# INDUCTION OF IL-12 USING IMMUNOTHERAPY

## FIELD

**[0001]** This invention relates to therapy using immune cells. More specifically, this invention relates to immune cell therapy that promotes IL-12 production in patients.

## BACKGROUND

**[0002]** The most precise, powerful and safest disease prevention and treatment mechanism known is the natural 'sterilizing' immune response which combines elements of both innate and adaptive immunity to clear the body of a large variety of foreign pathogens without medical intervention. The immune system is designed to 'remember' the cleared foreign antigens in order to quickly mount an immune response upon re-infection. Immune systems, even those of cancer patients, can recognize and mount a response to foreign antigens, such as found on viruses and bacteria, sufficiently enough to completely destroy and eliminate them from the body. The ferocity and specificity of this sterilizing immune response can be witnessed in the manner in which an inadequately suppressed immune system can completely destroy large transplanted organs, such as a kidney, liver or heart, while sparing self tissues. The destructive effect of this immunity against foreign antigens would be beneficial if this effect could be redirected to tumors and/or other antigens that escape due to an insufficient immune response by the patient.

**[0003]** Immunotherapy is dedicated to developing methods to harness, direct and control the immune response against a variety of infectious and noninfectious diseases including cancer. Therapeutic vaccines are a type of immunotherapy designed to educate the immune system. In patients with existing cancers, the vaccines are designed so the patient's immune system recognizes the tumor cells as foreign. If tumors are recognized by the immune system as a foreign pathogen, an immune response could theoretically be elicited which could cause immune cells to destroy large tumors and seek out and destroy metastatic tumor cells wherever they reside in the body. After successful immunotherapy, the ability of the immune system to 'remember' eliminated foreign cells would enable the immune system to eliminate any recurrent cancer cells without any additional treatment, much like the immune system protects against opportunistic infections.

**[0004]** An individual's immune system response to diseases or to disease organisms can be either a Th1 response or Th2 response. In a Th1 response, the CD4+ T cells become polarized toward Th1 cells and conversely, in a Th2 response, the CD4+ T cells become polarized toward Th2 cells. This increasingly popular classification method is referred to as the Th1/Th2 balance. Th1 cells promote cell-mediated immunity, while Th2 cells induce humoral immunity. Cellular immunity (Th1) directs natural killer cells (NK), T-cells and macrophages to attack abnormal cells and microorganisms at sites of infection. Humoral immunity (Th2) results in the production of antibodies used to neutralize foreign invaders. In general, Th2 polarization of CD4+ T cells has been shown to relate to cancer progression in most human and animal cancer studies, while Th1 polarization is correlated with tumor regression and anti-tumor immunity.

**[0005]** The immune response of an individual, Th1/Th2 balance, can be evaluated through the balance of cytokines in the individual. Cytokines are small cell-signaling protein molecules. The term cytokine is used as a generic name for a diverse group of soluble proteins and peptides that act as regulators normally at nano- to picomolar concentrations and which, either under normal or pathological conditions, modulate the functional activities of individual cells and tissues. These proteins also mediate interactions between cells directly and regulate processes taking place in the extracellular environment. Interleukins are a group of cytokines involved in immunomodulation and can be synthesized by a variety of cells in the immune system. There are a number of interleukins, such as IL-2, IL-4, IL-10 and IL-12, and each of these interleukins has a specific role within the immune system.

**[0006]** Th1 cells produce Type 1 cytokines that are involved in inflammatory responses. Type 1 cytokines include, for example, IL-2, IL-12, IL-15, IFN-gamma, TNF-alpha, TNF-beta, GM-CSF and C-C chemokines. Th2 cells produce Type 2 cytokines that are involved in humoral immune responses. Type 2 cytokines include, for example, IL-4, IL-5, IL-6, IL-10, IL-13 and TGF-beta. Th1 and Th2 immune responses are counter-regulatory, such that increased Type 1 responses downregulate Type 2 responses and increased Type 2 responses downregulate Type 1 responses.

**[0007]** IL-12 is a heterodimer composed of a p35 and a p40 subunit. It is produced primarily by Antigen Presenting Cells (APC). IL-12 can also be produced by monocytes and macrophages, dendritic cells and B-cells. IL-12 exerts immunomodulatory effects on T-cells and natural killer cells. Endogenous IL-12 is known to be involved in generating optimal Th1

responses and can play an important role in cell-mediated immunity against intracellular pathogens.

**[0008]** IL-12 has been the subject of intense investigation because it modulates important components of the immune system and has been demonstrated to have dramatic anti-tumor effects in the laboratory and in animal studies. IL-12 has been implicated, for example, in inhibiting growth of human lung adenocarcinoma and acute myeloid leukemia. However, the use of exogenous IL-12 in a therapeutic regimen has been limited by high toxicity in humans.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0009]** Fig. 1 is a graph illustrating the IL-12 level in a patient's plasma over more than a year. Allogeneic, activated Th-1 cells were administered to the patient at various times using various modes of administration.

## DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

**[0010]** The present invention relates to compositions and methods that lead to detectable levels of IL-12 in the plasma of a patient. The present invention includes a composition that, when administered to a patient, can lead to the production of detectable levels of endogenous IL-12 in the patient's plasma, without any significant toxicity. The endogenous IL-12 can surprisingly be detected in patients with cancer. The composition preferably includes allogeneic activated T-cells. T-cells are not capable of producing IL-12, therefore the T-cell composition administered to the patient elicits the production of IL-12 by the patient's own APC.

**[0011]** The present invention also includes methods of inducing production of endogenous IL-12 in a patient by the patient's own immune system. The method includes administering a composition of allogeneic material, preferably allogeneic activated T-cells. The composition may be administered as a single dose or multiple doses. Preferably, the allogeneic activated T-cells are administered in frequent, low doses. The allogeneic cells can be administered by intradermal, intravenous or intralesional routes. Preferably, the frequency is not less than every 3 days. When these compositions are administered, the patient's own immune system can be induced to produce detectable levels of endogenous IL-12 in the plasma, even in a patient with a cancer. Generally, IL-12 is not found in patients with cancer because tumors can inhibit expression of IL-12. Surprisingly, the methods described herein can overcome this inhibition

and create an environment sufficient to induce expression of IL-12 in the plasma for extended periods of time, for example, several months or even a year. Furthermore, the presence of endogenous IL-12 in plasma does not lead to significant toxicity in the patient as does the administration of exogenous IL-12 as medicant.

**[0012]** By endogenous IL-12, it is meant that the IL-12 is synthesized in the patient by the patient's own immune system. Specifically, the IL-12 can be synthesized by the patient's antigen presenting cells (APC). APC can include monocytes and macrophages, dendritic cells and B-cells. By exogenous IL-12, it is meant that the IL-12 is not synthesized by the patient's own immune system. Exogenous IL-12 includes IL-12 isolated and/or purified IL-12 from another individual or IL-12 that is expressed by a DNA construct(s) that includes a gene for IL-12.

**[0013]** Advantageously, the systemic production of endogenous IL-12 in the patient leads to minimal or no toxicity to the patient. The patient may experience transient symptoms such as transient flu-like symptoms. Generally, when exogenous IL-12 has been administered to the patient, toxic effects have limited the use in a therapeutic setting. The ability of the methods described herein to promote endogenous production of IL-12 that can lead to systemically detectable levels of IL-12 in the plasma without toxicity is surprising. This result enables the use of the patient's own immune system to harness the benefits created from the presence of IL-12 toward reduction and/or elimination of tumors and cancerous cells.

**[0014]** The use of these methods can also be applicable to reduction and/or elimination of other diseases that respond favorably to a Th1 environment, specifically to IL-12. Such diseases include cancer, infectious diseases, including chronic viral and intracellular bacterial or mycobacterial diseases, such as hepatitis B, hepatitis C, HIV1, HIV2, HTLV1, HTLV2, HPV, mycobacterium tuberculosis, periodontal disease, and allergic diseases like atopic asthma. In addition, methods to promote the endogenous production of IL-12 can have an anti-aging effects by maintaining cellular immunity. The balance of Th1 to Th2 cells in normal individuals decreases as part of the aging process, making the elderly more susceptible to infectious diseases and cancer. Promotion of endogenous IL-12 production can increase the Th1/Th2 ratio, thus protecting against vulnerability to disease.

**[0015]** The compositions of the present invention generally include foreign antigens, preferably alloantigens. The compositions also include at least one Th1 cytokine and/or at least one DC effector molecule capable of inducing the maturation of DC to produce IL-12. The

therapeutic composition generally includes the at least one Th1 cytokine, and/or the at least one DC effector molecule combined together with the alloantigen. The composition preferably contains living allogeneic activated T-cells that are capable of providing each of the components of the composition in a single cell type. In preferred embodiments, living allogeneic Th1 cells that are activated to produce Th1 cytokines, such as interferon-gamma, tumor necrosis factor-alpha and interleukin-2 and express the DC maturation effector molecule CD40L on the cell surface are used. Alternatively, the three components of the composition could be sourced from more than one cell type. For example, the Th1 cytokines may be sourced from one cell type in a composition and the alloantigen from a separate cell type and the DC effector molecules from a third cell type. Alternatively one cell type could contain any two of the components and a second cell type contain the third. The cell types do not need to be living as long as they provide a source of the necessary components of the composition.

**[0016]** Alternatively, the composition components can be sourced from natural or bioengineered proteins. For example, recombinant or purified Th1 cytokines or DC maturation molecules or alloantigens could be used together or in combination with living cell components. The composition components could be combined on a "chip" or biodegradable platform. The components do not need to be delivered at the same time to a patient, but can be delivered in any sequence.

**[0017]** The alloantigens in the therapeutic compositions must be provided in a manner that the antigen can be engulfed or presented to the immune system in order to be processed and presented to T-cells. The antigen can be a natural part of living cells or can be altered or bioengineered using molecular biological techniques. The antigen can be soluble or immobilized on a surface, an intact part of a living organism or cell, or a part of an attenuated organism. In preferred embodiments, the alloantigens are allogeneic T-cells and in more preferred embodiments, allogeneic activated T-cells.

**[0018]** In one exemplary embodiment, the therapeutic composition includes alloantigens expressed on T-cells. The T-cells are preferably CD4+ T-cells, and more preferably Th1 cells. The Th1 cells can be in-vitro differentiated, expanded and activated from naïve CD4+ precursor cells derived from normal blood donors. Preferably, the cells are in an activated state at the time of administration. Preferably, the cells are activated by cross-linking monoclonal antibodies directed to CD3/CD28 surface molecules. Crosslinking is preferably caused by immobilization

of the CD3/CD28 monoclonal antibodies on a surface. Preferably, the surface is a micro- or nanabead particle. The beads may be biodegradable beads. These cells can produce large amounts of inflammatory Th1 cytokines and express effector molecules on the cell surface, such as CD40L, which serve to promote the development of Th1 immunity by causing endogenous IL-12 production.

**[0019]** In preferred embodiments, the therapeutic composition includes activated allogeneic Th1 cells. These activated Th1 cells can be powerful inflammatory agents. These activated allogeneic Th1 cells and methods for preparing them are described, for example, in U.S. Patent Numbers 7,435,592, 7,678,572, 7,402,431 and 7,592,431 and are incorporated herein by reference. The activated allogeneic Th1 cells are intentionally mismatched to the patient.

**[0020]** A variety of Th1 inflammatory cytokines may be included in the therapeutic compositions. Examples of inflammatory Th1 cytokines include: IL-1, IL-2, IL-6, IL-12, IL-15, IFN-gamma, TNF-alpha, TNF-beta, GM-CSF and C-C chemokines and do not include TGF-beta, IL-4 or IL-10. The cytokine component can be natural or recombinant cytokines or can be bioengineered molecules designed to interact with the receptors for a cytokine. The cytokines may be directly included in the therapeutic compositions. Alternatively, the therapeutic compositions can include living cells or other components that produce and secrete the cytokines. Preferably the cytokines are provided naturally through an activated cell source, as exogenous cytokines tend to be very toxic to patients while endogenous cytokines are not. In some exemplary embodiments, the therapeutic compositions include T-cells in an activated state that are producing and secreting the inflammatory Th1 cytokines and thus, can serve as the source of these cytokines in the therapeutic compositions.

**[0021]** The therapeutic composition can include a factor or factors that cause the maturation of immature DCs. Specifically, maturation factors which promote DC1 cell maturation and IL-12 production leading to interferon-gamma production and Th1 adaptive immunity. DCs are capable of evolving from immature, antigen-capturing cells to mature, antigen-presenting, T cell-priming cells which convert antigens into immunogens and express cytokines, chemokines, costimulatory molecules necessary to initiate an immune response. The types of T cell-mediated immune responses (Th1 vs. Th2) induced varies depending on the activation signals received from the surrounding microenvironment. The ability of DCs to regulate immunity such as anti-tumor and anti-infectious disease immunity is dependent on DC maturation to promote Th1 immunity.

Human DCs are not a homogenous population. Besides inducing anti-tumor immunity, DCs can induce anergy or tolerance. DCs originate from CD34+ hematopoietic stem cells (HSC). Myeloid dendritic cells (DC1) and plasmacytoid DCs (DC2) are the two principal subpopulations of human DCs, and their characteristics vary greatly in phenotype, migration, and function. DC1 cells are effective T cell stimulators, inducing a tumor specific immune response. CD11c+DC1 cells primarily induce Th1 differentiation, whereas DC2 cells, which express the receptor for IL-3 (CD123), mainly promote a Th2 response. Both DC populations are significantly lower in patients with cancer than in healthy donors. DC1 cells produce IL-12 upon maturation and DC2 cells produce IL-10.

**[0022]** Production of cytokines such as IL-10 and IL-12 during the DC maturation process influences DC induction of a Th1 or Th2 immune response. In addition to expressing high levels of antigen-presenting molecules and costimulatory molecules, mature DC must release large amounts of IL-12 in order to stimulate a Th1 immune response. Release of IL-10, blocks the DC maturation process by interfering with up-regulation of costimulatory molecules and production of IL-12, subsequently limiting the ability of DCs to initiate a Th1 response.

**[0023]** A variety of factors can induce maturation of DC to become DC1, IL-12 producing cells following antigen uptake and processing, including: whole bacteria or bacterial-derived antigens (*e.g.* lipopolysaccharide, LPS), inflammatory cytokines such as IFN-gamma, TNF-alpha, IL-1, GM-CSF, ligation of select cell surface receptors (*e.g.* CD40), viral products (*e.g.* double-stranded RNA), Fas engagement on immature DCs, for example, induces both maturation and release of IL-1 beta and IFN-gamma. Ligation of CD40 promotes an up-regulation of the costimulatory molecules B7-1/CD80 and B7-2/CD86 and IL-12 secretion and release of chemokines (*e.g.* IL-8, MIP-1 alpha, MIP-1 beta).

**[0024]** In some preferred embodiments, CD40L is included as a factor for maturation of the DCs. Inclusion of other factors that cause maturation of the DCs is also within the scope of the invention. In some exemplary embodiments, the therapeutic compositions include T-cells in an activated state which express high density CD40L on the surface. CD40L is a potent effector molecule for DC maturation to produce IL-12.

**[0025]** In one exemplary embodiment, the therapeutic composition includes activated allogeneic T-cells, at least one type I cytokine and at least one factor that causes maturation of DCs. Compositions including these components are described, for example, in pending U.S. Patent Application Ser. No. 12/967,910 filed on Dec. 14, 2010 and incorporated herein by reference.

**[0026]** Intratumoral administration of the therapeutic compositions after ablation of some of the tumor cells in order to release tumor associated antigens into the microenvironment can provide a potent adjuvant effect for the maturation of DC to DC1 phenotype which produces IL-12 and promotes development of Type 1 anti-tumor immunity and the down regulation of tumor immunoavoidance mechanisms. Administration of the therapeutic composition can also be accomplished by other methods including, for example, intravenous, intradermal, intrathecal, intraperitoneal, intralesional, intrapleural administration and the like. Preferably, the composition is first administered intradermally, as the skin is rich in immature DC called Langerhans cells. In the presence of inflammatory Th1 cytokines, such as interferon-gamma, tumor necrosis factor-alpha, IL-2 and GM-CSF and a DC maturation factor, such as CD40L, the Langerhan's cells uptake the alloantigen and mature to DC1, IL-12 producing cells. These mature cells migrate to the lymphnodes and promote development of Th1 immunity.

**[0027]** Intradermal injections of the composition can "prime" a patient to become immune to the alloantigen in the composition. Multiple intradermal injections can increase the number of Th1 memory cells specific for the alloantigens in the circulation of the patient, which in turn changes the Th1/Th2 balance. Injection of 1 $x10^6$ cells to 1 x $10^7$ allogeneic activated Th1 cells is a preferred intradermal dose, 1 x $10^7$ cells in 1ml of fluid is the most preferred. The intradermal dosing is preferably repeated multiple times in order to build up the number of circulating Th1 memory cells. The intradermal dosing frequency is preferably about 3-4 injections every 7 days, more preferably every 3-4 days.

**[0028]** In preferred embodiments, the intradermal dosing is followed by an intratumoral dosing of the composition to create an in-situ vaccine. The intratumoral dosing is preferably conducted following the in-situ ablation of some of the tumor cells in the target lesion. The ablation is preferably caused by use of extreme cold (cryoablation) or heat (radiation), but can be also done using a variety of methods including alcohol ablation, chemotherapy and/or

monoclonal antibody drugs. A preferred intratumoral dose is between about 1 x $10^7$ and 1 x $10^8$ cells, most preferably about 3 x $10^7$ cells. It is preferred that a first intratumoral dose be injected immediately following the ablation and a second within about 7 days, preferably within about 3-4 days following the first injection. This process of ablation followed by intratumoral injection of the composition can be repeated as necessary.

**[0029]** The method also preferably includes administering the composition intravenously in order to cause the activation of host immune cells (both innate and adaptive) and their extravasation to sites of inflammation, including tumor locations. The intravenous dose of the composition of allogeneic activated Th1 cells preferably includes about 1 x $10^7$ to 1 x $10^9$ cells, more preferably about 5 x $10^7$ to 1 x $10^8$ cells. The intravenous infusions can be repeated several times, preferably on a monthly basis.

**[0030]** The allogeneic Th1 cells of the composition preferably produce large amounts of the Type 1 cytokines: IL2, IFN- γ, TNF- alpha and GM-CSF. The presence of inflammatory Th1 cytokines in a microenvironment where immature DC are engulfing and processing antigens can help promote maturation to DC1, IL-12 producing DC. IL-12 can stimulate the level of IFN- γ that in turn can lead to promotion of a Th1 immunity. IFN- γ is a pivotal Type 1 cytokine necessary to promote Type 1 anti-tumor immunity. IFN-γ can mediate anti-tumor effects by directly inhibiting tumor cell growth and inducing T cell-mediated anti-tumor responses. IFN-γ secretion can independently contribute to the NK cell response and enhance the NK cell response activated by IL-12.

**[0031]** The preferred medicament containing activated allogeneic Th1 cells can be derived from precursors purified from normal, screened blood donors. The cells should be supplied as a sterile, low endotoxin dosage form formulated for either intradermal or intratumoral injection, or intravenous infusion. The cells may also be formulated for intraperitoneal, intrapleural or epidural infusions. The donors are preferably tested to be negative for HIV1, HIV2, HTLV1, HTLV2, HBV, HCV, RPR (syphilis), and the cells are preferably tested to be negative for mycoplasma, EBV and CMV. In preferred embodiments, the activated allogeneic cells are HLA mismatched with the patient.

**[0032]** The methods of the present invention generally relate to producing detectable levels of endogenous IL-12 in the patient's plasma. The methods include administering the

compositions of the present invention in such a way as to engineer the patient's immune system to produce endogenous IL-12 at detectable levels in the patient's plasma. The methods described herein can increase the circulating numbers of Th1 immune cells in cancer patients, shifting the balance from Th2 environment to a Th1 environment. Additionally, the methods may also include steps that elicit an anti-tumor specific Th1 immunity and/or activate components of the innate and adaptive immune responses to generate a sustained Th1 cytokine environment in order to down-regulate tumor immunoavoidance.

**[0033]** The methods of the present invention can include administering a composition containing a foreign antigen to promote Th1 immunity in the patient against the foreign antigen. The method may also include ablating all or a portion of the tumor that results in at least some tumor necrosis. A variety of methods can be used to generate tumor necrosis in the patient. The method may also involve creating an inflammatory microenvironment in proximity to the site of tumor necrosis, i.e the site of the tumor lesion. In addition, the method can also include activating the adaptive and innate immune cells of the patient to maintain a prolonged Th1 environment. In preferred embodiments, a key component of the method includes the use of a medicant or composition containing activated allogeneic T cells as described above.

**[0034]** Since most human cancer patients generally present with polarized Th2 immunity, the objective of this method of treatment is generally to increase the amount of circulating Th1 cells in cancer patients. The number of circulating Th1 cells can be built up in the cancer patient by administering one of the therapeutic compositions described above to the patient that includes a foreign antigen.

**[0035]** In an exemplary embodiment, the patient is administered activated allogeneic Th1 cells that are injected intradermally. In preferred embodiments, intradermal injections are on a weekly schedule once a week for about 3-4 weeks. In other preferred embodiments, the intradermal injections may be administered multiple times about every 3-4 days. Intradermal injections may be administered every two days or up to a year apart. The injection schedule should be designed to enhance the footprint of Th1 memory cells in circulation. The alloantigens expressed on the foreign cells can stimulate a potent immune rejection response. In addition, the presence of Th1 cytokines in the composition or the expression of Th1 cytokines by the allogeneic cells can provide the inflammatory adjuvant environment necessary to steer the immune response to the alloantigens toward Th1 memory immunity. This can create an increased

pool of Th1 memory cells in circulation in the patient specific for the alloantigens contained within the allogeneic Th1 cells. Multiple administrations can act as booster shots, increasing the number of circulating memory Th1 cells specific for the alloantigens.

**[0036]** In some embodiments, the administration of allogeneic activated T cells may be followed by additional steps to enhance the patient response. These steps can include, for example, ablation of the tumor that causes tumor necrosis along with intratumoral administration of additional allogeneic activated T cells. Additional administration of the allogeneic activated cells intravenously may also be performed. These methods are described in the U.S. Patent No. 7,972,594 to Har-Noy incorporated herein by reference.

**[0037]** The administration of the therapeutic compositions or medicaments using the methods described herein can promote the systemic production of endogenous IL-12 in the patient by the patient's own immune system. The concentration of the endogenous IL-12 in the patient is sufficient that the IL-12 can be detected in the patient's plasma. The detectable levels of IL-12 are endogenous and not a result of any that might be present in the therapeutic composition because generally the components of the composition are eliminated by the patient's immune system in the rejection response elicited by the administration of allogeneic material. In preferred embodiments, the composition contains T-cells which can not produce IL-12. Thus, any of the IL-12 detected in the patient's plasma is a result of the IL-12 produced by the patient's own immune system.

**[0038]** Preferably, the IL-12 is produced by the patient's immune cells, for example, the patient's own monocytes, natural killer cells and dendritic cells. These cells will have matured under the influence of the inflammatory or Type I cytokines generated by the administration of the compositions described herein.

**[0039]** The concentration of the IL-12 in the patient's plasma can vary but is generally at least about 8000pg/ml. The concentration of the IL-12 in the patient's plasma is preferably between about 8000pg/ml to 200,000pg/ml. As described herein, the concentration of the IL-12 detected in the plasma of the patient does not lead to toxicity issues. However, administration of exogenous IL-12 has been known to be toxic to patients. Patients that seroconvert to IL-12 expression in the plasma have an increased survival compared to patients that do not express IL-12 in their serum. The level of IL-12 may not correlate with survival, only the presence of IL-12 is crucial.

**[0040]** The increase in IL-12 is generally detected after a period of time after the administration of the composition. Preferably, after about 3-4 weeks of dosing with therapeutic composition, the IL-12 can be detected in the plasma. There can be a delay in IL-12 seroconversion for about 90-120 days after the administration of the last composition.

**[0041]** The IL-12 in the plasma can be detected by using a variety of methods. IL-12 has two subunits called the p40 and p35 chains and antibodies specific to p40 are preferred for detection. Several methods are available to detect the presence of IL-12. Detection of IL-12 can include, for example, ELISA, and cytokine bead array.

**[0042]** The methods described herein can be suitable for a variety of patients, including humans. The methods may also be used on other mammals.

**[0043]** The present invention also includes methods of treating a disease in a patient. The diseases can include cancerous tumors as described above, hematological malignancies, as well as diseases caused by pathogenic agents. Other diseases that are susceptible to a Th1 response in a patient can also be treated using the methods described herein. The patient is administered the allogeneic composition according to the methods described herein. The patient's plasma is then monitored for the presence of IL-12. The detection of endogenous IL-12 can be indicative of the patient's immune response to the disease. Additional administrations of the therapeutic composition may be performed for maintenance of the IL-12 levels and thereby maintaining the patient's immune response against the disease antigens.

**[0044]** EXAMPLES

**[0045]** This study was performed to monitor the level of IL-12 in the plasma of a patient treated with allogeneic, activated Th1 cells. These activated allogeneic Th1 cells and methods for preparing them are described in U.S. Patent Numbers 7,435,592. The activated allogeneic Th1 cells were intentionally mismatched to the patient.

*Intradermal Injections*-Intradermal injections of activated allogeneic Th1 cells were administered. The cells were suspended in 1ml at a density of $1 \times 10^7$ cells/ml.

**[0046]** *Intratumoral Injections*-Intratumoral injection was administered in the necrotic center of an ablated tumor within one hour of ablation.

**[0047]** Cryoablation was done with the use of a CryoCare-28 Percutaneous Probe System (Endocare, CA, USA). This system used the Joule-Thomson effect to cool the end of a cryoprobe in a closed system. In accordance with the gas coefficient and the dimension of the

nozzle, different gaseous elements generate different thermal exchange events at the area close to the nozzle. Argon gas was used for cooling (-187$^0$C), and helium was used for heating (67$^0$C).

**[0048]** The planned target tumor lesion was identified and located under CT image guidance. A sterile field was created and local anesthesia administered to the planned probe insertion site. A guide probe was inserted percutaneously and verified by CT to be within the target tumor lesion. One or two freeze-thaw cycles were performed. A single probe of 2- or 5-mm was used according to the size of the target tumor. The time of freezing was approximately 5-20 minutes dependent on the achievement of an "ice-ball", visible on CT. Thawing was achieved by input of helium during a period equivalent to the freezing time before the second freezing process was initiated. The procedure requires ablation of a sample of the tumor lesion and does not require complete tumor ablation with tumor-free margins.

**[0049]** The lesion was allowed to cool following the second freezing cycle before injection of the allogeneic activated Th1 cells.

**[0050]** Tables 1, 2 and 3 show the timing of the specific treatments and the level of IL-12 in the patient's plasma on the indicated days. Fig. 1 is a graph illustrating the IL-12 expression by the patient's immune system during the study.

TABLE 1

pt#11

| weeks from BL | day from BL | treatment | IL12 pg/ml |
|---|---|---|---|
| 0 | 0 | Base | 0 |
| 3w+2d | 23 | Cryo+IT+IV | 0 |
| 4w+2d | 30 | post-2nd IV | 0 |
| 14w+2d | 100 | post-IV-B | 0 |
| 16w+1d | 113 | pre-1st ID in ID/IV prot. | 11,317 |
| | 136 | post-3rd IV in ID/IV prot. | 50,725 |
| 21w+1d | 148 | pre-IV-B in ID/IV prot. | 64,117 |
| | 149 | post-IV-B in ID/IV prot. | 60,301 |
| 25w+2d | 177 | Pre-IV-B 60D IV/ID (T) 14APR10 | 151,048 |
| | 178 | Post-IV-B 60D (T) 15APR10 | 88,362 |
| | 179 | 48h Post-IV-B 60D (T) 16APR10 | 135,169 |
| | 180 | 72h Post-IV-B 60D (T) 17APR10 | 79,476 |
| 27w+3d | 192 | F/U | 14,840 |
| | 197 | Pre-IV-B | 8,867 |
| | 198 | Post-IV-B | 10,610 |
| 37w+3d | 262 | F/U D240 | 32,188 |
| 42w+3d | 297 | pre-chemo | 35,115 |
| 46w+3d | 325 | | 35,552 |
| 49w+3d | 346 | | 16,265 |
| 50w+3d | 353 | | 15,584 |
| 52w+1d | 365 | | 16,546 |
| 52w+6d | 370 | Plasma* | 22,626 |

TABLE 2

| treatment | day from BL | IL12 pg/ml |
|---|---|---|
| Base | 0 | 0 |
| Cryo+IT+IV | 23 | 0 |
| post-2nd IV | 30 | 0 |
| post-IV-B | 100 | 0 |
| pre-1st ID in ID/IV prot. | 113 | 11,317 |
| post-3rd IV in ID/IV prot. | 136 | 50,725 |
| pre-IV-B in ID/IV prot. | 148 | 64,117 |
| post-IV-B in ID/IV prot. | 149 | 60,301 |
| Pre-IV-B 60D IV/ID (T) 14APR10 | 177 | 151,048 |
| Post-IV-B 60D (T) 15APR10 | 178 | 88,362 |
| 48h Post-IV-B 60D (T) 16APR10 | 179 | 135,169 |
| 72h Post-IV-B 60D (T) 17APR10 | 180 | 79,476 |
| F/U | 192 | 14,840 |
| Pre-IV-B | 197 | 8,867 |
| Post-IV-B | 198 | 10,610 |
| F/U D240 | 262 | 32,188 |
| pre-chemo | 297 | 35,115 |
| | 325 | 35,552 |
| | 346 | 16,265 |
| | 353 | 15,584 |
| | 365 | 16,546 |
| pre-IV-B | 370 | 22,626 |
| F/U | 374 | 26,405 |
| F/U | 388 | 219,275 |
| F/U | 390 | 155,023 |
| F/U | 394 | 336,141 |
| F/U | 401 | 113,513 |
| F/U | 408 | 92,122 |
| F/U | 417 | 63,357 |
| F/U | 423 | 79,075 |
| F/U | 429 | 48,038 |
| F/U | 436 | 59,471 |

TABLE 3

| Chemo | days fromBL | |
|---|---|---|
| 1st | 296 | start |
| | 301 | stop |
| 2nd | 324 | start |
| | 331 | stop |
| 3rd | 345 | start |
| | 352 | stop |
| 4th | 359 | start |
| | 364 | stop |
| 5th | 408 | start |
| | 415 | |
| | 422 | stop |
| 6th | 436 | start |

**[0051]** Although the present invention has been described with reference to preferred embodiments, workers skilled in the art will recognize that changes may be made in form and detail without departing from the spirit and scope of the invention.

WHAT IS CLAIMED IS:

1. A composition capable of causing the appearance of IL-12 in plasma, the composition essentially consisting of:

a foreign antigen;

at least one Th1 cytokine; and

a DC maturation molecule.

2. The composition of claim 1 where the foreign antigen is an alloantigen.

3. The composition of claim 1 where the Th1 cytokine is one or more of the following: IL-1, IL-2, IL-6, IL-8, IL-15, Interferon-gamma, TNF-alpha, GM-CSF.

4. The composition of claim 1 where the DC maturation molecule is CD40L and/or FasL.

5. The composition of claim 2 where the alloantigen is on a living T-cell.

6. The composition of claim 5 where the T-cells are CD4+ cells or Th1 cells.

7. The composition of claim 6 where the Th1 cell is activated.

8. The composition of claim 7 where the Th1 cells are activated by cross-linking CD3 and CD28.

9. The composition of claim 8 where the activated Th1 cells secrete one or more of the following Th1 cytokines: IL-2, IFN-gamma and GM-CSF.

10. The composition of claim 9 where the activated Th1 cells express the DC maturation molecule CD40L and/or FasL on their surface.

11. The composition of claim 1 where the components are immobilized on a surface.

12. The composition of claim 11 where the surface is biodegradable.

13. The composition of claim 5 where the living T-cells are packaged in a syringe or flexible container.

14. The composition of claim 13 where the cells are at a concentration of $1 \times 10^7$ cells/ml or greater.

15. The composition of claim 14 where the cells are suspended in a non-nutrient media.

16. A method of increasing plasma levels of IL-12 in a patient comprising:

administering a composition comprising of allogeneic cells wherein at least a portion of the cells are activated T-cells;

monitoring the level of IL-12 in the patient; and

readministering the composition if sufficient levels of IL-12 are not detected in the plasma of the patient.

17. The method of claim 16 wherein the detection of IL-12 is not toxic to the patient.

18. The method of claim 16 wherein the activated T-cells are CD4+ cells.

19. The method of claim 18 wherein the activated CD4 cells are Th1 cells.

20. The method of claim 19 wherein the Th1 cells are activated by cross-linking CD3 and CD28.

21. The method of claim 16 wherein administration of the composition increases the level of IFN-gamma in the patient and/or the Th1 response.

22. The method of claim 16 wherein the level of IL-12 in the plasma of the patient is at least about 5000pg/ml.

23. A method of treating a patient with a disease comprising:

administering a composition comprising allogeneic cells wherein at least a portion are activated T-cells, wherein the T-cells are activated by cross-linking one or more agents bound to the cell surface moieties on the T-cells;

monitoring the level of IL-12 in the patient's plasma; and

readministering the composition if IL-12 is not detected in the plasma of the patient.

24. The method of claim 23 wherein the disease is cancer or is a result of a pathogenic infection.

25. The method of claim 23 wherein the readministering is repeated until the concentration of the endogenous IL-12 is detectable.

26. A therapeutic composition for increasing the endogenous levels of IL-12 comprising activated allogeneic T cells wherein administration of the composition leads to detectable levels of endogenous IL-12 in the plasma of a patient without toxicity to the patient.

27. The composition of claim 26 wherein the patient has cancer or an infectious disease.

28. The composition of claim 26 wherein the T-cells are CD4+ cells.

29. The composition of claim 26 wherein the T-cells are activated by cross-linking one or more agents that are bound to the cell surface moieties on the T-cells.

30. The composition of claim 26 wherein the one or more agents are monoclonal antibodies such as anti-CD3 and anti-CD28 monoclonal antibodies.