of certain cytokine genes involved in T-cell activation and T-cell dependent immune response.

[0011] Cyclosporin is among the most frequently used drugs for immune suppression and the prevention of HVG and GVHD. It is strongly immunosuppressive in general. Although it can be effective in reducing adverse immune reactions in transplant patients, it also weakens the immune system so much that patients are left highly vulnerable to infections. Consequently, patients are much more easily infected by exposure pathogens, and have little capacity to mount an effective immune response to infections. Even mild pathogens then can be life-threatening. Cyclosporin also causes a variety of other undesirable side effects.

[0012] Methotrexate is also widely used in the prophylaxis and treatment of HVG and GVHD, by itself or in combination with other drugs. Studies have shown that, if it is effective at all, it is apparently less effective than cyclosporin. As with cyclosporin, methotrexate causes a variety of side effects, some of which can be deleterious to patient health.

[0013] FK-506 is a macrolide-like compound. Similar to cyclosporin, it is derived from fungal sources. The immunosuppressive effects of cyclosporin and FK-506 are similar. They block early events of T-cell activation by forming a heterodimeric complex with their respective cytoplasmic receptor proteins (i.e., cyclophilin and FK-binding protein). This then inhibits the phosphatase activity of calcineurin, thereby ultimately inhibiting the expression of nuclear regulatory proteins and T-cell activation genes.

[0014] Other drugs that have been used for immunosuppression include antithymocyte globulin, azathioprine, and cyclophosphamide. They have not proven to be advantageous. Rapamycin, another macrolide-like compound which interferes with the response of T-cells to IL-2, also has been used to block T-cell activated immune response. RS-61443, a derivative of mycophenolic acid, has been found to inhibit allograft rejection in experimental animals. Mizoribine, an imidazole nucleoside, blocks the purine biosynthetic pathway and inhibits mitogen stimulated T- and B-cell proliferation in a manner similar to azathioprine and RS-61443. Deoxyspergualin, a synthetic analog of spergualin, has been found to exert immunosuppressive properties in pre-clinical transplantation models. The anti-metabolite brequinar sodium is an inhibitor of dihydro-orotate dehydrogenase and blocks formation of the nucleotides uridine and cytidine via inhibition of pyrimidine synthesis. Berberine and its pharmacologically tolerable salts have been used as an immunosuppressant for treating autoimmune diseases such as rheumatism, for treating allergies, and for preventing graft rejection. It has been reported that berberine inhibits B-cell antibody production and generally suppresses humoral immune responses, but does not affect T-cell propagation. See Japanese Patent 07-316051 and US Patent No. 6,245,781.

[0015] None of these immunosuppressive drugs, whether used alone or in combination with other agents, are fully effective. All of them generally leave patients still susceptible to HVG and GVHD and weaken their ability to defend against infection. This renders them much more susceptible to infection and much less able to fight off infections when they do occur.

Furthermore, all of these drugs cause serious side effects, including, for instance, gastrointestinal toxicity, nephrotoxicity, hypertension, myelosuppression, hepatotoxicity, hypertension, and gum hypertrophy, among others. None of them have proven to be a fully acceptable or effective treatment. In sum, given these drawbacks, there is at present no entirely satisfactory pharmaceutically based treatment for adverse immune system dysfunction and/or responses such as HVG and GVHD.

[0016] It has long been thought that a more specific type of immune suppression might be developed without these drawbacks. For example, an agent that suppressed or eliminated alloreactive T-cells, specifically, would be effective against HVG and GVHD (at least for allogeneic grafts) without the deleterious side effects that occur with agents that globally attack and compromise the immune system. However, as yet, no such agent(s) have been developed.

Use of Restricted Stem Cells In Transplantation

[0017] The use of stem cells in lieu of or together with immunosuppressive agents has recently attracted interest. There have been some encouraging observations in this area. A variety of stem cells have been isolated and characterized in recent years. They range from those of highly restricted differentiation potential and limited ability to grow in culture to those with apparently unrestricted differentiation potential and unlimited ability to grow in culture. The former have generally been the easier to derive and can be obtained from a variety of adult tissues. The latter have had to be derived from germ cells and embryos, and are called embryonal stem ("ES") cells, embryonal germ ("EG") cells, and germ cells. The embryonal stem ("ES") cell has unlimited self-renewal and can differentiate into all tissue types. ES cells are derived from the inner cell mass of the blastocyst. Embryonal germ ("EG") cells are derived from primordial germ cells of a post-implantation embryo. Stem cells derived from adult tissue have been of limited value because they are immunogenic, have limited differentiation potential, and have limited ability to propagate in culture. ES, EG, and germ cells do not suffer from these disadvantages, but they have a marked propensity to form teratomas in allogeneic hosts, raising due concern for their use in medical treatments. For this reason, there is pessimism about their utility in clinical applications, despite their advantageously broad differentiation potential. Stem cells derived from embryos also are subject to ethical controversies that may impede their use in treating disease.

[0018] Some efforts to find an alternative to ES, EG, and germ cells have focused on cells derived from adult tissue. While adult stem cells have been identified in most tissues of mammals, their differentiation potential is restricted and considerably more narrow than that of ES, EG, and germ cells. Indeed many such cells can give rise only to one or a few differentiated cell types, and many others are restricted to a single embryonic lineage.

[0019] For instance, hematopoietic stem cells can differentiate only to form cells of the hematopoietic lineage, neural stem cells differentiate into cells only of neuroectodermal origin,

and mesenchymal stem cells ("MSCs") are limited to cells of mesenchymal origin. For the reasons noted above regarding the limitations, risks, and controversies of and relating to ES, EG, and germ cells, a substantial portion of work on the use of stem cells in transplantation therapies has utilized MSCs. Results of the last few years appear to show that allografts of MSCs do not engender a HVG immune reaction, which is a response invariably seen when other tissue is transplanted between allogeneic individuals. Moreover, the results suggest that MSCs weaken lymphocyte immune response, at least in some circumstances.

[0020] While these results immediately suggest that MSCs might be useful to decrease HVG and/or GVHD that ordinarily would accompany allogeneic transplantation, the observed immunosuppressive effects of MSCs were highly dose dependent, and relatively high doses were required to observe an immunosuppressive effect. In fact, decreased proliferation of lymphocytes in mixed lymphocyte assays *in vitro* was "marked" only at or above a 1:10 ratio of MSCs to lymphocytes. Furthermore, the observed inhibitory effect decreased and became unobservable as the dose of MSCs decreased, and at ratios below 1:100 the presence of MSCs actually seemed to stimulate proliferation of the T-cells. The same dose effects also were observed in mitogen-stimulated lymphocyte proliferation assays. See, for review, Ryan et al. (2005) "Mesenchymal stem cells avoid allogeneic rejection," J. Inflammation 2: 8; Le Blanc (2003) "Immunomodulatory effects of fetal and adult mesenchymal stem cells," Cytotherapy 5(6): 485-489, and Jorgensen et al. (2003) "Engineering mesenchymal stem cells for immunotherapy," Gene Therapy 10: 928-931. Additional results are summarized below.

[0021] For example, Bartholomew and co-workers found that baboon MSCs did not stimulate allogeneic lymphocytes to proliferate *in vitro* and that MSCs reduced proliferation of mitogen-stimulated lymphocytes by more than 50% in mixed lymphocyte assays *in vitro.* They further showed that administration of MSCs *in vivo* prolonged skin graft survival (relative to controls). Both the *in vitro* results and the *in vivo* results required a high dose of MSCs: 1:1 ratio with the lymphocytes for the *in vitro* results. The amount of MSCs that would be required to approach such a ratio *in vivo* in humans may be too high to achieve, as a practical matter. This may limit the utility of MSCs. See Bartholomew et al. (2002): "Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo," Experimental Hematology 30: 42-48.

[0022] Maitra and co-workers examined the effects of human MSCs on engraftment of allogeneic human umbilical cord blood cells after co-infusion into sub-lethally irradiated NOD-SCID mice. They found that human MSCs promoted engraftment and did not activate allogeneic T-cells in *in vitro* proliferation assays. They also found that human MSCs suppressed *in vitro* activation of allogeneic human T-cells by mitogens. The effects were dose dependent and relatively high ratios were required for suppression. (Maitra et al. (2004) Bone Marrow Transplantation 33: 597-604.)

[0023] Recently, Le Blanc and co-workers reported successfully treating one patient with Grade IV acute GVHD, which usually is fatal, by administration of "third party haploidentical" MSCs. The patient was a 9-year old boy with acute lymphoblastic leukemia, which was in its

third remission. Initially, the patient was treated with radiation and cyclophophamide and then given blood cells that were identical to his own cells at the HLA-A, HLA-B, and HLA-DRbeta1 loci. These had been obtained from an unrelated female donor. Despite aggressive treatment, including dosing with a variety of immunosuppressants, by 70 days after transplant the patient developed Grade IV acute GVHD. He was frequently afflicted by invasive bacterial, viral, and fungal infections.

[0024] Under these clearly dire circumstances, an alternative blood stem cell transplant was attempted. Haploidentical MSCs were isolated from the patient's mother and expanded *in vitro* for three weeks. The cells were harvested and 2 x 10^6 cells per kilogram were administered to the patient intravenously. There were no signs of toxicity associated with the MSCs, nor were there substantial side effects. Many symptoms resolved within a few days after the transplant; but, residual disease was apparent. After several additional intravenous injections of MSCs using the same methods, the patient's symptoms and GVHD were fully resolved. The patient was still disease free one year after discharge. According to the authors, in their experience, this patient is unique in surviving GVHD of this severity. The results reported by Le Blanc et al. are both promising and inspiring, and should be a spur to developing effective therapies that utilize stem cells. Le Blanc et al. (2004) "Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells," Lancet 363: 1439-41.

[0025] Nevertheless, these results, including those of Le Blanc and co-workers, reveal potential shortcomings of MSCs. The cells need to be administered with traditional immunosuppressive modalities which then will continue to engender deleterious immune responses. The dosing requirements for MSCs apparently will need to be very high to be effective, which will incur greater cost, more difficulty in administration, greater risk of toxicity and other harmful side effects, and other disadvantages.

[0026] In view of these limitations of current stem cell based transplantation-related therapies, there is clearly a strong need for progenitor cells that can be used for all - or at least most - recipient hosts without necessitating a host-recipient haplotype match. Further, there is a need for cells of greater "specific activity" so that they are therapeutically effective at lower doses and their administration does not pose the problems associated with the high dosing regimens required for beneficial results using MSCs. And, there is a need for cells that have essentially unlimited differentiation potential to form cells that occur in the organism of interest.

[0027] Accordingly, there has been a need for cells that have the self-renewing and differentiation capacity of ES, EG, and germ cells but are not immunogenic; do not form teratomas when allografted or xenografted to a host; do not pose other safety issues associated with ES, EG, and germ cells; retain the other advantages of ES, EG, and germ cells; are easy to isolate from readily available sources, such as placenta, umbilical cord, umbilical cord blood, blood, and bone marrow; can be stored safely for extended periods; can be obtained easily and without risk to volunteers, donors or patients, and others giving consent; and do not entail the technical and logistical difficulties involved in obtaining and working with ES, EG, and germ cells.

[0028] Recently, a type of cell, called herein multipotent adult progenitor cells ("MAPCs"), has been isolated and characterized (see, for instance, US Patent No. 7,015,037). ("MAPCs" also may be referred to as "MASCs.") These cells provide many of the advantages of ES, EG, and germ cells without many of their drawbacks. For example, MAPCs are capable of indefinite culture without loss of their differentiation potential. They show efficient, long term engraftment and differentiation along multiple developmental lineages in NOD-SCID mice and do so without evidence of teratoma formation (often seen with ES, EG, and germ cells) (Reyes, M. and C.M. Verfaillie (2001) Ann N Y Acad Sci. 938: 231-5).

Summary of the Invention

[0029] Aspects of the invention for which protection is sought are defined in the claims.

[0030] According to a first aspect, the invention provides cells for use in adjunctive prevention or treatment of a benign neoplasm of bone marrow cells, a myeloproliferative disorder, a myelo-dysplastic syndrome or an acute leukemia, an anemia or congenital immune deficiency, or an autoimmune dysfunction, disorder or disease, or an adverse immune reaction occurring in transplantation therapy, in a subject, wherein the cells are multipotent progenitor cells, characterised in that they express telomerase and are positive for oct-3/4; are not embryonic stem cells, embryonic germ cells, or germ cells; can differentiate into at least one cell type of each of at least two of the endodermal, ectodermal, and mesodermal embryonic lineages; do not provoke a deleterious immune response in the subject; are effective to treat the benign neoplasm of bone marrow cells, a myeloproliferative disorder, a myelo-dysplastic syndrome or an acute leukemia, an anemia or congenital immune deficiency, or an autoimmune dysfunction, disorder or disease, or adverse immune reaction occurring in transplantation therapy, in the subject; and are administered, by an effective route and in an effective amount, adjunctively to one or more other treatments.

[0031] According to a second aspect, the invention provides the use of cells in the manufacture of a medicament for adjunctive prevention or treatment of a benign neoplasm of bone marrow cells, a myeloproliferative disorder, a myelo-dysplastic syndrome or an acute leukemia, or an anemia or congenital immune deficiency, or an autoimmune dysfunction, disorder or disease, or an adverse immune reaction occurring in transplantation therapy, in a subject, wherein the cells are multipotent progenitor cells characterised in that they express telomerase and are positive for oct-3/4: are not embryonic stem cells, embryonic germ cells, or germ cells; can differentiate into at least one cell type of each of at least two of the endodermal, ectodermal, and mesodermal embryonic lineages; do not provoke a deleterious immune response in the subject; are effective to treat the benign neoplasm of bone marrow cells, myeloproliferative disorder, myelo-dysplastic syndrome or acute leukemia, or anemia or congenital immune deficiency, or autoimmune dysfunction, disorder or disease, or adverse immune reaction occurring in transplantation therapy, in the subject, and are administered, by an effective route and in an effective amount, adjunctively to one or more other treatments.

[0032] In either the first or the second aspect of the invention, the cells can optionally differentiate into at least one cell type of each of the endodermal, ectodermal and mesodermal embryonic lineages.

[0033] In either the first or the second aspect of the invention, the cells may have undergone at least 10 to 40 cell doublings in culture prior to their administration to the subj ect.

[0034] In either the first or the second aspect of the invention, the cells may be xenogeneic or allogeneic to the subject.

[0035] In either the first or the second aspect of the invention, the cells may be administered to the subject adjunctively to another treatment that is administered before, at the same time as, or after said cells are administered.

[0036] In either the first or the second aspect of the invention, the cells may be administered to the subject in one or more doses comprising 10^4 to 10^8 of said cells per kilogram of the subject's mass.

[0037] In either the first or the second aspect of the invention, optionally the subject will receive or has received a transplant and will be at risk for or has developed a host versus graft response or graft versus host disease, and said cells are administered adjunctively to the transplant.

[0038] In either the first or the second aspect of the invention, optionally the transplant is an organ, bone marrow or blood transplant, the subject is at risk for or has developed a host versus graft response, and said cells are administered adjunctively to the transplant to treat the host versus graft response.

[0039] In either the first or the second aspect of the invention, optionally the subject is or will be treated by radiation or chemotherapy or a combination of radiation and chemotherapy, weakening the subject's immune system, and treatment with said cells is adjunctive to the radiation, the chemotherapy, or the combination of the two.

[0040] In either the first or the second aspect of the invention, the cells may be administered to a subject having a compromised immune system.

[0041] In either the first or the second aspect of the invention, optionally the subject will be or is being treated with an immunosuppressive agent, and said treatment with said cells is adjunctive thereto.

[0042] In either the first or the second aspect of the invention, optionally the subject is at risk for or is suffering from one or more of a neoplasm, an anemia or other blood disorder, and an immune dysfunction, wherein treatment with said cells is adjunctive to a treatment thereof.

[0043] In either the first or the second aspect of the invention, optionally the subject is at risk for or is suffering from one or more of a myeloproliferative disorder, a myelodysplastic syndrome, leukemia, multiple myeloma, a lymphoma, a hemoglobinopathy, a thalassemia, a bone marrow failure syndrome, sickle cell anemia, aplastic anemia, Fanconi's anemia, an immune hemolytic anemia, a congenital immune deficiency, or an autoimmune dysfunction, wherein treatment with said cells is adjunctive to a treatment thereof.

[0044] In either the first or the second aspect of the invention, the cells may be allogeneic to the subject.

[0045] In either the first or the second aspect of the invention, the cells may be derived from bone marrow.

[0046] In either the first or the second aspect of the invention, the cells may be derived from human bone marrow.

[0047] Disclosed herein are cells that: (i) are not embryonic stem cells, not embryonic germ cells, and not germ cells; (ii) can differentiate into at least one cell type of each of at least two of the endodermal, ectodermal, and mesodermal embryonic lineages; (iii) do not provoke a deleterious immune response upon introduction to a non-syngeneic subject; and (iv) can modulate an immune response upon introduction into a subject. In addition to the foregoing, the cells may be immunosuppressive. Furthermore, cells in accordance with the foregoing may have immunomodulatory properties that are useful for treating, such as to preclude, prevent, ameliorate, lessen, decrease, minimize, eliminate, and/or cure deleterious immune responses and/or processes in a host. The cells may be used in this regard alone or together with other therapeutic agents and modalities as primary therapeutic modalities. The cells may be used in an adjunctive therapeutic modality in which they may be used either as the sole therapeutic agent or together with other therapeutic agents. The cells may be used, alone or with other therapeutic agents or modalities, both in one or more primary therapeutic modalities and in one or move adjunctive therapeutic modalities.

[0048] Cells described in greater detail herein generally are referred to herein as "multipotent adult progenitor cells" and by the acronyms "MAPC" (singular) and "MAPCs" (plural). It is to be appreciated that these cells are not ES, not EG, and not germ cells, and that they have the capacity to differentiate into cell types of at least two of the three primitive germ layer lineages (ectoderm, mesoderm, and endoderm), e.g., into cells of all three primitive lineages.

[0049] For instance, MAPCs can form the following cells and other cells of the lineages thereof: splanchnic mesodermal cells, muscle cells, bone cells, cartilage cells, endocrine cells, exocrine cells, endothelial cells, hair forming cells, teeth forming cells, visceral mesodermal cells, hematopoietic cells, stromal cells, marrow stromal cells, neuronal cells, neuroectodermal cells, epithelial cells, ocular cells, pancreatic cells, and hepatocyte-like cells, among many others. Among cells formed by MAPCs are osteoblasts, chondroblasts, adipocytes, skeletal

muscle cells, skeletal myocytes, biliary epithelial cells, pancreatic acinary cells, mesangial cells, smooth muscle cells, cardiac muscle cells, cardiomyocytes, osteocytes, vascular tube forming cells, oligodendrocytes, neurons, including serotonergic, GABAergic, dopaminergic neurons, glial cells, microglial cells, pancreatic epithelial cells, gut epithelial cells, liver epithelial cells, skin epithelial cells, kidney epithelial cells, renal epithelial cells, pancreatic islet cells, fibroblasts, hepatocytes, and other cells of the same lineages as the foregoing, among many others.

[0050] MAPCs have telomerase activity necessary for self-renewal and thought to be necessary for maintaining an undifferentiated state. Generally they also express oct-3/4. Oct-3/4 (oct-3A in humans) is otherwise specific to ES, EG, and germ cells. It is considered to be a marker of undifferentiated cells that have broad differentiation abilities. Oct-3/4 also is generally thought to have a role in maintaining a cell in an undifferentiated state. Oct-4 (oct-3 in humans) is a transcription factor expressed in the pregastrulation embryo, early cleavage stage embryo, cells of the inner cell mass of the blastocyst, and embryonic carcinoma ("EC") cells (Nichols, J. et al. (1998) Cell 95: 379-91), and is down-regulated when cells are induced to differentiate. The oct-4 gene (oct-3 in humans) is transcribed into at least two splice variants in humans, oct-3A and oct-3B. The oct-3B splice variant is found in many differentiated cells whereas the oct-3A splice variant (also previously designated oct-3/4) is reported to be specific for the undifferentiated embryonic stem cell. See Shimozaki et al. (2003) Development 130: 2505-12. Expression of oct-3/4 plays an important role in determining early steps in embryogenesis and differentiation. Oct-3/4, in combination with rox-1, causes transcriptional activation of the Zn-finger protein rex-1, which is also required for maintaining ES cells in an undifferentiated state (Rosfjord, E. and Rizzino, A. (1997) Biochem Biophys Res Commun 203: 1795-802; Ben-Shushan, E. et al. (1998) Mol Cell Biol 18: 1866-78).

[0051] MAPCs generally also express other markers thought to be specific to primitive stem cells. Among these are rex-1, rox-1, and sox-2. Rex-1 is controlled by oct-3/4, which activated downstream expression of rex-1. Rox-1 and sox-2 are expressed in non-ES cells.

[0052] Also disclosed herein are methods for using MAPCs for precluding, preventing, treating, ameliorating, lessening, decreasing, minimizing, eliminating, and/or curing a disease and/or an adverse immune response and/or processes in a subject. Also disclosed herein are methods for using the cells by themselves as a primary therapeutic modality. The cells may be used together with one or more other agents and/or therapeutic modalities as the primary therapeutic modality. The cells may be used as an adjunctive therapeutic modality, that is, as an adjunct to another, primary therapeutic modality. In some instances the cells are used as the sole active agent of an adjunctive therapeutic modality. In others the cells are used as an adjunctive therapeutic modality together with one or more other agents or therapeutic modalities. The cells may be used both as primary and as adjunctive therapeutic agents and/or modalities. In both regards, the cells can be used alone in the primary and/or in the adjunctive modality. They also can be used together with other therapeutic agents or modalities, in the primary or in the adjunctive modality or both.

[0053] As discussed above, a primary treatment, such as a therapeutic agent, therapy, and/or therapeutic modality, targets (that is, is intended to act on) the primary dysfunction, such as a disease, that is to be treated. An adjunctive treatment, such as a therapy and/or a therapeutic modality, can be administered in combination with a primary treatment, such as a therapeutic agent, therapy, and/or therapeutic modality, to act on the primary dysfunction, such as a disease, and supplement the effect of the primary treatment, thereby increasing the overall efficacy of the treatment regimen. An adjunctive treatment, such as an agent, therapy, and/or therapeutic modality, also can be administered to act on complications and/or side effects of a primary dysfunction, such as a disease, and/or those caused by a treatment, such as a therapeutic agent, therapy, and/or therapeutic modality. In regard to any of these uses, one, two, three, or more primary treatments may be used together with one, two, three, or more adjunctive treatments.

[0054] MAPCs may be administered to a subject prior to onset of a dysfunction, such as a disease, side effect, and/or deleterious immune response. The cells may be administered while the dysfunction is developing. The cells may be administered after the dysfunction has been established. Cells can be administered at any stage in the development, persistence, and/or propagation of the dysfunction or after it recedes.

[0055] As discussed above, disclosed herein are cells and methods for primary or adjunctive therapy. The cells may be administered to an allogeneic subject. They may be autologous to the subject. They may be syngeneic to the subject. The cells may be xenogeneic to a subject. Whether allogeneic, autologous, syngeneic, or xenogeneic, the MAPCs may be weakly immunogenic or are non-immunogenic in the subject. MAPCs may have sufficiently low immunogenicity or may be non-immunogenic and are sufficiently free of deleterious immune responses in general, that when administered to allogeneic subjects they can be used as "universal" donor cells without tissue typing and matching. MAPCs can also be stored and maintained in cell banks, and thus can be kept available for use when needed.

[0056] In all of these regards and others, MAPCs may be from mammals, including humans, and alternatively non-human primates, rats and mice, and dogs, pigs, goats, sheep, horses, and cows. MAPCs prepared from mammals as described above can be used in all of the methods and other aspects described herein.

[0057] MAPCs can be isolated from a variety of compartments and tissues of such mammals, including but not limited to, bone marrow, blood, spleen, liver, muscle, brain, and others discussed below. MAPCs may be cultured before use.

[0058] MAPCs may be genetically engineered, such as to improve their immunomodulatory properties. Genetically engineered MAPCs may be produced by *in vitro* culture. Genetically engineered MAPCs may be produced from a transgenic organism.

[0059] MAPCs may be administered to a subject by any route for effective delivery of cell therapeutics. The cells may be administered by injection, including local and/or systemic

injection. The cells may be administered within and/or in proximity to the site of the dysfunction they are intended to treat. The cells may be administered by injection at a location not in proximity to the site of the dysfunction. The cells may be administered by systemic injection, such as intravenous injection.

[0060] MAPCs may be administered one time, two times, three times, or more than three times until a desired therapeutic effect is achieved or administration no longer appears to be likely to provide a benefit to the subject. MAPCs may be administered continuously for a period of time, such as by intravenous drip. Administration of MAPCs may be for a short period of time, for days, for weeks, for months, for years, or for longer periods of time.

Brief Descriptions of the Figures

[0061]

Figure 1 is a schematic representation of the transcriptional profiling studies that were performed to generate (identify) gene and surface receptor-based markers that distinguish between MAPCs of the invention and other stem and progenitor cells that are more lineage committed. The experiments have resulted in a panel of 75 markers having 10-fold different expression between MSC cultures and the MAPCs.

Figure 2 is a set of graphs showing the tri-lineage differentiation of GFP-labeled rat MAPCs. The results show that MAPCs can differentiate into cells of all three embryonic lineages. As further described below, for endothelial differentiation, MAPCs were cultured on fibronectin-coated plates in the presence of vascular endothelial growth factor B (VEGF-B). For hepatocyte differentiation, cells were grown on matrigel-coated plates and treated with fibroblast growth factor-4 (FGF-4) and hepatocyte growth factor (HGF). Neuronal differentiation was induced by sequential treatment with basic-FGF (bFGF), with both FGF-8 and Sonic Hedgehog (SHH), and with brain-derived neurotrophic factor (BDNF). After two weeks, mRNA was extracted from cells and applied to qPCR analysis using primers specific for detection of various lineage markers. In all assays, cells cultured in the absence of lineage-inducing cytokines served as controls. The expression levels of lineage markers were first normalized to the expression level of an internal control gene (GAPDH) which is unaffected during differentiation. Differentiation success was then assessed by calculation of the relative expression in the differentiated or the control cells compared to the levels in the parental rat line, using an increase of more than 5-fold in the relative expression as a cut-off for successful differentiation. Differentiated rat MAPCs displayed significant expression of the endothelial markers, von Willebrand factor, and PECAM-1 (top panel); the hepatic markers albumin, cytokeratin-18, and HNF-1a (middle panel); and the neuronal/astrocyte markers GFAP, nestin, and NF-200 (bottom panel).

Figure 3 is a pair of bar charts showing the low immunogenicity (top panel) and immunosuppressivity (bottom panel) of MAPCs in mixed lymphocyte reactions (MLR), as described further below. In the top panel: B + B = donor B + donor B; B + A = donor B + donor

A; B + K = donor B + donor K; B + R = donor B + donor R; B + T = donor B + donor T; donor B + PHA; B + BMPC = donor B + MAPC. The same result was achieved with twelve different donors. In the bottom panel: donor W + donor W; donor W + donor A; donor W + donor T; donor W + MSC; donor W + MAPC (17); donor W + PHA; donor W + donor A + MSC; donor W + donor A + MAPC (17); donor W + donor T + MSC; donor W + donor T + MAPC (17); donor W + donor P + MSC; donor W + donor P + MAPC (17). PHA is phytohemagglutinin (positive control for T-cell activation).

Figure 4 is a chart showing that MAPCs can suppress the proliferation of ConA stimulated T-cells as described in Example 6. The caption "LN Only" designates the results for control reactions omitting MAPCs. The numbers for MAPCs indicate how many cells were used in the assays.

Figure 5A is a chart showing the immunosuppressive effects of Lewis MAPCs in mixed lymphocyte reactions, as described in Example 7. The box in Figure 5A enumerates the number of MAPCs in each reaction. In the box, R designates responder cells and S designates stimulator cells (irradiated splenocytes from DA rats).

Figure 5B is a chart showing the immunosuppressive effects of Sprague-Dawley MAPCs in mixed lymphocyte reactions, as described in Example 7. Captions and abbreviations are the same as in Figure 5A.

Figure 6 is a graph showing that infusion of MAPCs does not adversely affect the health of recipients as determined by their respiratory rate. The graph is further described in Example 8.

Figure 7 is a bar chart that depicts the results of an experiment demonstrating the ability of MAPCs to suppress an on-going immune response. The chart shows that MAPCs are strongly immunosuppressive in MLRs, both when they are added at the same time as the T-cell activator (stimulator) (Day 0, left side of chart), and when they are added 3 days after addition of the T-cell activator (stimulator) (Day 3, right side of chart). Details of the experiments are further described in Example 10.

Figure 8 is a bar graph showing that MAPC inhibition of T cell proliferation in MLRs is reversible. Results are exposed as mean cpm +/-SD of triplicate culture. The graph is further described in Example 11.

Figure 9 is a chart showing that MAPCs inhibit GVHD as described in Example 13.

Definitions

[0062] As used herein, certain terms have the meanings set out below.

"A" or "an" means one or more; at least one.

"Adjunctive" means jointly, together with, in addition to, in conjunction with, and the like.

"Co-administer" can include simultaneous or sequential administration of two or more agents.

"Cytokines" refer to cellular factors that induce or enhance cellular movement, such as homing of MAPCs or other stem cells, progenitor cells, or differentiated cells. Cytokines may also stimulate such cells to divide.

"Deleterious" means, as used herein, harmful. By way of illustration, "deleterious immune response" means, as used herein, a harmful immune response, such as those that are lacking or are too weak, those that are too strong, and/or those that are misdirected. Among deleterious immune responses are the harmful immune responses that occur in immune diseases. Examples include the lack of immune responses in immunodeficiency diseases, and the exaggerated and/or misdirected immune responses in autoimmune diseases. Also among deleterious immune responses are immune responses that interfere with medical treatment, including otherwise normal immune responses. Examples include immune responses involved in rejecting transplants and grafts, and the response of immunocompetent cells in transplants and grafts that cause graft versus host disease.

"Differentiation factors" refer to cellular factors, such as growth factors, that induce lineage commitment.

"Dysfunction" means, as used herein, a disorder, disease, or deleterious effect of an otherwise normal process. By way of illustration, an immune dysfunction includes immune diseases, such as autoimmune diseases and immune deficiencies. It also includes immune responses that interfere with medical treatment, including otherwise normal immune responses that interfere with medical treatment. Examples of such dysfunctions include immune responses involved in rejecting transplants and grafts, and the response of immunocompetent cells in transplants and grafts that cause graft versus host disease.

"EC cells" refers to embryonic carcinoma cells.

"Effective amount" generally means an amount which provides the desired local or systemic effect. For example, an effective amount is an amount sufficient to effectuate a beneficial or desired clinical result. The effective amounts can be provided all at once in a single administration or in fractional amounts that provide the effective amount in several administrations. For instance, an effective amount of MAPCs could be administered in one or more administrations and could include any preselected amount of cells. The precise determination of what would be considered an effective amount may be based on factors individual to each subject, including their size, age, injury, and/or disease or injury being treated, and amount of time since the injury occurred or the disease began. One skilled in the art will be able to determine the effective amount for a given subject based on these considerations which are routine in the art. Thus, for instance, the skilled artisan in this art, such as a physician, based on the known properties of MAPCs as disclosed herein and in the art, together with a consideration of the foregoing factors, will be able to determine the effective amount of MAPCs for a given subject. As used herein, "effective dose" means the

same as "effective amount."

"EG cells" refers to embryonal germ cells.

"Engraft" refers to the process of cellular contact and incorporation into an existing tissue of interest *in vivo.*

"Enriched population" means a relative increase in numbers of MAPCs relative to other cells or constituents in an initial population, such as an increase in numbers of MAPCs relative to one or more non-MAPC cell types in culture, such as primary culture, or *in vivo.*

"ES cells" refers to embryonal stem cells.

"Expansion" refers to the propagation of a cell or cells without differentiation.

"Fanconi's anemia" as used herein means the same as Fanconi anemia, an inherited disease.

"GVHD" refers to graft versus host disease, which means processes that occur primarily in an immunocompromised host when it is recognized as non-self by immunocompetent cells of a graft.

"HVG" refers to host versus graft response, which means processes which occur when a host rejects a graft. Typically, HVG is triggered when a graft is recognized as foreign (non-self) by immunocompetent cells of the host.

"Isolated" refers to a cell or cells which are not associated with one or more cells or one or more cellular components that are associated with the cell or cells *in vivo.*

"MAPC" is an acronym for "multipotent adult progenitor cell." It refers to a non-ES, non-EG, non-germ cell that can give rise to cell lineages of more than one germ layer, such as all three germ layers (i.e., endoderm, mesoderm, and ectoderm). MAPCs also have telomerase activity. They may be positive for oct-3/4 (e.g., human oct-3A). They also may express rex-1 and rox-1. Further, they may express sox-2, SSEA-4, and/or nanog. The term "adult" in MAPC is not restrictive. It only denotes that these cells are not ES, EG, or germ cells. Typically, as used herein, MAPC is singular and MAPCs is plural. MAPCs also have been referred to as multipotent adult stem cells (MASCs). See U.S. Patent No. 7,015,037 for disclosure of MAPC/MASC and methods of isolation and growth thereof.

"MASC," see MAPC.

"MNC" refers to mononuclear cells.

"Modality" means a type, approach, avenue, or method, such as, a therapeutic modality; i.e., a type of therapy.

"MSC" is an acronym for mesenchymal stem cells.

"Multipotent" with respect to MAPCs, refers to the ability to give rise to cell lineages of more than one germ layer, such as all three primitive germ layers (i.e., endoderm, mesoderm, and

ectoderm) upon differentiation.

"Persistence" refers to the ability of cells to resist rejection and remain and/or increase in number over time (e.g., days, weeks, months, or years) *in vivo.*

"Progenitor" as used in multipotent adult progenitor cells (MAPCs) indicates that these cells can give rise to other cells such as further differentiated cells. The term is not limitative and does not limit these cells to a particular lineage.

"Self-renewal" refers to the ability to produce replicate daughter stem cells having differentiation potential that is identical to those from which they arose. A similar term used in this context is "proliferation."

A "subject" is a vertebrate, such as a mammal, such as a human. Mammals include, but are not limited to, humans, farm animals, sport animals, and pets. Subjects in need of treatment by methods of the present invention include those suffering from a disorder, dysfunction, or disease (such as an immune deficiency or dysfunction, such as HVG and GVHD), or a side effect of the same, or a treatment thereof, that can benefit from administration of MAPCs either as a primary or an adjunctive treatment.

"Transplant" as used herein means to introduce into a subject, cells, tissues, or organs. The transplant can be derived from the subject, from culture, or from a non-subject source.

"Treat," "treating," or "treatment" includes treating, preventing, ameliorating, inhibiting, or curing a deficiency, dysfunction, disease, or other process resulting in a deleterious effect, such as an immune system deficiency, dysfunction, disease, or other process that deleteriously affects immune system functions or properties or that interferes with a therapy.

Detailed Description of the Invention

Introduction

[0063] MAPCs are very promising for treating disease by cell transplantation techniques, such as for tissue and organ regeneration, both when used alone and when used in combination with other treatments. Among the potential obstacles to realizing the promise of MAPCs for treating diseases, and for tissue and organ regeneration, are the adverse immune reactions that typically complicate or prevent success in transplantation therapies, such as blood and bone marrow transplantation therapies and solid organ transplantation. Prominent among these immune complications are graft rejection by a host's immune system (referred to herein as host versus graft response and as "HVG") and systemic damage to an immunocompromised host that results when immunocompetent cells in a graft are activated by contact with non-self components of the host (referred to herein as graft versus host disease

and as "GVHD").

[0064] It has been found (as described in greater detail elsewhere herein) that MAPCs do not provoke an immune response in allogeneic hosts. Thus, transplantation of MAPCs to an allogeneic host should not engender allogeneic graft rejection (i.e., HVG).

[0065] Furthermore, it has also been found that allogeneic MAPCs can be administered to a host at high concentration without deleterious effects on respiration, suggesting that undue clumping and/or deposition in the lungs does not occur.

[0066] In addition, it has been found (as described in greater detail elsewhere herein) that MAPCs can modulate immune responses. In particular in this regard, it has been found that MAPCs can suppress immune responses, including but not limited to immune responses involved in, for example, HVG and GVHD, to name just two. In an even more detailed particular in this regard, it has been found that MAPCs can suppress proliferation of T-cells, even in the presence of potent T-cell stimulators, such as Concanavalin A and allogeneic stimulator cells.

[0067] Moreover, it has been found that even relatively small amounts of MAPCs can suppress these responses. Indeed, only 3% MAPCs in mixed lymphocyte reactions is sufficient to reduce T-cell response to potent stimulators by 50% *in vitro.*

[0068] Accordingly, this disclosure provides compositions and methods and the like for treating, ameliorating, and/or curing or eliminating, adverse immune reactions, such as those that occur in transplantation therapies.

[0069] The low immunogenicity of allogeneic MAPCs, their ability to suppress adverse immune responses, and their high specific activity makes them particularly valuable for adjunctive therapies in the treatment of diseases with an adverse immune component. Among such diseases are autoimmune diseases in which, typically, dysfunction of the subject's own immune system causes disease. MAPCs also are useful as immunosuppressive adjunctive therapeutics for treating adverse immune responses that occur in transplantation therapy. Examples include HVG in immunocompetent hosts and GVHD in immunocompromised hosts. MAPCs further can be useful in adjunctive immunosuppressive therapy in the treatment of a variety of neoplasms, anemias and blood disorders, and in the treatment of certain inflammatory diseases. Diseases in this regard are discussed in greater detail below.

[0070] Using the methods described herein for MAPC isolation, characterization, and expansion, together with the disclosure herein on immune-suppressing properties of MAPCs, MAPCs can be used to prevent, suppress, or diminish immune disorders, dysfunctions, or diseases, including, for example, adverse immune reactions, such as those that result from other therapies, including those that complicate transplantation therapies, such as HVG and GVHD. Such disorders, dysfunctions, and diseases also include congenital immune disorders and autoimmune diseases, among others.

[0071] MAPCs are useful in these regards and others both as primary and adjunctive therapeutic agents and modalities. MAPCs can be used therapeutically alone or together with other agents. MAPCs can be administered before, during, and/or after such agents. Likewise, whether used alone or with other agents, MAPCs can be administered before, during, and/or after a transplant. If administered during transplant, MAPCs can be administered together with the transplant material or separately. If separately administered, the MAPCs can be administered sequentially or simultaneously with the transplant. Furthermore, MAPCs may be administered in advance of the transplant and/or after the transplant.

[0072] Other agents that can be used in conjunction with MAPCs, in transplantation therapies in particular, include immunomodulatory agents. A variety of such agents are described elsewhere herein. The immunomodulatory agents may be immunosuppressive agents, such as those described elsewhere herein. Among such agents are corticosteroids, cyclosporin A, cyclosporin-like immunosuppressive compounds, azathioprine, cyclophosphamide, and methotrexate.

[0073] MAPCs can be administered to hosts by a variety of methods as discussed elsewhere herein. MAPCs may be administered by injection, such as by intravenous injection. MAPCs may be encapsulated for administration. MAPCs may be administered *in situ.* Examples include *in situ* administration of MAPCs in solid organ transplantation and in organ repair. These and other forms of administration are discussed below.

[0074] MAPCs may be administered in doses measured by the ratio of MAPCs (cells) to body mass (weight). Alternatively, MAPCs can be administered in doses of a fixed number of cells. Dosing, routes of administration, formulations, and the like are discussed in greater detail elsewhere herein.

Mechanisms of Action

[0075] Without being limited to any one or more explanatory mechanisms for the immunomodulatory and other properties, activities, and effects of MAPCs, it is worth noting that they can modulate immune responses through a variety of modalities. For instance, MAPCs can have direct effects on a graft or host. Such direct effects are primarily a matter of direct contact between MAPCs and cells of the host or graft. The contact may be with structural members of the cells or with constituents in their immediate environment. Such direct mechanisms may involve direct contact, diffusion, uptake, or other processes well known to those skilled in the art. The direct activities and effects of the MAPCs may be limited spatially, such as to an area of local deposition or to a bodily compartment accessed by injection.

[0076] MAPCs also can "home" in response to "homing" signals, such as those released at sites of injury or disease. Since homing often is mediated by signals whose natural function is to recruit cells to the sites where repairs are needed, the homing behavior can be a powerful tool for concentrating MAPCs to therapeutic targets. This effect can be stimulated by specific

factors, as discussed below.

[0077] MAPCs may also modulate immune processes by their response to factors. This may occur additionally or alternatively to direct modulation. Such factors may include homing factors, mitogens, and other stimulatory factors. They may also include differentiation factors, and factors that trigger particular cellular processes. Among the latter are factors that cause the secretion by cells of other specific factors, such as those that are involved in recruiting cells, such as stem cells (including MAPCs), to a site of injury or disease.

[0078] MAPCs may, in addition to the foregoing or alternatively thereto, secrete factors that act on endogenous cells, such as stem cells or progenitor cells. The factors may act on other cells to engender, enhance, decrease, or suppress their activities. MAPCs may secrete factors that act on stem, progenitor, or differentiated cells causing those cells to divide and/or differentiate. MAPCs that home to a site where repair is needed may secrete trophic factors that attract other cells to the site. In this way, MAPCs may attract stem, progenitor, or differentiated cells to a site where they are needed. MAPCs also may secrete factors that cause such cells to divide or differentiate.

[0079] Secretion of such factors, including trophic factors, can contribute to the efficacy of MAPCs in, for instance, limiting inflammatory damage, limiting vascular permeability, improving cell survival, and engendering and/or augmenting homing of repair cells to sites of damage. Such factors also may affect T-cell proliferation directly. Such factors also may affect dendritic cells, by decreasing their phagocytic and antigen presenting activities, which also may affect T-cell activity

[0080] By these and other mechanisms, MAPCs can provide beneficial immunomodulatory effects, including, but not limited to, suppression of undesirable and/or deleterious immune reactions, responses, functions, diseases, and the like. MAPCs may provide beneficial immunomodulatory properties and effects that are useful by themselves or in adjunctive therapy for precluding, preventing, lessening, decreasing, ameliorating, mitigating, treating, eliminating and/or curing deleterious immune processes and/or conditions. Such processes and conditions include, for instance, autoimmune diseases, anemias, neoplasms, HVG, GVHD, and certain inflammatory disorders, as described in greeter detail elsewhere herein. MAPCs are useful in these other regards particularly in mammals. MAPCs may be used therapeutically in human patients, often adjunctively to other therapies.

MAPC Administration

MAPC Preparations

[0081] MAPCs can be prepared from a variety of tissues, such as bone marrow cells, as

discussed in greater detail elsewhere herein.

[0082] MAPC preparations may be clonally derived. In principle, the MAPCs in these preparations are genetically identical to one another and, if properly prepared and maintained, are free of other cells.

[0083] MAPC preparations that are less pure than these may be used. While rare, less pure populations may arise when the initial cloning step requires more than one cell. If these are not all MAPCs, expansion will produce a mixed population in which MAPCs are only one of at least two types of cells. More often mixed populations arise when MAPCs are administered in admixture with one or more other types of cells.

[0084] The purity of MAPCs for administration to a subject may be about 100%. It may be 95% to 100%. It may be 85% to 95%. Particularly in the case of admixtures with other cells, the percentage of MAPCs can be 25%-30%, 30%-35%, 35%-40%, 40%-45%, 45%-50%, 60%-70%, 70%-80%, 80%-90%, or 90%-95%.

[0085] The number of MAPCs in a given volume can be determined by well known and routine procedures and instrumentation. The percentage of MAPCs in a given volume of a mixture of cells can be determined by much the same procedures. Cells can be readily counted manually or by using an automatic cell counter. Specific cells can be determined in a given volume using specific staining and visual examination and by automated methods using specific binding reagent, typically antibodies, fluorescent tags, and a fluorescence activated cell sorter.

[0086] MAPC immunomodulation may involve undifferentiated MAPCs. It may involve MAPCs that are committed to a differentiation pathway. Such immunomodulation also may involve MAPCs that have differentiated into a less potent stem cell with limited differentiation potential. It also may involve MAPCs that have differentiated into a terminally differentiated cell type. The best type or mixture of MAPCs will be determined by the particular circumstances of their use, and it will be a matter of routine design for those skilled in the art to determine an effective type or combination of MAPCs.

Formulations

[0087] The choice of formulation for administering MAPCs for a given application will depend on a variety of factors. Prominent among these will be the species of subject, the nature of the disorder, dysfunction, or disease being treated and its state and distribution in the subject, the nature of other therapies and agents that are being administered, the optimum route for administration of the MAPCs, survivability of MAPCs via the route, the dosing regimen, and other factors that will be apparent to those skilled in the art. In particular, for instance, the choice of suitable carriers and other additives will depend on the exact route of administration and the nature of the particular dosage form, for example, liquid dosage form (e.g., whether the composition is to be formulated into a solution, a suspension, gel or another liquid form,

such as a time release form or liquid-filled form).

[0088] For example, cell survival can be an important determinant of the efficacy of cell-based therapies. This is true for both primary and adjunctive therapies. Another concern arises when target sites are inhospitable to cell seeding and cell growth. This may impede access to the site and/or engraftment there of therapeutic MAPCs. Also disclosed are measures to increase cell survival and/or to overcome problems posed by barriers to seeding and/or growth.

[0089] Examples of compositions comprising MAPCs include liquid preparations, including suspensions and preparations for intramuscular or intravenous administration (e.g., injectable administration), such as sterile suspensions or emulsions. Such compositions may comprise an admixture of MAPCs with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, dextrose, or the like. The compositions can also be lyophilized. The compositions can contain auxiliary substances such as wetting or emulsifying agents, pH buffering agents, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard texts, such as "REMINGTON'S PHARMACEUTICAL SCIENCE," 17th edition, 1985, may be consulted to prepare suitable preparations, without undue experimentation.

[0090] Compositions of the invention often are conveniently provided as liquid preparations, e.g., isotonic aqueous solutions, suspensions, emulsions, or viscous compositions, which may be buffered to a selected pH. Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection. Viscous compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with specific tissues.

[0091] Various additives often will be included to enhance the stability, sterility, and isotonicity of the compositions, such as antimicrobial preservatives, antioxidants, chelating agents, and buffers, among others. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. In many cases, it will be desirable to include isotonic agents, for example, sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents that delay absorption, for example, aluminum monostearate, and gelatin. According to the present invention, however, any vehicle, diluent, or additive used would have to be compatible with the cells.

[0092] MAPC solutions, suspensions, and gels normally contain a major amount of water (preferably purified, sterilized water) in addition to the cells. Minor amounts of other ingredients such as pH adjusters (e.g., a base such as NaOH), emulsifiers or dispersing agents, buffering agents, preservatives, wetting agents and jelling agents (e.g., methylcellulose) may also be present.

[0093] Typically, the compositions will be isotonic, i.e., they will have the same osmotic

pressure as blood and lacrimal fluid when properly prepared for administration.

[0094] The desired isotonicity of the compositions of this invention may be accomplished using sodium chloride, or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate, propylene glycol, or other inorganic or organic solutes. Sodium chloride is preferred particularly for buffers containing sodium ions.

[0095] Viscosity of the compositions, if desired, can be maintained at the selected level using a pharmaceutically acceptable thickening agent. Methylcellulose is preferred because it is readily and economically available and is easy to work with. Other suitable thickening agents include, for example, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, carbomer, and the like. The preferred concentration of the thickener will depend upon the agent selected. The important point is to use an amount, which will achieve the selected viscosity. Viscous compositions are normally prepared from solutions by the addition of such thickening agents.

[0096] A pharmaceutically acceptable preservative or cell stabilizer can be employed to increase the life of MAPC compositions. If such preservatives are included, it is well within the purview of the skilled artisan to select compositions that will not affect the viability or efficacy of the MAPCs.

[0097] Those skilled in the art will recognize that the components of the compositions should be chemically inert. This will present no problem to those skilled in chemical and pharmaceutical principles. Problems can be readily avoided by reference to standard texts or by simple experiments (not involving undue experimentation) using information provided by the disclosure, the documents cited herein, and generally available in the art.

[0098] Sterile injectable solutions can be prepared by incorporating the cells utilized in practicing the present invention in the required amount of the appropriate solvent with various amounts of the other ingredients, as desired.

[0099] MAPCs may be formulated in a unit dosage injectable form, such as a solution, suspension, or emulsion. Pharmaceutical formulations suitable for injection of MAPCs typically are sterile aqueous solutions and dispersions. Carriers for injectable formulations can be a solvent or dispersing medium containing, for example, water, saline, phosphate buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like), and suitable mixtures thereof.

[0100] The skilled artisan can readily determine the amount of cells and optional additives, vehicles, and/or carrier in compositions to be administered in methods of the invention. Typically, any additives (in addition to the cells) are present in an amount of 0.001 to 50 wt % in solution, such as in phosphate buffered saline. The active ingredient is present in the order of micrograms to milligrams, such as about 0.0001 to about 5 wt %, preferably about 0.0001 to about 1 wt %, most preferably about 0.0001 to about 0.05 wt % or about 0.001 to about 20 wt %, preferably about 0.01 to about 10 wt %, and most preferably about 0.05 to about 5 wt %.

[0101] For any composition to be administered to an animal or human, and for any particular method of administration, it is preferred to determine therefore: toxicity, such as by determining the lethal dose (LD) and LD50 in a suitable animal model, e.g., rodent such as mouse or rat; and, the dosage of the composition(s), concentration of components therein, and timing of administering the composition(s), which elicit a suitable response. Such determinations do not require undue experimentation from the knowledge of the skilled artisan, this disclosure, and the documents cited herein. And, the time for sequential administrations can be ascertained without undue experimentation.

[0102] MAPCs may be encapsulated for administration, particularly where encapsulation enhances the effectiveness of the therapy, or provides advantages in handling and/or shelf life. Encapsulation where it increases the efficacy of MAPC mediated immunosuppression may, as a result, also reduce the need for immunosuppressive drug therapy.

[0103] Also, encapsulation may provide a barrier to a subject's immune system that may further reduce a subject's immune response to the MAPCs (which generally are not immunogenic or are only weakly immunogenic in allogeneic transplants), thereby reducing any graft rejection or inflammation that might occur upon administration of the cells.

[0104] Where MAPCs are administered in admixture with cells of another type, which are more typically immunogenic in an allogeneic or xenogeneic setting, encapsulation may reduce or eliminate adverse host immune responses to the non-MAPC cells and/or GVHD that might occur in an immunocompromised host if the admixed cells are immunocompetent and recognize the host as non-self.

[0105] MAPCs may be encapsulated by membranes, as well as capsules, prior to implantation. It is contemplated that any of the many methods of cell encapsulation available may be employed. Cells may be individually encapsulated. Many cells may be encapsulated within the same membrane. When the cells are to be removed following implantation, a relatively large size structure encapsulating many cells, such as within a single membrane, may provide a convenient means for retrieval.

[0106] A wide variety of materials may be used for microencapsulation of MAPCs. Such materials include, for example, polymer capsules, alginate-poly-L-lysine-alginate microcapsules, barium poly-L-lysine alginate capsules, barium alginate capsules, polyacrylonitrile/polyvinylchloride (PAN/PVC) hollow fibers, and polyethersulfone (PES) hollow fibers.

[0107] Techniques for microencapsulation of cells that may be used for administration of MAPCs are known to those of skill in the art and are described, for example, in ,,,, U.S. Patent No. 5,639,275 (which, for example, describes a biocompatible capsule for long-term maintenance of cells that stably express biologically active molecules. Additional methods of encapsulation are in European Patent Publication No. 301,777 and U.S. Pat. Nos. 4,353,888;

4,744,933; 4,749,620; 4,814,274; 5,084,350; 5,089,272; 5,578,442; 5,639,275; and 5,676,943. All of the foregoing are in parts pertinent to encapsulation of MAPCs.

[0108] MAPCs may be incorporated into a polymer, such as a biopolymer or synthetic polymer. Examples of biopolymers include, but are not limited to, fibronectin, fibin, fibrinogen, thrombin, collagen, and proteoglycans. Other factors, such as the cytokines discussed above, can also be incorporated into the polymer. MAPCs may be incorporated in the interstices of a three-dimensional gel. A large polymer or gel, typically, will be surgically implanted. A polymer or gel that can be formulated in small enough particles or fibers can be administered by other common, more convenient, non-surgical routes.

[0109] Pharmaceutical compositions of the invention may be prepared in many forms that include tablets, hard or soft gelatin capsules, aqueous solutions, suspensions, and liposomes and other slow-release formulations, such as shaped polymeric gels. Oral liquid pharmaceutical compositions may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups, or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid pharmaceutical compositions may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives. An oral dosage form may be formulated such that cells are released into the intestine after passing through the stomach. Such formulations are described in U.S. Patent No. 6,306,434 and in the references contained therein.

[0110] Pharmaceutical compositions suitable for rectal administration can be prepared as unit dose suppositories. Suitable carriers include saline solution and other materials commonly used in the art.

[0111] For administration by inhalation, cells can be conveniently delivered from an insufflator, nebulizer or a pressurized pack or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide, or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

[0112] Alternatively, for administration by inhalation or insufflation, a means may take the form of a dry powder composition, for example, a powder mix of a modulator and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules or cartridges or, e.g., gelatin or blister packs from which the powder may be administered with the aid of an inhalator or insufflator. For intra-nasal administration, cells may be administered via a liquid spray, such as via a plastic bottle atomizer.

Other Active Ingredients

[0113] MAPCs may be administered with other pharmaceutically active agents. One or more of such agents may be formulated together with MAPCs for administration. MAPCs and the one or more agents may be in separate formulations. The compositions comprising the MAPCs and/or the one or more agents may be formulated with regard to adjunctive use with one another.

[0114] MAPCs may be administered in a formulation comprising a immunosuppressive agents, such as any combination of any number of a corticosteroid, cyclosporin A, a cyclosporin-like immunosuppressive agent, cyclophosphamide, antithymocyte globulin, azathioprine, rapamycin, FK-506, and a macrolide-like immunosuppressive agent other than FK-506 and rapamycin. Such agents may include a corticosteroid, cyclosporin A, azathioprine, cyclophosphamide, rapamycin, and/or FK-506. Immunosuppressive agents in accordance with the foregoing may be the only such additional agents or may be combined with other agents, such as other agents noted herein. Other immunosuppressive agents include Tacrolimus, Mycophenolate mofetil, and Sirolimus.

[0115] Such agents also include antibiotic agents, antifungal agents, and antiviral agents, to name just a few other pharmacologically active substances and compositions that may be used.

[0116] Typical antibiotics or anti-mycotic compounds include, but are not limited to, penicillin, streptomycin, amphotericin, ampicillin, gentamicin, kanamycin, mycophenolic acid, nalidixic acid, neomycin, nystatin, paromomycin, polymyxin, puromycin, rifampicin, spectinomycin, tetracycline, tylosin, zeocin, and cephalosporins, aminoglycosides, and echinocandins.

[0117] Further additives of this type relate to the fact that MAPCs, like other stems cells, following administration to a subject may "home" to an environment favorable to their growth and function. Such "homing" often concentrates the cells at sites where they are needed, such as sites of immune disorder, dysfunction, or disease. A number of substances are known to stimulate homing. They include growth factors and trophic signaling agents, such as cytokines. They may be used to promote homing of MAPCs to therapeutically targeted sites. They may be administered to a subject prior to treatment with MAPCs, together with MAPCs, or after MAPCs are administered.

[0118] Certain cytokines, for instance, alter or affect the migration of MAPCs or their differentiated counterparts to sites in need of therapy, such as immunocompromised sites. Cytokines that may be used in this regard include, but are not limited to, stromal cell derived factor-1 (SDF-1), stem cell factor (SCF), angiopoietin-1, placenta-derived growth factor (PlGF), granulocyte-colony stimulating factor (G-CSF), cytokines that stimulate expression of endothelial adhesion molecules such as ICAMs and VCAMs, and cytokines that engender or facilitate homing.

[0119] They may be administered to a subject as a pre-treatment, along with MAPCs, or after

MAPCs have been administered, to promote homing to desired sites and to achieve improved therapeutic effect, either by improved homing or by other mechanisms. Such factors may be combined with MAPCs in a formulation suitable for them to be administered together. Alternatively, such factors may be formulated and administered separately.

[0120] Order of administration, formulations, doses, frequency of dosing, and routes of administration of factors (such as the cytokines discussed above) and MAPCs generally will vary with the disorder or disease being treated, its severity, the subject, other therapies that are being administered, the stage of the disorder or disease, and prognostic factors, among others. General regimens that have been established for other treatments provide a framework for determining appropriate dosing in MAPC-mediated direct or adjunctive therapy. These, together with the additional information provided herein, will enable the skilled artisan to determine appropriate administration procedures, without undue experimentation.

Routes

[0121] MAPCs can be administered to a subject by any of a variety of routes known to those skilled in the art that may be used to administer cells to a subject.

[0122] Among methods that may be used in this regard are methods for administering MAPCs by a parenteral route. Parenteral routes of administration include, among others, administration by intravenous, intraarterial, intracardiac, intraspinal, intrathecal, intraosseous, intraarticular, intrasynovial, intracutaneous, intradermal, subcutaneous, and/or intramuscular injection Intravenous, intraarterial, intracutaneous, intradermal, subcutaneous and/or intramuscular injection may be used. Intravenous, intraarterial, intracutaneous, subcutaneous, and/or intramuscular injection may be used.

[0123] MAPCs may be administered by systemic injection. Systemic injection, such as intravenous injection, offers one of the simplest and least invasive routes for administering MAPCs. In some cases, these routes may require high MAPC doses for optimal effectiveness and/or homing by the MAPCs to the target sites. MAPCs may be administered by targeted and/or localized injections to ensure optimum effect at the target sites.

[0124] MAPCs may be administered to the subject through a hypodermic needle by a syringe. MAPCs may be administered to the subject through a catheter. MAPCs may be administered by surgical implantation. Further in this regard, MAPCs may be administered to the subject by implantation using an arthroscopic procedure. MAPCs may be administered to the subject in or on a solid support, such as a polymer or gel. MAPCs may be administered to the subject in an encapsulated form.

[0125] MAPCs suitably formulated for oral, rectal, epicutaneous, ocular, nasal, and/or pulmonary delivery and are administered accordingly.

Dosing

[0126] Compositions can be administered in dosages and by techniques well known to those skilled in the medical and veterinary arts taking into consideration such factors as the age, sex, weight, and condition of the particular patient, and the formulation that will be administered (e.g., solid vs. liquid). Doses for humans or other mammals can be determined without undue experimentation by the skilled artisan, from this disclosure, the documents cited herein, and the knowledge in the art.

[0127] The dose of MAPCs appropriate to be used will depend on numerous factors. It may vary considerably for different circumstances. The parameters that will determine optimal doses of MAPCs to be administered for primary and adjunctive therapy generally will include some or all of the following: the disease being treated and its stage; the species of the subject, their health, gender, age, weight, and metabolic rate; the subject's immunocompetence; other therapies being administered; and expected potential complications from the subject's history or genotype. The parameters may also include: whether the MAPCs are syngeneic, autologous, allogeneic, or xenogeneic; their potency (specific activity); the site and/or distribution that must be targeted for the MAPCs to be effective; and such characteristics of the site such as accessibility to MAPCs and/or engraftment of MAPCs. Additional parameters include coadministration with MAPCs of other factors (such as growth factors and cytokines). The optimal dose in a given situation also will take into consideration the way in which the cells are formulated, the way they are administered, and the degree to which the cells will be localized at the target sites following administration. Finally, the determination of optimal dosing necessarily will provide an effective dose that is neither below the threshold of maximal beneficial effect nor above the threshold where the deleterious effects associated with the dose of MAPCs outweighs the advantages of the increased dose.

[0128] The optimal dose of MAPCs may be in the range of doses used for autologous, mononuclear bone marrow transplantation. For fairly pure preparations of MAPCs, optimal doses may range from 10^4 to 10^8 MAPC cells/kg of recipient mass per administration. The optimal dose per administration may be between 10^5 to 10^7 MAPC cells/kg. The optimal dose per administration may be 5×10^5 to 5×10^6 MAPC cells/kg. By way of reference, higher doses in the foregoing are analogous to the doses of nucleated cells used in autologous mononuclear bone marrow transplantation. Some of the lower doses are analogous to the number of $CD34^+$ cells/kg used in autologous mononuclear bone marrow transplantation.

[0129] It is to be appreciated that a single dose may be delivered all at once, fractionally, or continuously over a period of time. The entire dose also may be delivered to a single location or spread fractionally over several locations.

[0130] MAPCs may be administered in an initial dose, and thereafter maintained by further

administration of MAPCs. MAPCs may be administered by one method initially, and thereafter administered by the same method or one or more different methods. The subject's MAPC levels can be maintained by the ongoing administration of the cells. MAPCs may be administered either initially or to maintain their level in the subject or both by intravenous injection. Other forms of administration, may be used, dependent upon the patient's condition and other factors, discussed elsewhere herein.

[0131] It is noted that human subjects are treated generally longer than experimental animals; but, treatment generally has a length proportional to the length of the disease process and the effectiveness of the treatment. Those skilled in the art will take this into account in using the results of other procedures carried out in humans and/or in animals, such as rats, mice, non-human primates, and the like, to determine appropriate doses for humans. Such determinations, based on these considerations and taking into account guidance provided by the present disclosure and the prior art will enable the skilled artisan to do so without undue experimentation.

[0132] Suitable regimens for initial administration and further doses or for sequential administrations may all be the same or may be variable. Appropriate regiments can be ascertained by the skilled artisan, from this disclosure, the documents cited herein, and the knowledge in the art.

[0133] The dose, frequency, and duration of treatment will depend on many factors, including the nature of the disease, the subject, and other therapies that may be administered. Accordingly, a wide variety of regimens may be used to administer MAPCs.

[0134] MAPCs may be administered to a subject in one dose. In others MAPCs are administered to a subject in a series of two or more doses in succession. MAPCs may be administered in a single dose, in two doses, and/or more than two doses, the doses may be the same or different, and they are administered with equal or with unequal intervals between them.

[0135] MAPCs may be administered in many frequencies over a wide range of times. MAPCs may be administered over a period of less than one day. MAPCs may be administered over two, three, four, five, or six days. MAPCs may be administered one or more times per week, over a period of weeks. MAPCs may be administered over a period of weeks for one to several months. They may be administered over a period of months. In others they may be administered over a period of one or more years. Generally lengths of treatment will be proportional to the length of the disease process, the effectiveness of the therapies being applied, and the condition and response of the subject being treated.

Therapeutic Uses of Immunomodulating MAPCs

[0136] The immunomodulatory properties of MAPCs may be used in treating a wide variety of

disorders, dysfunctions and diseases, such as those that, intrinsically, as a secondary effect or as a side effect of treatment, present with deleterious immune system processes and effects. Several illustrations are discussed below.

[0137] Also disclosed herein is administering MAPCs to a subject having a weakened (or compromised) immune system, either as the sole therapy or as adjunctive therapy with another treatment. MAPCs may be administered to a subject adjunctively to radiation therapy or chemotherapy or a combination of radiation and chemotherapies that either have been, are being, or will be administered to the subject. The radiation therapy, chemotherapy, or a combination of radiation and chemotherapies may be part of a transplant therapy. MAPCs may be administered to treat a deleterious immune response, such as HVG or GVHD.

[0138] The subject may be the recipient of a non-syngeneic, typically allogeneic, blood cell or bone marrow cell transplant, the immune system of the subject has been weakened or ablated by radiation therapy, chemotherapy, or a combination of radiation and chemotherapy, immunosuppressive drugs are being administered to the subject, the subject is at risk to develop or has developed graft versus host disease, and MAPCs are administered to the subject adjunctively to any one or more of the transplant, the radiation therapy and/or the chemotherapy, and the immunosuppressive drugs to treat, such as ameliorate, arrest, or eliminate, graft versus host disease in the subject.

Neoplasms

[0139] The term "neoplasm" generally denotes disorders involving the clonal proliferation of cells. Neoplasms may be benign, which is to say, not progressive and non-recurrent, and, if so, generally are not life-threatening. Neoplasms also may be malignant, which is to say, that they progressively get worse, spread, and, as a rule, are life threatening and often fatal.

[0140] MAPCs may be administered to a subject suffering from a neoplasm, adjunctive to a treatment thereof. For example, the subject is at risk for or is suffering from a neoplasm of blood or bone marrow cells and has undergone or will undergo a blood or bone marrow transplant. Using the methods described herein for MAPC isolation, characterization, and expansion, together with the disclosures herein on immune-suppressing properties of MAPCs, MAPCs are administered to treat, such as to prevent, suppress, or diminish, the deleterious immune reactions, such as HVG and GVHD, that may complicate the transplantation therapy.

[0141] MAPCs can be used alone for an immunosuppressive purpose, or together with other agents. MAPCs can be administered before, during, or after one or more transplants. If administered during transplant, MAPCs can be administered separately or together with transplant material. If separately administered, the MAPCs can be administered sequentially or simultaneously with the other transplant materials. Furthermore, MAPCs may be administered well in advance of the transplant and/or well after, alternatively to or in addition to administration at or about the same time as administration of the transplant.

[0142] Other agents that can be used in conjunction with MAPCs, in transplantation therapies in particular, include immunomodulatory agents, such as those described elsewhere herein, particularly immunosuppressive agents, more particularly those described elsewhere herein, especially in this regard, one or more of a corticosteroid, cyclosporin A, a cyclosporin-like immunosuppressive compound, azathioprine, cyclophosphamide, methotrexate, and an immunosuppressive monoclonal antibody agent.

[0143] Among neoplastic disorders of bone marrow that are treated with MAPCs in this regard are myeloproliferative disorders ("MPDs"); myelodysplastic syndromes (or states) ("MDSs"), leukemias, and lymphoproliferative disorders including multiple myeloma and lymphomas.

[0144] MPDs are distinguished by aberrant and autonomous proliferation of cells in blood marrow. The disorder may involve only one type of cell or several. Typically, MPDs involve three cell lineages and are erythrocytic, granulocytic, and thrombocytic. Involvement of the three lineages varies from one MPD to another and between occurrences of the individual types. Typically, they are differently affected and one cell lineage is affected predominately in a given neoplasm. MPDs are not clearly malignant; but, they are classified as neoplasms and are characterized by aberrant, self-replication of hematopoietic precursor cells in blood marrow. MPDs have the potential, nonetheless, to develop into acute leukemias.

[0145] MDSs like MPDs are clonal disorders, and they are characterized by aberrant, self-replication of hematopoietic precursor cells in blood marrow. Like MPDs, they can develop into acute leukemias. Most, but not all, MDSs manifest peripheral blood cytopenias (chronic myelomonocytic leukemia is the exception), whereas MPDs do not.

[0146] The laboratory and clinical manifestations of these disorders may vary with their course and between individual occurrences. Manifestations can overlap, and it can be difficult to make a certain diagnosis that distinguishes one disease from all the others. Diagnosis of neoplasms of bone marrow hematopoietic cells thus requires special caution, so as to not misdiagnose as a benign disorder one that is, in reality, deadly malignant.

[0147] The following diseases are among the myeloproliferative disorders (MPDs) that may be treated with MAPCs, typically or adjunctively: chronic myelocytic leukemia ("CML")/chronic granulocytic leukemia ("CGL"), agnogenic myelofibrosis, essential thrombocythemia, and polycythemia vera

[0148] The following diseases are among the myelodysplastic syndromes (MDSs) that may be treated with MAPCs, typically or adjunctively: refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, and chronic myelomonocytic leukemia.

[0149] The following diseases are among the lymphoproliferative disorders, including multiple myelomas and lymphomas that may be treated with MAPCs, typically adjunctively,: pre-B acute

lymphoblastic leukemia, chronic lymphocytic leukemia ("CLL"), B-cell lymphoma, hairy cell leukemia, myeloma, multiple myeloma, T-acute lymphoblastic leukemia, peripheral T-cell lymphoma, other lymphoid leukemias, and other lymphomas.

[0150] Also among neoplasms that may be treated with MAPCs, typically adjunctively, are the following: a benign neoplasm of bone marrow cells, a myeloproliferative disorder, a myelodysplastic syndrome, or an acute leukemia; chronic myelocytic leukemia ("CML") (also called chronic granulocytic leukemia ("CGL")), agnogenic myelofibrosis, essential thrombocythemia, polycythemia vera, other myeloproliferative disorders, acute multiple myeloma, myeloblastic leukemia, acute promyelocytic leukemia, pre-B acute lymphoblastic leukemia, chronic lymphocytic leukemia ("CLL"), B-cell lymphoma, hairy cell leukemia, myeloma, T-acute lymphoblastic leukemia, peripheral T-cell lymphoma, other lymphoid leukemias, other lymphomas, or other acute leukemia.

[0151] MAPCs may be administered adjunctively to a treatment for any of the foregoing diseases.

Treatments Involving Immunoablation or Compromise

[0152] Acute leukemias often are difficult to treat by methods that have been effective for other malignancies. This is partly due to the mobility of cells from bone marrow, including those of a neoplasm. Partly it may be due to the diffuse distribution of bone marrow throughout the skeleton. And partly it is due, no doubt, to the nature of the cells themselves and their transformation.

[0153] At present, a standard treatment for hematologic malignancies involves ablating all hematopoietic cells in the patient. There is no way to do this without also ablating the patient's healthy hematopoietic cells. Typically, the patient is treated using chemo-radiotherapy in sufficiently high doses to kill virtually all bone marrow cells, both normal and neoplastic. The treatment's side effects are severe, and its effects on patients are unpleasant, painful, and physically and emotionally debilitating. The treatment not only ablates the diseased tissue and cells, it also eviscerates the patient's hematopoietic system and immune system. The treatment leaves them compromised, dependent on transfusions, and so highly susceptible to infection that even an otherwise minor exposure to an infectious agent can be fatal.

[0154] Normal hematopoietic capacity is restored thereafter by either autologous or allogeneic peripheral blood or bone marrow transplants. Unfortunately, the patient's immune system not only is severely compromised by the ablative treatment, but, also in the case of allogeneic transplantation, by intentional immune suppression to prevent rejection of the transplant and ensure engraftment and proliferation of the new hematopoietic stem cells that will repopulate the patient's marrow and regenerate the patient's hematopoietic and immune systems.

[0155] Many complications have been encountered in carrying out such transplants to

regenerate the hematopoietic and immune systems in an immunocompromised host. One is rejection by residual immune competent cells and processes in the host, referred to herein as HVG response. Another is triggered by immunocompetent cells in the graft, referred to herein as GVHD.

[0156] These complications might be avoided by using syngeneic or autologous donor material. However, syngeneic donors generally are rare and autologous transplants have a high risk of disease recurrence. Hence, transplants generally use allogeneic cells and tissues obtained from an HLA compatible donor. Unfortunately this procedure results in GVHD, ranging from mild to severe in the preponderance of those receiving this form of therapy. If not at least ameliorated, these immune reactions will result in failure of the transplant therapy, and may themselves be fatal to the patient.

[0157] A variety of agents have been developed to suppress immune responses that ameliorate graft complications, such as HVG and GVHD, as discussed herein above. Some are sufficiently effective to reduce adverse immune reactions to a manageable level in some transplant therapies, such as bone marrow and peripheral blood transplantation. These agents have improved the prognosis for transplant patients, to some extent; but, none of them is fully effective, and all of them have rather substantial shortcomings.

[0158] It has been found (as described in greater detail elsewhere herein) that MAPCs do not provoke an immune response in allogeneic hosts. Transplantation of MAPCs to an allogeneic host does not, thus, engender allogeneic graft rejection (i.e., HVG).

[0159] Furthermore, it has also been found that allogeneic MAPCs can be administered to a host at high concentration without deleterious effects on respiration.

[0160] In addition, it has been found, (as described in greater detail elsewhere herein) that MAPCs can modulate immune responses. In particular in this regard, it has been found that MAPCs can suppress immune responses, including but not limited to immune responses involved in, for example, HVG response and GVHD, to name just two. In an even more detailed particular in this regard, it has been found that MAPCs can suppress proliferation of T-cells, even in the presence of potent T-cell stimulators, such as Concanavalin A and allogeneic or xenogeneic stimulator cells.

[0161] Moreover, it has been found that even relatively small amounts of MAPCs can suppress these responses. Indeed, only 3% MAPCs in mixed lymphocyte reactions is sufficient to reduce T-cell response by 50% *in vitro.*

[0162] Accordingly, disclosed herein are compositions and methods and the like for treating, such as for ameliorating, and/or curing or eliminating, neoplasms, such as neoplasms of hematopoietic cells, particularly those of bone marrow.

[0163] Among these are those that are myeloproliferative disorders (MPDs), such as chronic

myelocytic leukemia (also called "chronic granulocytic leukemia" and "CGL"), agnogenic myelofibrosis, essential thrombocythemia, polycythemia vera; myelodysplastic syndromes (MDSs), such as refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, chronic myelomonocytic leukemia; and clearly malignant neoplasms - the acute leukemias - such as acute myeloblastic leukemia, chronic myelogenous leukemia (CML), acute promyelocytic leukemia, B-acute lymphoblastic leukemia, CLL, B-cell lymphoma, hairy cell leukemia, myeloma, T-acute lymphoblastic leukemia, peripheral T-cell lymphoma, and other lymphoid leukemias and lymphomas.

[0164] MAPCs may be administered adjunctively to a treatment for any of the foregoing diseases.

Anemias and Other Disorders of the Blood

[0165] MAPCs may be used to treat an anemia or other blood disorder, often adjunctively. MAPCs may be used to treat the following anemias and/or blood disorders, either solely or, typically, adjunctively: hemoglobinopathies, thalassemia, bone marrow failure syndrome, sickle cell anemia, aplastic anemia, or an immune hemolytic anemia. Also, disorders include refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, chronic myelomonocytic leukemia, or other myelodysplastic syndrome, and Fanconi's anemia.

[0166] MAPCs may be administered adjunctively to a treatment for any of the foregoing diseases.

Immune Diseases

[0167] MAPC immunomodulation may be used to treat an immune dysfunction, disorder, or disease, either solely, or as an adjunctive therapy. This may also relate to congenital immune deficiencies and autoimmune dysfunctions, disorders, and diseases. This may also relate to using MAPCs to treat, solely or adjunctively, Crohn's disease, Guillain-Barré syndrome, lupus erythematosus (also called "SLE" and systemic lupus erythematosus), multiple sclerosis, myasthenia gravis, optic neuritis, psoriasis, rheumatoid arthritis, Graves' disease, Hashimoto's disease, Ord's thyroiditis, diabetes mellitus (type 1), Reiter's syndrome, autoimmune hepatitis, primary biliary cirrhosis, antiphospholipid antibody syndrome ("APS"), opsoclonus-myoclonus syndrome ("OMS"), temporal arteritis, acute disseminated encephalomyelitis ("ADEM" and "ADE"), Goodpasture's, syndrome, Wegener's granulomatosis, celiac disease, pemphigus, polyarthritis, and warm autoimmune hemolytic anemia.

[0168] These may also relate to Crohn's disease, lupus erythematosus (also called "SLE" and

systemic lupus erythematosus), multiple sclerosis, myasthenia gravis, psoriasis, rheumatoid arthritis, Graves' disease, Hashimoto's disease, diabetes mellitus (type 1), Reiter's syndrome, primary biliary cirrhosis, celiac disease, polyarthritis, and warm autoimmune hemolytic anemia.

[0169] In addition, MAPCs may be used in this regard, solely and, typically, adjunctively, to treat a variety of diseases thought to have an autoimmune component, including but not limited to treating endometriosis, interstitial cystitis, neuromyotonia, scleroderma, progressive systemic scleroderma, vitiligo, vulvodynia, Chagas' disease, sarcoidosis, chronic fatigue syndrome, and dysautonomia.

[0170] Inherited immune system disorders include Severe Combined Immunodeficiency (SCID) including but not limited to SCID with Adenosine Deaminase Deficiency (ADA-SCID), SCID which is X-linked, SCID with absence of T & B Cells, SCID with absence of T Cells, Normal B Cells, Omenn Syndrome, Neutropenias including but not limited to Kostmann Syndrome, Myelokathexis; Ataxia-Telangiectasia, Bare Lymphocyte Syndrome, Common Variable Immunodeficiency, DiGeorge Syndrome, Leukocyte Adhesion Deficiency; and phagocyte Disorders (phagocytes are immune system cells that can engulf and kill foreign organisms) including but not limited to Chediak-Higashi Syndrome, Chronic Granulomatous Disease, Neutrophil Actin Deficiency, Reticular Dysgenesis.

[0171] MAPCs may be administered adjunctively to a treatment for any of the foregoing diseases.

Inflammatory Diseases

[0172] Additionally, MAPCs may be used to treat inflammatory diseases, either as a sole agent or adjunctively. MAPCs may be used to treat serious inflammatory states, such as those that arise from acute allergic reaction, or ancillary to other diseases or treatments. For the most part, at present, the use of MAPCs in this regard is limited to acute cases in which the subject is at risk of great incapacity or loss or life.

[0173] MAPCs may be administered adjunctively to a treatment for any of the foregoing diseases.

MAPCs as Described in U.S. Patent No. 7,015,037

[0174] Human MAPCs are described in the art. Methods of MAPC isolation for humans and mouse are known in the art. It is therefore now possible for one of skill in the art to obtain bone marrow aspirates, brain or liver biopsies, and other organs, and isolate the cells using positive or negative selection techniques available to those of skill in the art, relying upon the genes that are expressed (or not expressed) in these cells (e.g., by functional or morphological

assays such as those disclosed in the above-referenced applications). Such methods are described in, for instance, U.S. Patent No. 7,015,037, which contains description of MAPCs and methods of preparation.

Isolation and Growth of MAPCs as described in PCT/US00/21387

[0175] Methods of MAPC isolation for humans and mouse are known in the art. They are described in, for instance, US Patent No. 7,015,037, PCT/US00/21387 (published as WO 01/11011), and PCT/US02/04652 (published as WO 02/064748).

[0176] MAPCs were initially isolated from bone marrow, but were subsequently established from other tissues, including brain and muscle. Thus, MAPCs can be isolated from multiple sources, including bone marrow, placenta, umbilical cord and cord blood, muscle, brain, liver, spinal cord, blood or skin. For example, MAPCs can be derived from bone marrow aspirates, which can be obtained by standard means available to those of skill in the art.

Phenotype of Human MAPCs Under Conditions Described in U.S. Patent No. 7,015,037

[0177] Immunophenotypic analysis by FACS of human MAPCs obtained after 22-25 cell doublings indicated that the cells do not express CD31, CD34, CD36, CD38, CD45, CD50, CD62E and -P, HLA-DR, Mucl8, STRO-1, cKit, Tie/Tek; and express low levels of CD44, HLA-class I, and β2-microglobulin, but express CD10, CD13, CD49b, CD49e, CDw90, Flk1 (N>10).

[0178] Once cells underwent >40 doublings in cultures re-seeded at about 2 x $10^3/cm^2$, the phenotype became more homogenous, and no cell expressed HLA class-I or CD44 (n=6). When cells were grown at higher confluence, they expressed high levels of Muc18, CD44, HLA class I and β2-microglobulin.

[0179] Immunohistochemistry showed that human MAPCs grown at about 2 x $10^3/cm^2$ seeding density expressed EGF-R, TGF-R1 and -2, BMP-R1A, PDGF-R1a and -B, and that a small subpopulation (between 1 and 10%) of MAPCs stained with anti-SSEA4 antibodies.

[0180] Using Clontech cDNA arrays the expressed gene profile of human MAPCs cultured at seeding densities of about 2 x 10^3 cells/cm^2 for 22 and 26 cell doublings was determined:

1. A. MAPCs did not express CD31, CD36, CD62E, CD62P, CD44-H, cKit, Tie, receptors for IL1, IL3, IL6, IL11, G CSF, GM-CSF, Epo, Flt3-L, or CNTF, and low levels of HLA-class-I, CD44-E and Muc-18 mRNA.
2. B. MAPCs expressed mRNA for the cytokines BMP1, BMP5, VEGF, HGF, KGF, MCP1; the cytokine receptors Flk1, EGF-R, PDGF-R1α, gp130, LIF-R, activin-R1 and -R2, TGFR-2, BMP-R1A; the adhesion receptors CD49c, CD49d, CD29; and CD10.

3. C. MAPCs expressed mRNA for hTRT and TRF1; the POU domain transcription factor oct-4, sox-2 (required with oct-4 to maintain undifferentiated state of ES/EC, Uwanogho D., 1995 Embryonic expression of the chicken Sox2, Sox3 and Soxl1 genes suggests an interactive role in neuronal development, Mech. Dev. 49 (1-2) 23-36), sox 11 (neural development), sox 9 (chondrogenesis) homeodeomain transcription factors: Hox-a4 and -a5 (cervical and thoracic skeleton specification; organogenesis of respiratory tract), Hox-a9 (myelopoiesis), Dlx4 (specification of forebrain and peripheral structures of head), MSX1 (embryonic mesoderm, adult heart and muscle, chondro- and osteogenesis) (Foerst-Potts L. 1997, Disruption of Msx-1 and Msx-2 reveals roles for these genes in craniofacial, eye and axial development, Dev. Dyn. 209 (1) 70-84), PDX1 (pancreas) (Offield MF, 1996, PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum, Development 122 (3) 983-95).
4. D. Presence of oct-4, LIF-R, and hTRT mRNA was confirmed by RT-PCR.
5. E. In addition, RT-PCR showed that rex-1 mRNA and rox-1 mRNA were expressed in MAPCs.

[0181] Oct-4, rex-1 and rox-1 were expressed in MAPCs derived from human and murine marrow and from murine liver and brain. Human MAPCs expressed LIF-R and stained positive with SSEA-4. Finally, oct-4, LIF-R, rex-1 and rox-1 mRNA levels were found to increase in human MAPCs cultured beyond 30 cell doublings, which resulted in phenotypically more homogenous cells. In contrast, MAPCs cultured at high density lost expression of these markers. This was associated with senescence before 40 cell doublings and loss of differentiation to cells other than chondroblasts, osteoblasts, and adipocytes. Thus, the presence of oct-4, combined with rex-1, rox-1, and sox-2, correlated with the presence of the most primitive cells in MAPCs cultures.

[0182] Methods for culturing MAPCs are well-known in the art. (See for instance, U.S. Patent No. 7,015,037) The density for culturing MAPCs can vary from about 100 cells/cm^2 or about 150 cells/cm^2 to about 10,000 cells/cm^2, including about 200 cells/cm^2 to about 1500 cells/cm^2 to about 2000 cells/cm^2. The density can vary between species. Additionally, optimal density can vary depending on culture conditions and source of cells. It is within the skill of the ordinary artisan to determine the optimal density for a given set of culture conditions and cells.

[0183] Also, effective atmospheric oxygen concentrations of less than about 10%, including about 3 - 5%, can be used at any time during the isolation, growth, and differentiation of MAPCs in culture.

[0184] The present invention is additionally described by way of the following illustrative, non-limiting examples.

Examples

EXAMPLE 1: Human MAPCs (from Bone Marrow Mononuclear Cells)

[0185] Bone marrow mononuclear cells were obtained from bone marrow aspirates from the posterior iliac crest of >80 healthy human volunteers. Ten to 100 cubic centimeters of bone marrow was obtained from each subject. Mononuclear cells ("MNCs") were obtained from bone marrow by centrifugation over a Ficoll-Paque density gradient (Sigma Chemical Co., St Louis, MO). Bone marrow MNCs were incubated with CD45 and Glycophorin A microbeads (Miltenyi Biotec, Sunnyvale, CA) for 15 minutes and $CD45^+GlyA^+$ cells removed by placing the sample in front of a SuperMACS magnet. The eluted cells are 99.5% $CD45^-GlyA^-$.

[0186] Depletion of $CD45^+GlyA^+$ cells resulted in recovery of $CD45^-GlyA^-$ cells which constituted approximately 0.05 to 0.10% of the total bone marrow mononuclear cells. Cells were selected that do not express the common leukocyte antigen CD45, or the erythroid precursor marker glycophorin-A (GlyA). $CD45^-GlyA^-$ cells constitute $1/10^3$ marrow mononuclear cells. $CD45^-GlyA^-$ cells were plated in wells coated with fibronectin in 2% FCS, EGF, PDGF-BB, dexamethasone, insulin, linoleic acid, and ascorbic acid. After 7-21 days, small clusters of adherent cells developed. Using limiting dilution assays, the frequency of cells giving rise to these adherent clusters is $1/5 \times 10^3$ $CD45^-GlyA^-$ cells. When colonies appeared (about 10^3 cells), cells were recovered by trypsinization and re-plated every 3-5 days at a 1:4 dilution under the same culture conditions. Cell densities were maintained between $2\text{-}8 \times 10^3$ cells/cm^2.

EXAMPLE 2: Mouse MAPCs

[0187] All tissues were obtained according to guidelines from the University of Minnesota IACUC. BM mononuclear cells (BMMNC) were obtained by Ficoll Hypaque separation. BM was obtained from 5-6 week old ROSA26 mice or C57/BL6 mice. Alternatively, muscle and brain tissue was obtained from 3-day old 129 mice. Muscles from the proximal parts of fore and hind limbs were excised and thoroughly minced. The tissue was treated with 0.2% collagenase (Sigma Chemical Co., St Louis, MO) for 1 hour at 37°C, followed by 0.1% trypsin (Invitrogen, Grand Island, NY) for 45 minutes. Cells were then triturated vigorously and passed through a 70 µm filter. Cell suspensions were collected and centrifuged for 10 minutes at 1600 rpm. Brain tissue was dissected and minced thoroughly. Cells were dissociated by incubation with 0.1% trypsin and 0.1% DNAse (Sigma) for 30 minutes at 37 °C. Cells were then triturated vigorously and passed through a 70 µm filter. Cell suspensions were collected and centrifuged for 10 minutes at 1600 rpm.

[0188] BMMNC or muscle or brain suspensions were plated at $1 \times 10^5/cm^2$ in expansion medium [2% FCS in low glucose Dulbecco's minimal essential medium (LG-DMEM), 10 ng/ml

each of platelet derived growth factor (PDGF), epidermal growth factor (EGF) and leukemia inhibitory factor (LIF)] and maintained at 5 x 10^3/cm^2. After 3-4 weeks, cells recovered by trypsin/EDTA were depleted of $CD45^+GlyA^+$ cells with micromagnetic beads. Resulting $CD45^-$ $GlyA^-$ cells were replated at 10 cells/well in 96 well plates coated with FN and were expanded at cell densities between 0.5 and 1.5 x 10^3/cm^2. The expansion potential of MAPCs was similar regardless of the tissue from which they were derived.

EXAMPLE 3: Rat MAPCs

[0189] BM and MNCs from Sprague Dawley, Lewis, or Wistar rats were obtained and plated under conditions similar to those described for mMAPCs.

EXAMPLE 4: Clonality of Mouse and Rat MAPCs

[0190] To demonstrate that differentiated cells were single cell-derived and MAPCs are indeed "clonal" multipotent cells, cultures were made in which MAPCs had been transduced with a retroviral vector. Undifferentiated MAPCs and their progeny were found to have the retrovirus inserted in the same site in the genome.

[0191] Studies were done using two independently derived ROSA26 MAPCs, two C57BL/6 MAPCs, and one rMAPC population expanded for 40 to >90 PDs, as well as with the eGFP transduced "clonal" mouse and "clonal" rMAPCs. No differences were seen between eGFP transduced and untransduced cells. Of note, eGFP expression persisted in differentiated MAPCs.

[0192] Specifically, murine and rat BMMNCs cultured on FN with EGF, PDGF-BB, and LIF for three weeks were transduced on two sequential days with an eGFP oncoretroviral vector. Afterwards, $CD45^+$ and $GlyA^+$ cells were depleted and cells subcultured at 10 cells/well. eGFP-transduced rat BMMNCs were expanded for 85 PDs. Alternatively, mouse MAPCs expanded for 80 PDS were used. Subcultures of undifferentiated MAPCs were generated by plating 100 MAPCs from cultures maintained for 75 PDs and re-expanding them to > 5 x 10^6 cells. Expanded MAPCs were induced to differentiate *in vitro* to endothelium, neuroectoderm, and endoderm. Lineage differentiation was shown by staining with antibodies specific for these cell types.

EXAMPLE 5: Human MAPCs Are Not Immunogenic

[0193] Mesenchymal stem cells have demonstrated low *in vitro* immunogenicity and the ability to engraft across allogeneic recipients (Di Nicola, M. et al. (2002) Blood 99: 3838-3843;

Jorgensen, C. et al. (2002) Gene Therapy 10: 928-931; Le Blanc, K. et al. (2003) Scandinavian Journal of Immunology 57: 11-20; McIntosh, K. et al. (2000) Graft 6: 324-328; Tse, W. et al. (2003) Transplantation 75: 389-397).

[0194] Figure 3 shows that human MAPCs exhibit low *in vitro* immunogenicity and are immunosuppressive when added to otherwise potent T-cell MLRs. Results were consistent across all donor and responder pairs tested.

[0195] Responder and stimulator cells were prepared for these experiments and the MLRs were performed.

EXAMPLE 6: MAPCs Modulate T-Cell Responses

[0196] The ability of MAPCs to modulate, and in this case suppress, immune responsiveness is illustrated by T-cell proliferation assays, for example, which can be carried out as follows.

Preparation of Responder T-Cells

[0197] Responder cells were prepared from lymph nodes of Lewis rats. Lymph nodes were surgically removed from the rats and immediately placed into 3 to 5 ml of media (complete RPMI or complete DMEM) in 60 x 15 mm Petri plates. The lymph nodes were dispersed through a nylon filter (using the hand plunger of a syringe). The dispersions were loaded into 50 ml tubes and centrifuged at 1,250 rpm for 8 minutes. The resulting supernatants (media) were discarded. The pellets (containing the cells) were resuspended in fresh media (complete RPMI or complete DMEM). The cells were washed three times, and then resuspended in fresh media. Resuspended cell densities were determined by counting the number of cells in a known volume thereof. The cells were maintained on ice. Prior to use, the cells were resuspended in media (complete RPMI or complete DMEM) at a density of 1.25×10^6 cells/ml.

Preparation of MAPCs

[0198] MAPCs were prepared from Lewis or from Sprague-Dawley rats and then frozen as described above. They were thawed and then irradiated at 3000 rad. The irradiated cells were then resuspended in media (complete RPMI or complete DMEM) to densities of 0.4×10^6 cells/ml, 0.8×10^6 cells/ml, and 1.6×10^6 cells/ml.

Preparation of Concanavalin A

[0199] Concanavalin A ("ConA") was used to activate the T-cells. The ConA was dissolved in PBS (complete RPMI or complete DMEM) to final concentrations of 0 (PBS only) 10, 30, and 100 µg/ml.

Assay Procedure

[0200] Each data point is based on at least three determinations.

[0201] 20 µl of each of the ConA solutions was added to wells of microtiter plates (96 well, flat bottom), followed by 80 µl/well of the responder cells and 100 µl/well of the MAPCs. The plates were incubated at 37°C in humidified incubators under 5% CO_2 for 4-5 days. The plates were pulsed with 1 µCi/well ^{3}H-thymidine during the last 14-18 hours of culture. Thereafter, the cells were automatically harvested on glass fiber filters using a Tomtec harvesting machine. The thymidine uptake was quantified in a microplate scintillation counter. Results were expressed as mean counts per minute (CPM) +/- SD.

[0202] Final concentrations of ConA in the growth media in the wells were 0, 1, 3.16, and 10 µg/ml. MAPCs were present in the wells in the amounts of 0, 0.4, 0.8, and 1.6 x 10^5 cells/well.

[0203] The results, described below, are illustrated in Figure 4.

Results

[0204] Increasing amounts of ConA resulted in a dose-dependent stimulation of T-cell proliferation (Figure 4, LN only). Lewis MAPCs inhibited proliferation of the ConA stimulated T-cells. The inhibition depended on the dose of MAPCs. The maximum inhibition, 50%, occurred at the highest dose of MAPCs used in these experiments and with low and intermediate doses of ConA. These results are displayed graphically in Figure 4 and show that MAPCs suppress the proliferation of activated T-cells.

EXAMPLE 7: MAPCs Suppress Proliferation of Stimulated T-Cells

[0205] The ability of MAPCs to suppress proliferation of syngeneic and non-matched (allogeneic) T-cell proliferative responses is demonstrated by the results of mixed lymphocyte reactions. The example below shows the suppressive effects of MAPCs on T-cells from Lewis rats stimulated by irradiated splenocytes from DA rats. MAPCs from syngeneic Lewis rats and non-matched (allogeneic) Sprague-Dawley rats both inhibited T-cell responses in a dose-dependent manner. The experiments were carried out as follows.

Preparation of Responder T-Cells

[0206] Responder cells were prepared from the lymph nodes of Lewis rats as described above.

Irradiated Spleen Stimulator Cells

[0207] Spleens were surgically removed from DA rats. Splenocytes then were isolated from the spleens essentially as described above for the isolation of responder cells from the lymph nodes of Lewis rats. The isolated spleen cells were irradiated at 1800 rad. The cells then were resuspended to 4 x 10^6/ml and kept on ice until they were used.

Preparation of MAPCs

[0208] Syngeneic MAPCs were prepared from Lewis rats as described above. Non-matched (allogeneic) MAPCs were prepared from Sprague-Dawley rats in the same way. Both Lewis and Sprague-Dawley MAPCs were irradiated at 3000 rad, then resuspended in complete RPMI media at densities of 0.03 x 10^6/ml, 0.06 x 10^6/ml, 0.125 x 10^6/ml, 0.25 x 10^6/ml, 0.5 x 10^6/ml, 1 x 10^6/ml, and 2 x 10^6/ml.

Assay Procedure

[0209] 96 well microtiter plate wells were loaded with: 100 µl of MAPCs (at the dilutions indicated above) or 100 µl of media; 50 µl of a stimulator cell stock or control; 50 µl each of a responder cell stock or control; and media (complete RPMI or complete DMEM) as required to equalize total volumes to a final volume of 200 µl/well. 96 well flat bottom microtiter plates were used for all assays.

[0210] Each data point is based on at least three determinations.

[0211] The plates were incubated at 37°C in humidified incubators under 5% CO_2 for 4-5 days. The plates were pulsed with 1µCi/well ^{3}H-thymidine during the last 14-18 hours of culture. Thereafter, the cells were automatically harvested on glass fiber filters using a Tomtec harvesting machine. The thymidine uptake was quantified in a microplate scintillation counter. Results were expressed as mean counts per minute (CPM) +/- SD.

Results

[0212] Exposure of T-cells derived from the lymph nodes of Lewis rats (Responders) to stimulator cells consisting of irradiated splenocytes from DA rats (Stimulators) resulted in very robust proliferative responses of responder cells, as shown in Figures 5A and 5B, for "no MAPC".

[0213] Addition of increasing doses of syngeneic Lewis MAPCs (Figure 5A) and non-matched (allogeneic) third-party Sprague-Dawley MAPCs (Figure 5B) resulted in a significant and dose-dependent inhibition of T-cell activation. Maximal levels of inhibition were ~80%. Even at the lowest doses of MAPCs, there was 40-50% inhibition.

[0214] There was no ^{3}H-thymidine incorporation in the controls, showing that incorporation was due solely to proliferation of activated responder T-cells, as shown in Figures 5A and 5B.

[0215] In summary, the results show that syngeneic and third-party (allogeneic) MAPCs suppress T-cell proliferation even in the presence of potent activator splenocytes from non-matched rats. In these experiments, inhibition peaked when there were similar numbers (200,000 cells) each of stimulators, responders, and MAPCs in the reaction. Under these conditions, there was ~80% inhibition. There was very substantial inhibition at much lower ratios of MAPCs to the other cells in the reaction. For instance, at 1.5% MAPCs, there was 50% inhibition (3,000 MAPCs versus 200,000 of each of the types of cells). The results demonstrate not only that MAPCs have a strong immunosuppressive effect, but also that a relatively small number of MAPCs is sufficient to inhibit a relatively large number of competent T-cells even in the presence of very potent T-cell activators.

EXAMPLE 8: MAPCs Are Safe

[0216] The main immediate risk of intravenous injection of large numbers of cells is the accumulation of cell clumps in the lungs which results in respiratory distress and can cause cardiac arrest. To show the safety of MAPCs in this regard, we measured the effects of intravenous injection of MAPCs on respiratory rates in Buffalo rats.

[0217] MAPCs were prepared from Lewis rats as described above ("Lewis MAPCs"). Splenocytes also were prepared from Lewis rats as described above for use as controls ("Lewis Splenocytes").

[0218] One female Lewis rat served as the splenocyte donor for each group (experimental condition). Two female Buffalo rats were used as recipients for each group (experimental condition).

[0219] The cells were administered to the rats as indicated below. The data are displayed graphically in Figure 6. The concordance of data points to the individual rats as numbered below is enumerated in the vertical legend at the right of Figure 6. All rats were Buffalo rats.

1.1, 1.2	10 x 10^6 Lewis MAPC per rat
2.1, 2.2	5 x 10^6 Lewis MAPC per rat
3.1, 3.2	2.5 x 10^6 Lewis MAPC per rat
4.1, 4.2	1.2 x 10^6 Lewis MAPC per rat
5.1, 5.2	10 x 10^6 Lewis Splenocytes per rat
6.1, 6.2	5 x 10^6 Lewis Splenocytes per rat
7.1, 7.2	2.5 x 10^6 Lewis Splenocytes per rat
8.1,8.2	1.2 x 10^6 Lewis Splenocytes per rat

[0220] As indicated above, rats were injected with 1.2, 2.5, 5, or 10 million MAPCs or 1.2, 2.5, 5, or 10 million splenocytes. This corresponded to 5, 10, 25, or 50 million cells/kg. Respiratory rates were measured before (0 min) and at 1, 5, and 10 min after intravenous injection of the MAPCs or splenocytes. Respiratory rates were measured for 20 seconds and the counts were multiplied by 3 to derive the per minute respiratory rates. Normal rat respiratory rates are between 60 and 140/min.

Results

[0221] All animals survived after intravenous cell injections. No significant differences or trends were observed in the respiratory rates under the different conditions. The results are shown in Figure 6. Initial respiratory rates (0 min) were slightly reduced because the animals were anesthetized before the cells were injected. At each time-point, measurements were clustered without any apparent trends.

[0222] In summary, intravenous injection of 5-50 million MAPCs/kg did not cause changes in respiratory rates or mortality in any of the rats under any of the conditions. The results show that intravenous injection of MAPCs is safe even at high doses.

EXAMPLE 9: MAPC Expression of Immune Markers

[0223] The immunomodulatory nature of MAPCs was further characterized by determining immune regulatory markers in MAPCs. The markers were determined using marker-specific antibodies and FACS analysis.

[0224] Rat bone marrow MAPCs were isolated, cultured, and harvested as described above. For FACS analysis, the cells were suspended at 1-2 x 10^8 cells/ml in PBS. 200 µl of the cell suspension was added to each of a series of 12 x 75 polypropylene tubes. A marker-specific

antibody or a control was added to each of the tubes, and they were then incubated for 15-20 minutes at room temperature. At the end of the incubation period, 2 ml of PBS was added to each tube and they were then centrifuged at 400 x g for 5 minutes. Supernatants were discarded and the cells were resuspended in each tube in 100 µl of PBS. A fluorescent labeled secondary antibody was added to each tube in an appropriate volume, and the tubes were again incubated for 15-20 minutes at room temperature, this time in the dark. Thereafter, 2 ml of PBS was added to each tube and they were again centrifuged at 400 x g for 5 minutes. Supernatants were discarded, and the cells in each tube were resuspended in 200 µl of PBS and were then kept on ice until analyzed by FACS.

[0225] Results are enumerated and presented graphically in Table 1. As shown in the table, rat MAPCs are: (a) positive for MHC class I, CD11c, CD29, and CD90, and (b) negative for MHC class II, CD11b, CD31, CD40, CD45, CD54, CD80, CD86, CD 95, and CD 106. Negative results were validated for each antibody by positive staining of control cells, including rat peripheral blood and endothelial cells. These patterns of marker expression, as to both the markers that are detected in MAPCs and those that were not detected, are fully consistent with the low immunostimulatory cross-section of MAPCs.

Table 1 - Detection of Immune Cell-Related Markers in MAPCs

Marker	Name/Function of Marker	Commercial Anti-Rat-Marker Antibody	FACS Detection
MHC class I		yes	Positive
MHC class II		yes	Negative
CD11b	Mac-1	yes	Negative
CD11c	Integrin αX	yes	Positive
CD29	Integrin β1	yes	Positive
CD31	PECAM-1	yes	Negative
CD40	TNFRSF	yes	Negative
CD45	Leukocyte common antigen	yes	Negative
CD54	ICAM-1	yes	Negative
CD80	B7-1	yes	Negative
CD86	B7-2	yes	Negative
CD90	Thy-1	yes	Positive
CD95	Apo-1	yes	Negative
CD 106	VCAM-1	yes	Negative

EXAMPLE 10: MAPCs Suppress Previously Stimulated T-Cells in MLRs

Cells

[0226] Responder cells were prepared from the lymph nodes of Lewis rats as described above. Splenocytes, prepared from Buffalo or DA rats, as described above, were used as stimulators. MAPCs were prepared as described above.

Procedures

[0227] MAPCs were added to a first group of MLRs at the same time as splenocytes (as was done in the foregoing examples). In addition, MAPCs were added to a second group of MLRs that were set-up identically to the first group. However, the MAPCs in the second group were added 3 days after the addition of the splenocytes (or control). Thus, in the first group, MAPCs were added before the T-cells began proliferating in response to the splenocytes. In the second group, the MAPCs were added when the T-cell response to the splenocytes had been underway for 3 days. All plates were incubated for a total of 4 days, then pulsed with ^{3}H-thymidine and harvested, as described in the foregoing examples. The experiments otherwise were carried out as described for the MLRs in the foregoing examples.

[0228] Each data point is based on at least three determinations.

Results

[0229] As can be seen from the right side of Figure 7, MAPCs strongly suppress T-cell proliferation in MLRs when they are added three days after stimulation by allogeneic splenocytes. Comparison of the left and right sides of Figure 7 shows that MAPCs strongly suppress the on-going proliferation reaction. Quantitatively, the results show for Buffalo cells that MAPCs added 3 days post-stimulation suppressed T-cell proliferation 50% compared to controls (right side of the figure), and 75% when added at the same time as the stimulatory splenocytes (left side of the figure). Similarly, for DA cells, MAPCs added 3 days post-stimulation suppressed T-cell proliferation 33% compared to controls (right side of the figure), and 70% when added at the same time as the stimulatory splenocytes (left side of the figure).

[0230] In all cases, the degree of immunosuppression by MAPCs depended, in general, on the number of MAPCs that were added to the reaction. In other words, immunosuppression depended on the dose of MAPCs added to the MLRs.

EXAMPLE 11: MAPC Inhibition of T Lymphocytes is Reversible

[0231] T cells were cultured with allogeneic splenocyte stimulators for 13 days in the presence or absence of irradiated MAPCs. Following the 13-day culture, T cells were recovered and replated with irradiated allogeneic splenocytes, with or without MAPCs, and cultured for 4 days.

[0232] Proliferation of T cells that was inhibited by the presence of irradiated MAPCs during the primary culture period was restored when MAPCs were absent during the secondary culture. The presence of MAPCs in the secondary culture inhibited T cell proliferation comparably to that in the primary culture, with up to 90% inhibition at a 1:2 ratio of MAPCs to T cells. The results, presented in Figure 8, clearly demonstrate that inhibition of T cell proliferation by MAPCs in MLRs is reversible, and that MAPCs inhibit both primary and secondary T cell proliferative responses.

EXAMPLE 12: MAPCs Prevent GVHD

[0233] The ability of MAPCs to suppress GVHD was demonstrated in a rat model as follows. T cells from Buffalo rat donors served as the "graft" in Lewis x Buffalo rat recipients. The Buffalo graft cells were activated by the "foreign" Lewis cells in the host, leading to GVHD in the model.

[0234] F1 recipient rats (Lewis x Buffalo) were sublethally irradiated on Day 0 with a single 600 rad dose. The same day they were injected intravenously with 2×10^7 bone marrow cells and 10×10^7 T cells (splenocytes) from donor Buffalo rats. The rats were monitored 3x/week for signs of GVHD, evaluated by posture, activity, fur texture, skin, and weight loss.

[0235] Group 1 received no MAPCs. Group 2 received 2.5×10^6 MAPCs on Day 1. Group 3 received 2.5×10^6 MAPCs on each of Day 1 and Day 8. There were five rats in each group. The results are shown in Figure 9. MAPC administration clearly improved survival rates. Forty percent of the rats in Group 2 and sixty percent of the rats in Group 3 survived to the end of the experiment (42 days), whereas all of the rats not receiving MAPCs (Group 1) succumbed to GVHD by day 21.

REFERENCES CITED IN THE DESCRIPTION

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PATENTKRAV

1. Celler til anvendelse i supplerende forebyggelse eller behandling af en benign neoplasma i knoglemarvsceller, en myeloproliferativ lidelse, et myelo-dysplastisk syndrom eller en akut leukæmi, en anæmi eller medfødt immundefekt eller en autoimmun dysfunktion, lidelse eller sygdom, eller en ugunstig immunreaktion forekommende i transplantationsterapi i et individ, hvor cellerne er multipotente progenitorceller, **kendetegnet ved at** de eksprimere telomerase og er positive for oct-3/4; ikke er embryonale stamceller, embryonale kimceller eller kimceller; kan differentiere til mindst en celletype af hver af mindst to af de endodermale, ektodermale og mesodermale embryonale linjer; ikke provokere ikke et skadeligt immunrespons i individet; er effektive til behandling af den benigne neoplasma i knoglemarvsceller, en myeloproliferativ lidelse, et myelo-dysplastisk syndrom eller en akut leukæmi, en anæmi eller medfødt immundefekt eller en autoimmun dysfunktion, lidelse eller sygdom eller en ugunstig immunreaktion forekommende i transplantationsterapi i individet; og administreres ad en effektiv vej og i en effektiv mængde, supplerende med en eller flere andre behandlinger.

2. Anvendelse af celler til fremstilling af et medikament til supplerende forebyggelse eller behandling af en benign neoplasma af knoglemarvsceller, en myeloproliferativ lidelse, et myelo-dysplastisk syndrom eller en akut leukæmi eller en anæmi eller medfødt immundefekt eller en autoimmun dysfunktion, lidelse eller sygdom eller en ugunstig immunreaktion forekommende i transplantationsterapi, hvor cellerne er multipotente progenitorceller, **kendetegnet ved at** de eksprimere telomerase og er positive for oct-3/4: ikke er embryonale stamceller, embryonale kimceller eller kimceller; kan differentiere til mindst en celletype af hver af mindst to af endodermale, ektodermale og mesodermale embryonale linjer; provokere ikke et skadeligt immunrespons i individet; er effektive til behandling af den benigne neoplasma i knoglemarvsceller, myeloproliferativ lidelse, myelo-dysplastisk syndrom eller akut leukæmi eller en anæmi eller medfødt immundefekt eller autoimmun dysfunktion, lidelse eller sygdom eller ugunstig immunreaktion forekomende i transplantationsterapi i individ og administreres ad en effektiv vej og i en effektiv mængde, supplerende med en eller flere andre behandlinger.

3. Celler til anvendelse ifølge krav 1 eller anvendelsen af celler ifølge krav 2, hvor nævnte celler kan differentiere til mindst en celletype af hver af de endodermale, ektodermale og mesodermale embryonale linjer.

4. Celler til anvendelse ifølge et hvilket som helst af kravene 1 eller 3, eller anvendelsen af celler ifølge et hvilket som helst af kravene 2 til 3, hvor nævnte celler har gennemgået mindst 10 til 40 celledubleringer i kultur inden deres administration til individet.

5. Celler til anvendelse ifølge et hvilket som helst af kravene 1 eller 3 til 4, eller anvendelse af celler ifølge et hvilket som helst af kravene 2 til 4, hvor nævnte celler er xenogene eller allogene for individet.

6. Celler til anvendelse ifølge et hvilket som helst af kravene 1 eller 3 til 5, eller anvendelse af celler ifølge et hvilket som helst af kravene 2 til 5, hvor nævnte celler administreres til individet supplerende til en anden behandling der administreres før, på samme tid som eller efter at nævnte celler administreres.

7. Celler til anvendelse ifølge et hvilket som helst af kravene 1 eller 3 til 6, eller anvendelse af celler ifølge et hvilket som helst af kravene 2 til 6, hvor nævnte celler administreres til individet i en eller flere doser omfattende 10^4 til 10^8 af nævnte celler pr. kg af individets masse.

8. Celler til anvendelse ifølge et hvilket som helst af kravene 1 eller 3 til 7, eller anvendelse af celler ifølge et hvilket som helst af kravene 2 til 7, hvor individet vil modtager eller har modtaget en transplantation og vil være i fare for eller har udviklede en vært mod transplantatrespons eller transplantat mod værtssygdom, og nævnte celler administreres supplerende til transplantationen.

9. Celler til anvendelse ifølge krav 8 eller anvendelse af celler ifølge krav 8, hvor transplantationen er et organ, knoglemarv eller blodtransplantation, individet er i fare for eller har udviklet en vært mod transplantatrespons og nævnte celler administreres supplerende til transplantationen til behandling af vært mod transplantatresponset.

10. Celler til anvendelse ifølge krav 8 eller anvendelse af celler ifølge krav 8, hvor individet er eller vil blive behandlet med stråling eller kemoterapi eller en kombination af stråling og kemoterapi, svækkelse af individets immunsystem og behandling med nævnte celler er supplerende til stråling, kemoterapi eller kombinationen af de to.

11. Celler til anvendelse ifølge krav 8 eller anvendelse af celler ifølge krav 8, hvor nævnte celler administreres til et individ med et kompromitteret immunsystem.

12. Celler til anvendelse ifølge krav 9, eller anvendelse af celler ifølge krav 9, hvor nævnte individ vil blive eller er blevet behandlet med et immunsuppressivt middel, og behandlingen med nævnte celler er supplerende hertil.

13. Celler til anvendelse ifølge et hvilket som helst af kravene 1 eller 3 til 12, eller anvendelse af celler ifølge et hvilket som helst af kravene 2 til 12, hvor individet er i fare for eller lider af en eller flere af en neoplasma, en anæmi eller anden blodlidelse og en immundysfunktion, hvor behandling med nævnte celler er supplerende til en behandling deraf.

14. Celler til anvendelse ifølge krav 13 eller anvendelse af celler ifølge krav 13, hvor individet er i fare for eller lider af en eller flere af en myeloproliferativ lidelse, et myelodysplastisk syndrom, leukæmi, multiple myelom, et lymfom, en hæmoglobinopati, en thalassæmi, et knoglemarvssyndrom, seglcelleanæmi, aplastisk anæmi, Fanconis anæmi, en immun hæmolytisk anæmi, en medfødt immundefekt eller en autoimmun dysfunktion, hvor behandling med nævnte celler er supplerende til en behandling deraf.

15. Celler til anvendelse ifølge et hvilket som helst af kravene 1 eller 3 til 14, eller anvendelse af celler ifølge et hvilket som helst af kravene 2 til 14, hvor nævnte celler er allogene for individet.

16. Celler til anvendelse ifølge et hvilket som helst af kravene 1 eller 3 til 15, eller anvendelse af celler ifølge et hvilket som helst af kravene 2 til 15, hvor nævnte celler er udledt fra knoglemarv.

17. Celler til anvendelse ifølge krav 16 eller anvendelse af celler ifølge krav 16, hvor nævnte celler er udledt af human knoglemarv.

DRAWINGS

FIGURE 1

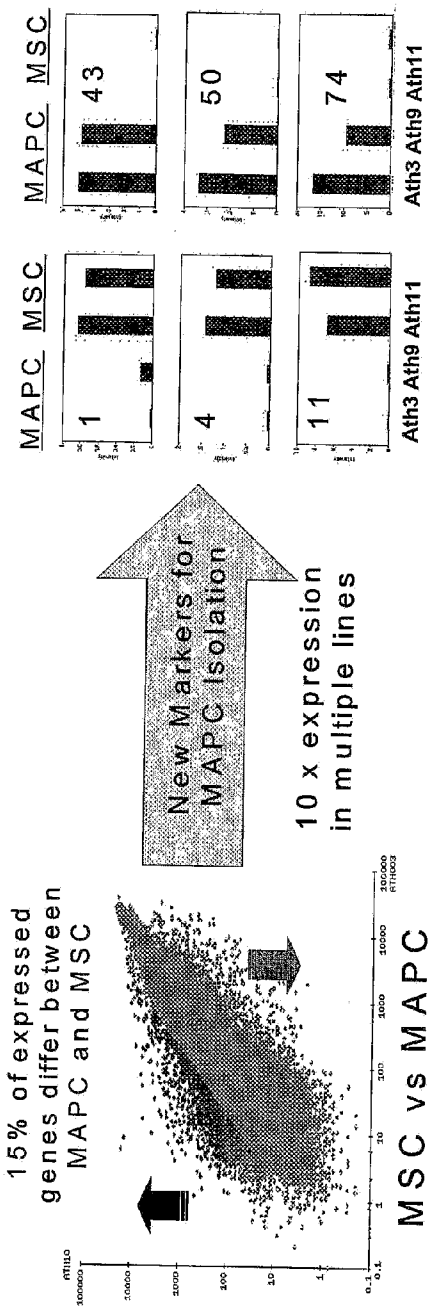


FIGURE 2

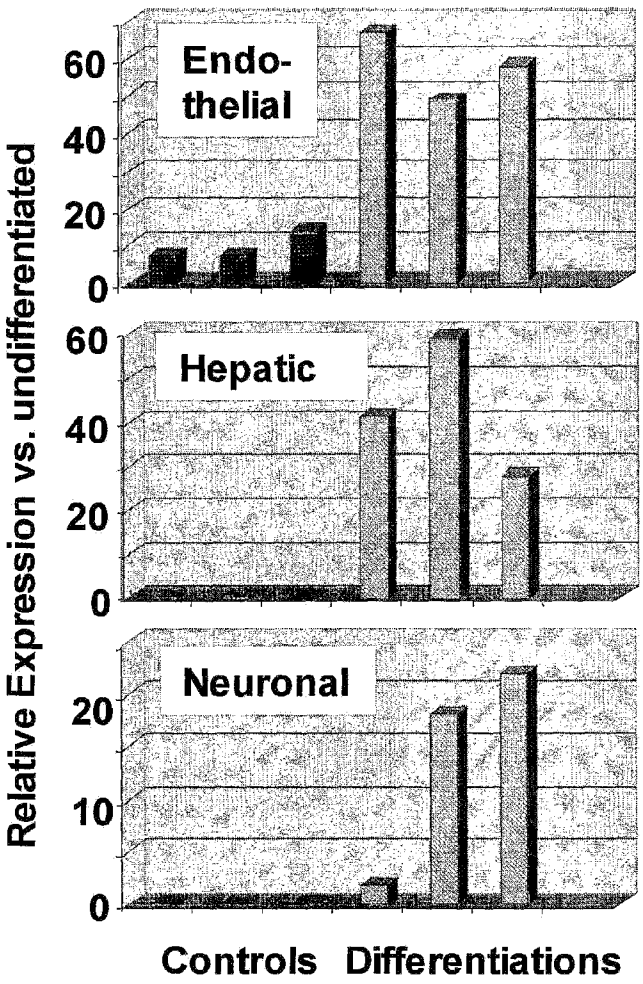

Endo-
thelial
60
40
20
0
Hepatic
60
40
20
0
Neuronal
20
10
0
Relative Expression vs. undifferentiated
Controls
Differentiations

FIGURE 3

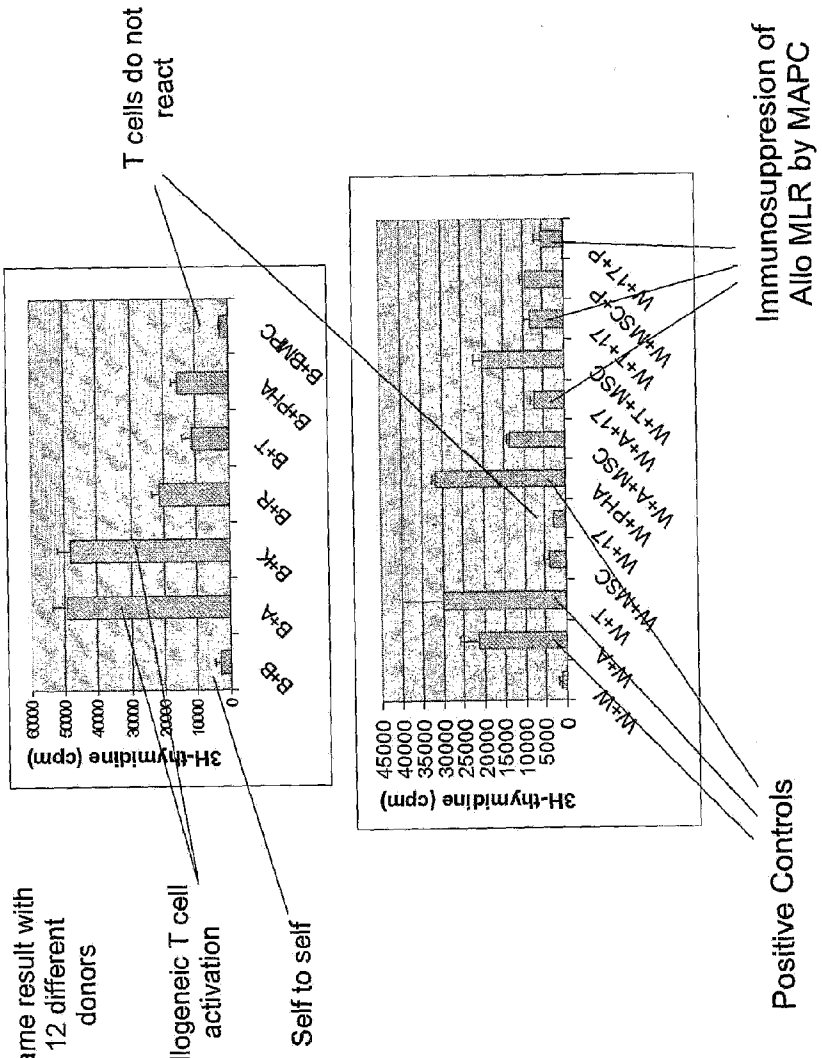

T cells do not react
Immunosuppresion of Allo MLR by MAPC
Same result with 12 different donors
Allogeneic T cell activation
Self to self
Positive Controls
3H-thymidine (cpm)
B+B
B+A
B+K
B+R
B+T
B+PHA
B+BMPC
W+W
W+A
W+T
W+ASC
W+T7
W+PHA
W+A+MSC
W+A+T7
W+T+MSC
W+T+T7
W+MSC+P
W+T7+P

FIGURE 4

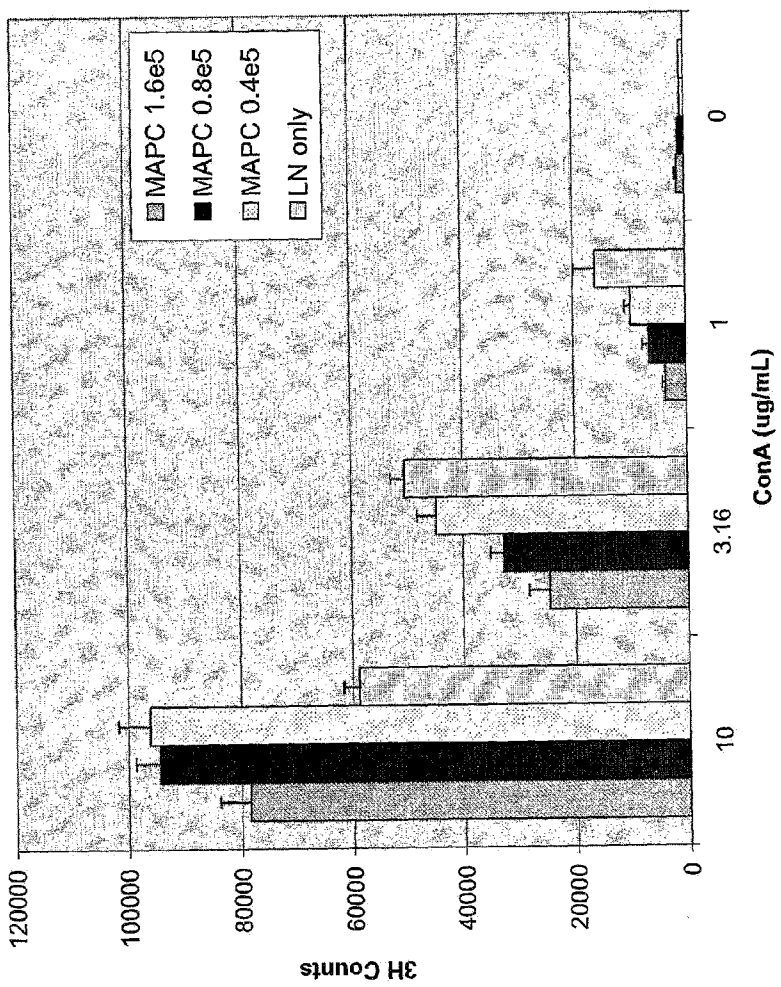

MAPC 1.6e5
MAPC 0.8e5
MAPC 0.4e5
LN only
3H Counts
120000
100000
80000
60000
40000
20000
0
10
3.16
1
0
ConA (ug/mL)

Figure 5A

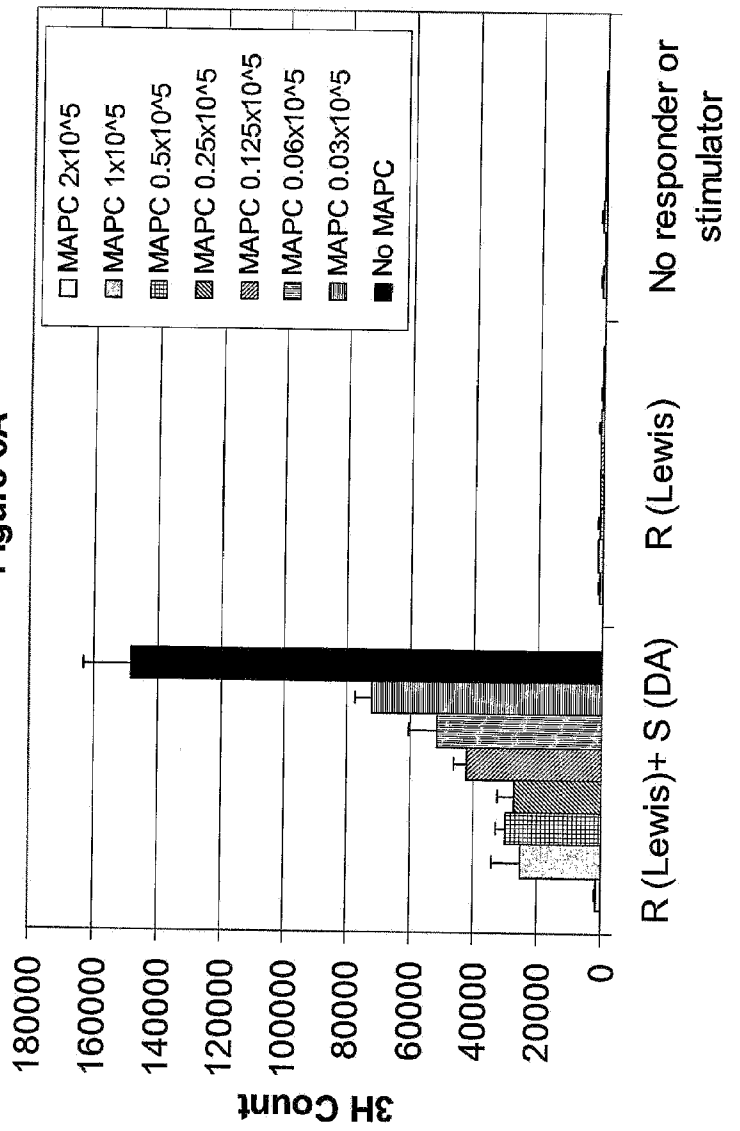
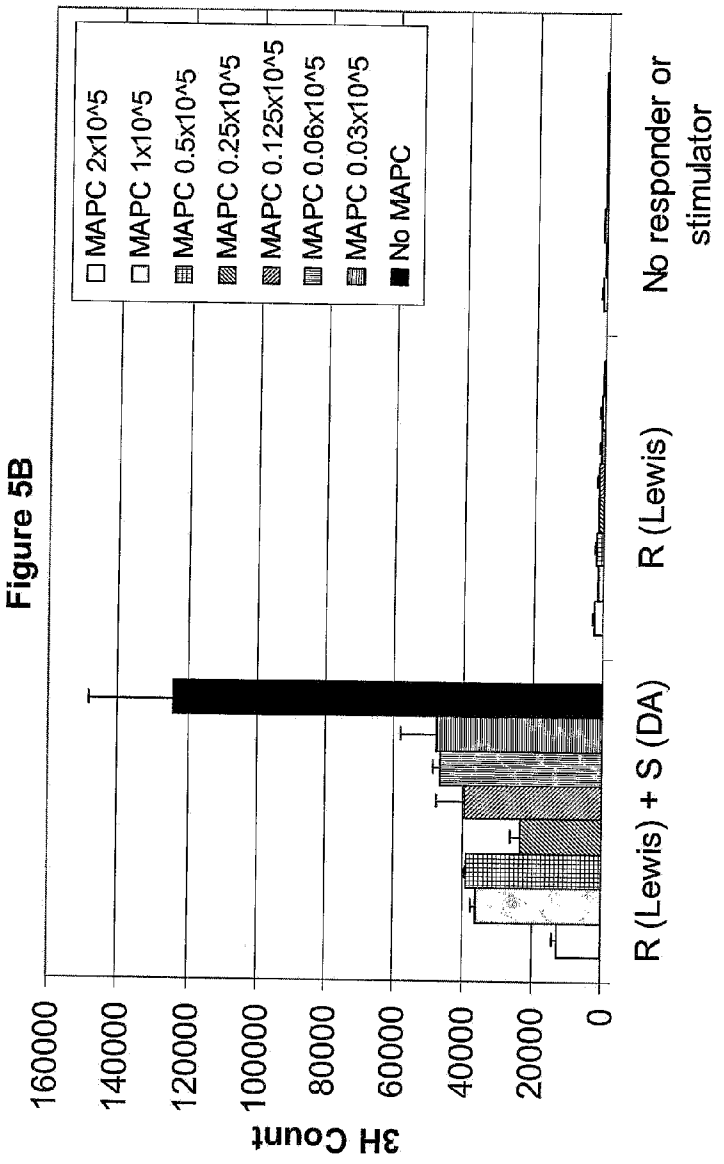

3H Count
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
MAPC 2x10^5
MAPC 1x10^5
MAPC 0.5x10^5
MAPC 0.25x10^5
MAPC 0.125x10^5
MAPC 0.06x10^5
MAPC 0.03x10^5
No MAPC
R (Lewis)+ S (DA)
R (Lewis)
No responder or stimulator

Figure 5B


MAPC 2x10^5
MAPC 1x10^5
MAPC 0.5x10^5
MAPC 0.25x10^5
MAPC 0.125x10^5
MAPC 0.06x10^5
MAPC 0.03x10^5
No MAPC
3H Count
160000
140000
120000
100000
80000
60000
40000
20000
0
R (Lewis) + S (DA)
R (Lewis)
No responder or stimulator

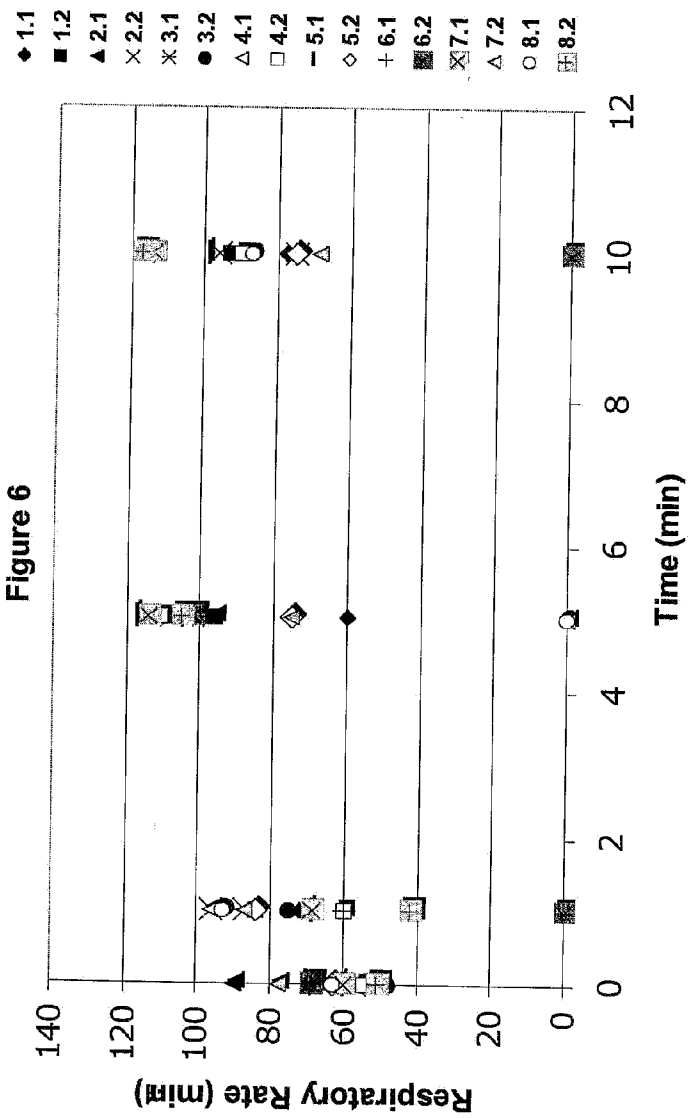

1.1
1.2
2.1
2.2
3.1
3.2
4.1
4.2
5.1
5.2
6.1
6.2
7.1
7.2
8.1
8.2
Respiratory Rate (min)
140
120
100
80
60
40
20
0
0
2
4
6
8
10
12
Time (min)

Figure 6

FIGURE 7

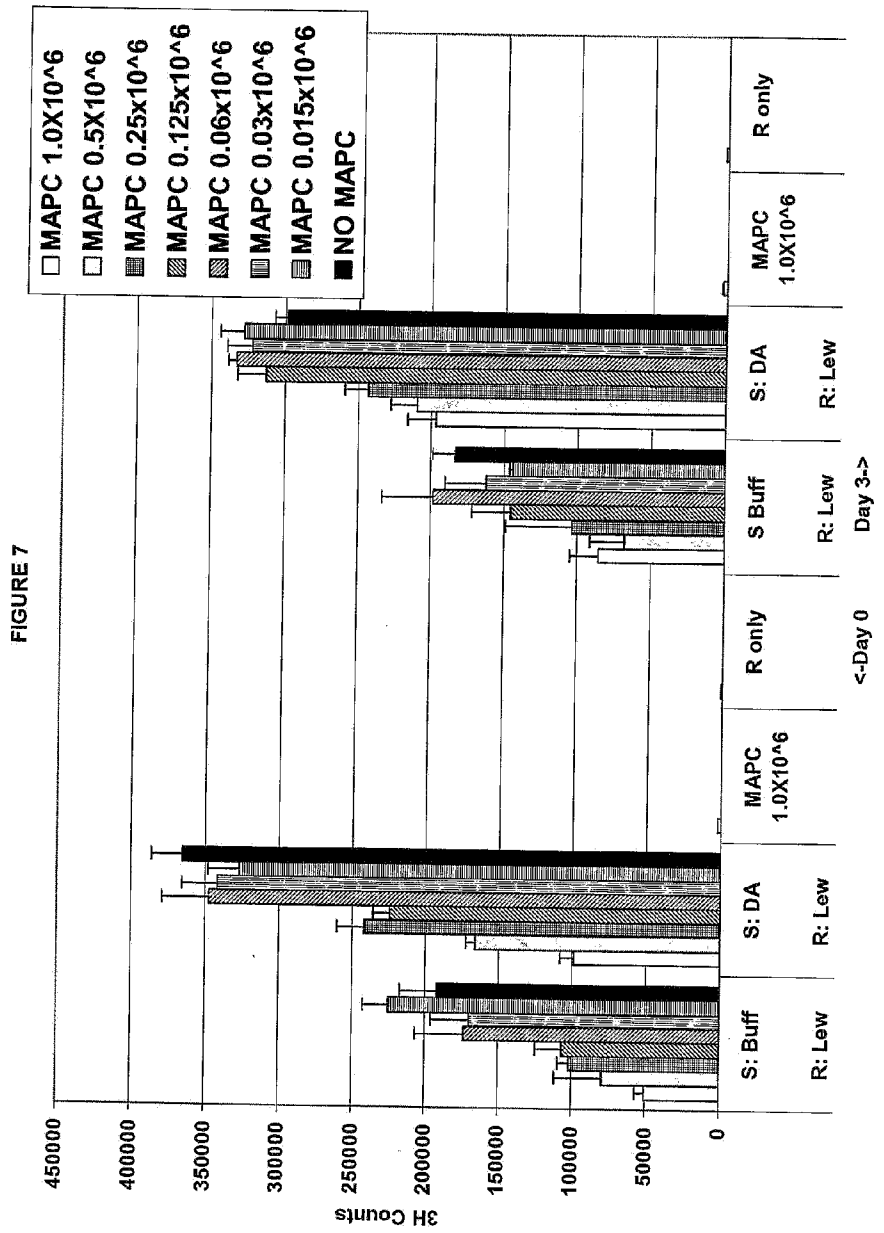

MAPC 1.0X10^6
MAPC 0.5X10^6
MAPC 0.25x10^6
MAPC 0.125x10^6
MAPC 0.06x10^6
MAPC 0.03x10^6
MAPC 0.015x10^6
NO MAPC
3H Counts
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
S: Buff
R: Lew
S: DA
R: Lew
MAPC 1.0X10^6
R only
S Buff
R: Lew
S: DA
R: Lew
MAPC 1.0X10^6
R only
<-Day 0
Day 3->
S: Buff
R: Lew
S: DA
R: Lew
MAPC 1.0X10^6
R only

Figure 8

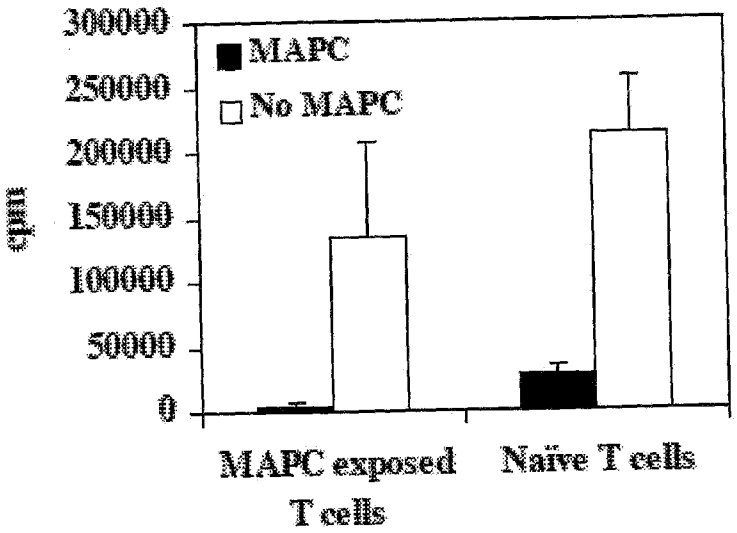
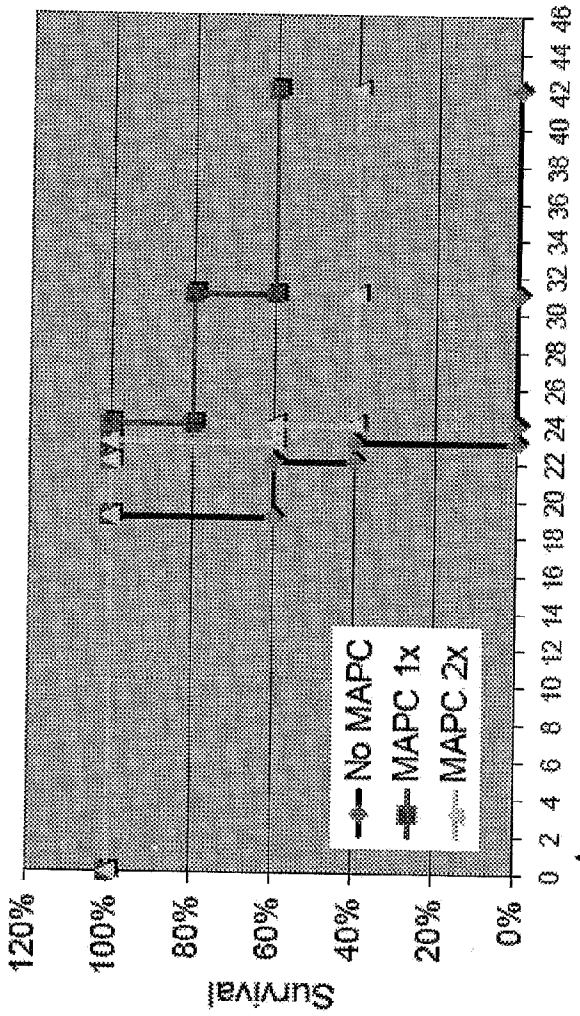

300000
250000
200000
150000
100000
50000
0
cpm
MAPC
No MAPC
MAPC exposed T cells
Naïve T cells

Figure 9


120%
100%
80%
60%
40%
20%
0%
Survival
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days
No MAPC
MAPC 1x
MAPC 2x
MAPC