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Review

Regulatory T Cells, a Potent Immunoregulatory Target for CAM Researchers: Modulating Tumor Immunity, Autoimmunity and Alloreactive Immunity (III)

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Regulatory T (T_{reg}) cells are the major arbiter of immune responses, mediating actions through the suppression of inflammatory and destructive immune reactions. Inappropriate T_{reg} cell frequency or functionality potentiates the pathogenesis of myriad diseases with ranging magnitudes of severity. Lack of suppressive capability hinders restraint on immune responses involved in autoimmunity and alloreactivity, while excessive suppressive capacity effectively blocks processes necessary for tumor destruction. Although the etiology of dysfunctional T_{reg} cell populations is under debate, the ramifications, and their mechanisms, are increasingly brought to light in the medical community. Methods that compensate for aberrant immune regulation may not address the underlying complications; however, they hold promise for the alleviation of debilitating immune system-related disorders. The dominant immunoregulatory nature of T_{reg} cells, coupled with recent mechanistic knowledge of natural immunomodulatory compounds, highlights the importance of T_{reg} cells to practitioners and researchers of complementary and alternative medicine (CAM).

Keywords: alloreactive immunity – autoimmunity – CAM – Cancer – regulatory T Cells – T_{reg}

Behind the Line of Defense: T_{reg} Cell Relations to Self and Allogeneic Bodies

The intricacies of immune system constituents and interrelationships have been recognized, along with a descriptive appraisal of regulatory T (T_{reg}) cell function in relation to allergy and infection (1,2). The objective of this article is (i) to explain T_{reg} cell function in cancer, autoimmunity and alloresponses and (ii) to examine the pathological costs of irregular T_{reg} cell activity. Due to the high frequency and established knowledge of cancer, autoimmunity and allogeneic immunity, these three afflictions will be utilized as models to communicate the significance and relevance of T_{reg} cells to complementary and alternative medicine (CAM).

Enhanced cell- and humoral-mediated inflammatory responses, resulting from autoimmune and allogeneic diseases, destroy tissues, while depressed immune responses to tumor tissue allow for tumor immunity. Recent evidence has served to elucidate the mechanism of action and substantiate the usage of a wide array of traditional herbs, folk medicines, plant-derived polyphenols and other compounds found in nature, that are employed to attenuate complications related to aberrant functioning of immune responses in these diseases (3–9). Of interest to practitioners, researchers and patients of CAM modalities are those compounds that maintain powerful immunoregulatory capacity via direct or indirect action on T_{reg} cells (see Table 1).

Cancer manifestation and severity depends on a number of factors including the location and character of the malignancy as well as occurrence of metastasis. It is mainly a disease of the later years and one of the leading causes of death in developed nations. Traditional therapies for cancer including surgery, chemotherapy and radiotherapy are losing popularity due to gradual development of tumor resistance to therapy and non-specific toxicity toward normal cells (19). New therapeutic options and possibilities with higher specificity, efficacy and safety are desirable.

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Table 1. Biologically active CAM products with immunomodulatory capacity

Natural product	Plant source	Medicinal system	Utility	Mechanism	Reference
Triptolide	*Tripterygium wilfordii* Hook F.	Chinese	Immunosuppression in graft acceptance	Inhibition of NF-κB activation Inhibit maturation and trafficking of DCs and effector cells	(10,11)
Berbamine	*Berberis julianae*, *Berberis poiretii*	Chinese	Immunosuppression in graft acceptance	Decrease ratio of $CD4^+$ to $CD8^+$ cells	(12)
Piperine	*Piper longum* Linn.	Asian and the Pacific Island	Immunostimulation in cancer, especially melanoma	Increase white blood cell count Increase bone marrow cellularity and α-esterase cell population Increase circulating antibodies and antibody cells Inhibit NF-κB	(13,14)
Andrographolide	*Andrographis paniculata*	Indian Ayurveda	Immunostimulation in cancer	Increase lymphocyte proliferation Increase production of IL-2 and TNF-α	(15,16)
Herbkines	Eight species of Oriental herbs	Oriental Medicine	Immunostimulation in cancer.	Enhance T_H1 and T_H2 cytokine production of IFN-γ, TNF-α, IL-2, IL-4 and IL-12	(17)
Sairei-to	Twelve species of medical herbs	Japanese Kampo	Immunosuppression in autoimmune diseases, especially rheumatoid arthritis systemic lupus ethythematosus	Decrease T_H1 cell-mediated inflammation Enhance T_H2 cell functionality Decrease IgG1 levels	(18)

Andrographis paniculata is a medicinal plant of Ayurveda, known as 'kalmegh', which grows abundantly in India and is cultivated in China and Thailand. The phytochemical extracts from the leaves and stems include diterpenes, flavonoids and stigmasterols, granting it a variety of pharmacological activities and potential for usage in traditional systems (16). Andrographolide, a biologically active constituent of *A. paniculata*, is a potential anticancer agent, mediating these effects through the inhibition of cancer cell proliferation and the destruction of cancer cells (16). The proposed mechanisms by which Andrographolide exerts its anticancer effects include direct cell cycle arrest and indirect stimulation of immune system cells. Immunostimulatory activity of andrographolide is evidenced by increased IL-2 and TNF-α production and enhancement of lymphocyte proliferation, resulting in strengthened response and cytotoxic activity of lymphocytes against cancer cells (15,16). The pharmacological activity suggests that andrographolide is good candidate for development as a therapeutic agent or a lead compound in anticancer and immunomodulatory therapeutics (16).

Therapeutics designated for immune suppression in autoimmunity and alloresponses in Graft-versus-Host-Disease (GvHD) and Host-versus-Graft-Disease (HvGD) include total body irradiation, chemotherapy and immunosuppression, via corticosteroids. Each of these treatments is weighted down with a variety of deleterious side effects, primarily increased incidence and severity of infection and abnormal tissue growth (2,20).

Triptolide (TPT) is a biologically active compound that is isolated from the Chinese medicinal plant, *Tripterygium wilfordii* Hook F. (10,11). TPT demonstrates potent anti-inflammatory and immunosuppressive actions inhibiting autoimmunity, allograft rejection and GvHD (11). These effects were previously attributed to the suppression of T cells; however, recent studies of its functions on dendritic cells (DCs), in T cell-mediated immunity, has been explored. Usage of TPT in a model for skin graft rejection in mice, demonstrated that TPT impairs allostimulatory functions, i.e. inhibition of maturation and trafficking of DCs, resulted in the prevention of graft rejection (10). Chemoattraction of neutrophils and T cells by DCs may favor their interactions and the initiation of immune response; therefore, the attenuation of DC frequency and activity by TPT significantly impairs chemoattraction of effector T cells (11).

T_{reg} cells have been demonstrated to suppress antigen-specific T cell responses against tumors and allografts, and implicated in the control of autoimmune diseases (21–24).

CAM benefits from the research done to establish a scientific basis for various CAM treatment modalities, as it lends credibility and, most importantly, offers efficacious treatment options to a large segment of the population afflicted with cancer, autoimmune disease and alloreactive responses. Knowledge of the dynamic relationship between T_{reg} cells and immune system responses to self and allogeneic antigens is essential in order to approach T_{reg} cells as a clinical target for the alleviation of severe complications arising from immune system dysregulation.

Too Much of a Good Thing: Excessive T_{reg} Cell Suppression in Tumor Immunity

Cancer is a category of diseases characterized by the uncontrolled division of cells and the ability of these cells to

invade other tissues through implantation or metastasis. Excessive and uncontrollable cell division is due to mutations in the gene's encoding for protein regulators of cell cycle and mitosis, e.g. proto-oncogenes and tumor suppressor genes (TSG), such that signals for cell growth overwhelm regulatory signals. Mutations may be passed down through genetic inheritance or can be caused by carcinogens, radioactive materials and viral genome insertions. The rate of mutations increases with age, leading to an accumulating reservoir of damaged DNA sufficient to transform a normal cell into a malignant one.

Activation of the aryl hydrocarbon receptor (AhR) on T_{reg} cells induces proliferation and subsequent T_{reg} cell-mediated immune suppression. Carcinogenic hydrocarbons found in cigarette smoke, broiled meats and elsewhere in the environment have the capacity to act as potent activating ligands for AhR. The induction of profound immune suppression via AhR activation can result in tumor development (25,26). Although, these compounds are of great toxicological concern, they offer researchers the ability to elucidate T_{reg} cell manipulation through AhRs.

T cells are essential for the destruction of cancer cells; therefore, inefficient immune responses to cancer cells allow for their preservation (27). Many tumor-associated antigens are normal self-constituents; therefore, they are presumably under the control of T_{reg} cells (28). Since T_{reg} cells are involved in the suppression of T_H1 cell-mediated immune system function, it follows that T_{reg} cells protect tumors from attack. Elevated levels of tumor-specific T_{reg} cells have been found in tumor sites as well as tumor-infiltrating lymphocyte populations, in lung, breast and ovarian tumors and implicated in Hodgkin's lymphoma (27,29). The mechanism for sequestration of T_{reg} cells to tumor tissue, which yields the increased suppression of immune system attack, is apparently due to chemokine ligand 22 (CCL22). CCL22 is secreted by tumor cells and attracts T_{reg} cells from their normal residence, in the lymph nodes, to the tumor tissue area (30–34). The result is significant suppression of $CD8^+$ cells, allowing for tumor immunity and progression (35). Measuring the ratio of T_{reg} cells to total T cells present, in tumor tissue, showed that the higher the ratio, the farther the cancer had progressed and the more dire the prognosis (28). Interestingly, TGF-β secreted by most melanomas could play a critical role, as it is one of the suppressive mechanisms of T_{reg} cells (29). Also naive Th0 cells may develop into T_{reg} cells when exposed to TGF-β (29).

Misidentification of Self as Foe: Dysfunctional Immunoregulation in Autoimmune Responses

Autoimmune disease occurs when there is a breech in the normal processes producing tolerance to self, i.e. the failure to respond to specific autoantigens (28). Antigen presenting cells (APC), either DCs or macrophages, process specific autoantigens that migrate to the draining lymph nodes. In the lymph node region, APCs can present these autoantigens to autoreactive T cells, which have escaped negative selection by the thymus and have evaded peripheral tolerance. This activation of autoreactive T cells leads to their clonal expansion and migration to the specific tissues, where they induce inflammation and tissue destruction (36).

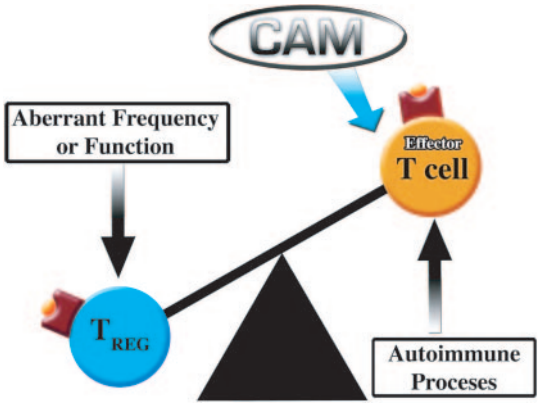

Figure 1. Decrease in T_{reg} cell frequency and/or function leaves autoimmune development and progression unregulated. A number of CAM therapeutics may restore balance through suppression of the autoreactive processes directly or indirectly through T_{reg} cell manipulation.

Central tolerance and peripheral tolerance comprise the two mechanisms by which the immune system hinders the autoreactive T cells from inducing their deleterious functions. Central tolerance is mediated through negative selection. This process entails the elimination, through clonal deletion of autoreactive T cells, during ontogenic development in the thymus (28).

Peripheral tolerance is the backup available if central tolerance fails and autoreactive cells escape the thymus. This mechanism of tolerance occurs continuously throughout life, keeping autoreactive cells in check through immune ignorance, peripheral deletion and active suppression. T_{reg} cells maintain peripheral tolerance and regulate autoimmunity (28,37). Thymically derived T_{reg} cells (nT_{reg}) are the main regulatory cells involved, utilizing an array of TCRs targeted towards autoantigen recognition in order to maintain immune homeostasis in the periphery, and regulate autoimmunity and pathogenic immune responses (22–24). The pathological response of autoreactive effector cells can be suppressed by actions of nT_{reg} cells and the peripherally induced T_r1 and T_H3 cells, each using different mechanisms (28,38,39).

T_{reg} cells mediate the prevention of autoimmunity in two ways. The first involves the prevention of autoreactive T cell priming and differentiation in the draining lymph nodes. Located around DCs in the lymph nodes, T_{reg} cells can prevent the early stages of T cell activation (40). If this early stage of T cell activation is not suppressed, autoreactive T cells migrate to target tissue inducing inflammation and tissue destruction (36,41). The second of T_{reg} cell suppression involves the activation, proliferation and trafficking of T_{reg} cells to the affected tissue to suppress effector cell functions locally. T_{reg} cell activity is confined to the microenvironment where they are activated, due to their antigen-specific nature (23).

Dysregulation in T_{reg} cell frequency or functioning may lead to a number of debilitating autoimmune diseases including, multiple sclerosis (MS), rheumatoid arthritis (RA), myasthenia gravis (MG), autoimmune polyglandular syndrome type II (APS-II), Hashimoto's thyroiditis (HT), type-1 diabetes (T1D), systemic lupus erythematosus (SLE) and autoimmune lymphoproliferative syndrome (ALS) (37,38,42–46) (Fig. 1).

Multiple Sclerosis

Multiple sclerosis is a chronic inflammatory disease characterized by lymphocyte infiltration and inflammation of the central nervous system white matter. Effector T cells specific for myelin protein peptides are involved. Decreased numbers and dysfunction, e.g. low cloning potential in the presence of IL-2, in T_{reg} cells may allow for the over stimulation of $CD4^+$ effector cells upon antigenic challenge, resulting in the production of proinflammatory cytokines and neuronal damage (24,37,42,47).

Experimental autoimmune encephalomyelitis (EAE) is a T_H1 cell-mediated inflammatory disease of the central nervous system, and provides a model of human MS from which exploration of T_{reg} cell functionality in autoimmune detriment is possible. Activation of T_{reg} cells with cognate antigen, proteolipid protein-1 (PLP_1), carried on a proteolipid protein produced an antigen-specific T_{reg} cell capable of suppressing PLP_1 peptide induced EAE in a mouse model (48–50). Thus, there is the suggestion that the induction and activation of peptide-specific T_{reg} cells, by a cognate autoantigen, is essential for the broad immune suppressive functions integral to the attenuation of autoimmunity. Demonstration of the potency of T_{reg} cells, expanded with a single epitope, against autoimmunity is encouraging for CAM researchers to apply this knowledge to patients with MS and other autoimmune diseases.

Rheumatoid Arthritis

Rheumatoid arthritis is a chronic inflammatory disorder leading to the destruction of joint architecture. The pathogenic events leading to the development of RA is not well understood; however, the presence of proinflammatory cytokines plays a key role in the development and maintenance of RA. RA patients' nT_{reg} cells are able to suppress effector T cell proliferation, yet incapable of suppressing TNF-α and IFN-γ production. Suggesting that dysfunction of nT_{reg} cells ability to suppress cytokine production contributes to etiology of RA (42). However, in a number of cases peripheral T_{reg} cells suppress the proliferation of effector T cells, but do not effectively limit proinflammatory cytokine secretion, e.g. anti-TNF-α or anti-IL-17 therapy suppresses inflammation in afflicted patients (43,51).

An increased frequency of T_{reg} cells in directly related to reduced severity of RA. Thus, T_{reg} cell proliferation and activity in the periphery and joints is essential for prevention of rheumatic disease and their dysfunction is implicated in pathogenesis (43).

Autoimmune Myasthenia Gravis

Autoimmune myasthenia gravis is a well characterized autoimmune disease affecting neuromuscular transmission. MG is $CD4^+$ T cell dependent, mediated by anti-acetylcholine receptor (anti-AChR) autoantibodies. MG patients show no difference in frequency and proliferation from normal controls, but exhibit a markedly attenuated ability to suppress effector T cell proliferation. Therefore, the pathogenesis and progression of MG may be dependent upon aberrant T_{reg} cell functioning due to abnormally low levels of FoXP3 production, and subsequent decrease in T_{reg} cell regulatory capacity (43).

Autoimmune Polyglandular Syndrome Type II

Autoimmune polyglandular syndrome type II is a multiple endocrine disease initiated by an autoimmune process. The hallmarks of APS-II include the occurrence of two or more of the following diseases: Addison's disease, T1D or autoimmune thyroid disease. No difference is seen in frequency, surface markers, death rates or FoXP3 expression of T_{reg} cells in APS-II patients; however, there is a significant decrease in suppressor function, resulting in failure to suppress proliferation of effector T cells (42).

Autoimmune Thyroiditis

Hashimoto thyroiditis (HT) is an organ-specific autoimmune disease characterized by lymphocyte infiltration of the thyroid that leads to follicular destruction. Thyroglobulin (Tg)-specific T cells are generated and migrate to the thyroid where they produce IFN-γ, facilitating apoptosis of thyrocytes through caspase activation. The magnitude of the attack on the thyroid increases by means of further expansion and accumulation of activated Tg-specific T cells (52). IL-10 produced by T_{reg} cells, induced by DCs, is essential for suppression of Tg-specific T cell responses, targeted lymphocyte infiltration and follicular destruction (53,54).

Type-1 Diabetes

Type-1 diabetes (T1D) is a chronic T_H1 cell-mediated autoimmune disease that destroys the insulin-producing β cells in the islets of Langerhans within the pancreas, in genetically prone individuals (36,42). A decrease in several immunoregulatory lineages, including natural killer T cells and T_{reg} cells is found in T1D, leaving little suppression of the effector antigen-specific $CD4^+$ and $CD8^+$ T cells involved in the pathogenesis of T1D (36). The deficit of T_{reg} cells is observed in both newly diagnosed individuals and those with long-term conditions. Functional capacity of T_{reg} cells is also needed (42).

Two methodologies have proven effective in the pursuit of utilizing T_{reg} cells to prevent or reverse T1D. Boosting the regulation of T cells via healthy T_{reg} cells has been accomplished through the activation of T_{reg} cells that respond to the

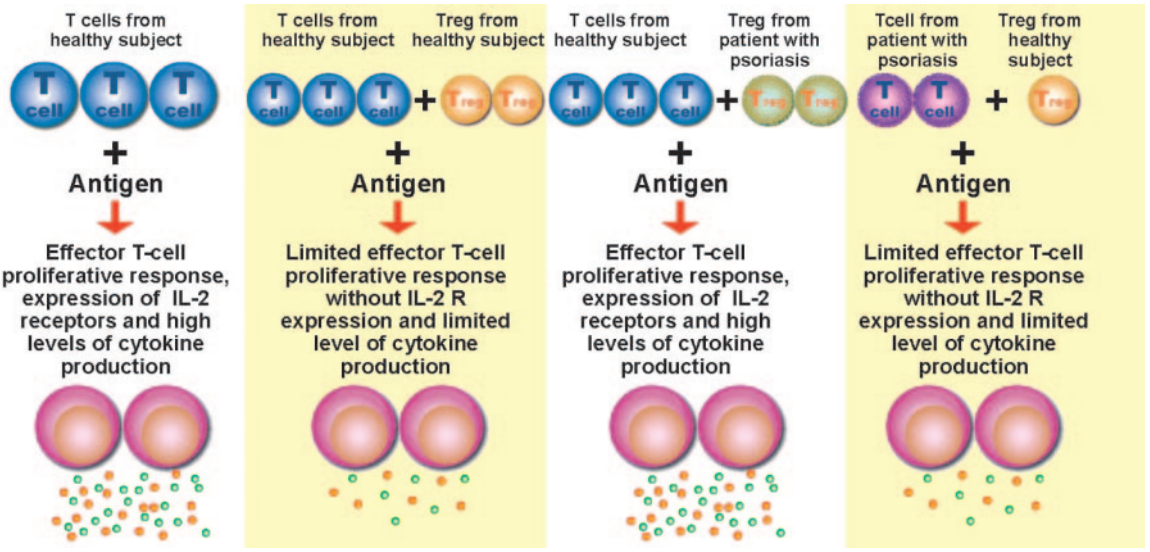

Figure 2. The importance of regulatory T cells in controlling lymphoproliferative response of $CD4^+$ effector cells, expression of IL-2 receptor and level of cytokine production in healthy subjects and patients with psoriasis. These criss-cross experiments were set for testing psoriatic T_{reg} cells in their ability to inhibit normal T cell response, and vice versa.

antigen of islet cells and the adoptive transfer of T_{reg} cells from a non-T1D mouse into a diabetes-prone mouse (55,56).

Systemic Lupus Erythematosus and Autoimmune Lymphoproliferative Syndrome

Systemic lupus erythematosus (SLE) and autoimmune lymphoproliferative syndrome are chronic, systemic, autoimmune diseases demonstrated to involve a decreased frequency of nT_{reg} cells. SLE patients also display B cell hyperreactivity, defects in lymphoid activation processes, aberrant cytokine production and a lower percent of T_{reg} cells in population of peripheral blood mononuclear cells (PBMCs). It is unclear whether T_{reg} cell function is involved in SLE or ALS etiology and pathogenesis (42).

Psoriasis

Psoriasis is an inflammatory skin disease which has many characteristics of a T_H1 cell-mediated autoimmune disease. Activation of autoreactive T cells, and their cytokine secretions, trigger keratinocytes to proliferate and produce psoriasis (57–61). Dysfunctional T_{reg} cells, with decreased capacity for $CD4^+$ suppression, may be the culprit for the unrestrained T cell proliferation observed (62–65). These findings represent a critical component of organ-specific autoimmune disease and their implications for possible therapeutic manipulation of T_{reg} cells (see Fig. 1).

The role of T_{reg} cells in the active suppression of psoriasis, seen in Fig. 2, may be applied to other autoimmune diseases discussed previously. The great potentiality of adoptive antigen-specific T_{reg} cell transfer impels the research of CAM modalities and remedies to facilitate such processes with efficiency and safety.

Rejecting Valuable Donations: Insufficient T_{reg} Cell Suppression of Allogeneic Reactions

Bone marrow (BM) transplants are utilized to correct myriad afflictions ranging from primary immunodeficiencies to hematologic malignancies. In order for a BMT to be successful it must overcome two alloreactive obstacles: GvHD and graft rejection, also known as HvGD.

GvHD is common to allogeneic BM transplantations. It involves the immunologic attack on the cells and tissues of the recipient by the T cell contaminants contained in the donor BM. GvHD primarily affects the skin, liver and gastrointestinal tract through T cell infiltration of epithelia of these areas. T cells present in the graft recognize the host tissues as antigenically foreign and begin an offensive proinflammatory reaction utilizing TNF-α and IL-1, the conditioning of T cell activation with cytokine production, adhesion molecule expression and maturation and trafficking of effector T cells to the area (66). A variety of host antigens presented to donor T cell major histocompatibility complexes (MHC), in particular human lymphocyte antigens (HLAs), are responsible for the initiation of GvHD. Donor T cells are undesirable as effector cells; however, they are beneficial for graft acceptance by preventing the recipients immune system from rejecting the graft. The dual quality of donor T cells implores research in order to maximize graft acceptance through downplaying GvHD aspects of donor T cells.

Graft rejection is mediated by the host immune system in response to the foreign graft cells (20). Dominant transplantation tolerance to BM and tissue grafts has been induced in mice. The presence or addition of T_{reg} cells can induce antigen-specific tolerance to BM grafts, while reduction of T_{reg} cells may accelerate GvHD and graft rejection. The proposed mechanisms of T_{reg} cell functionality in graft acceptance

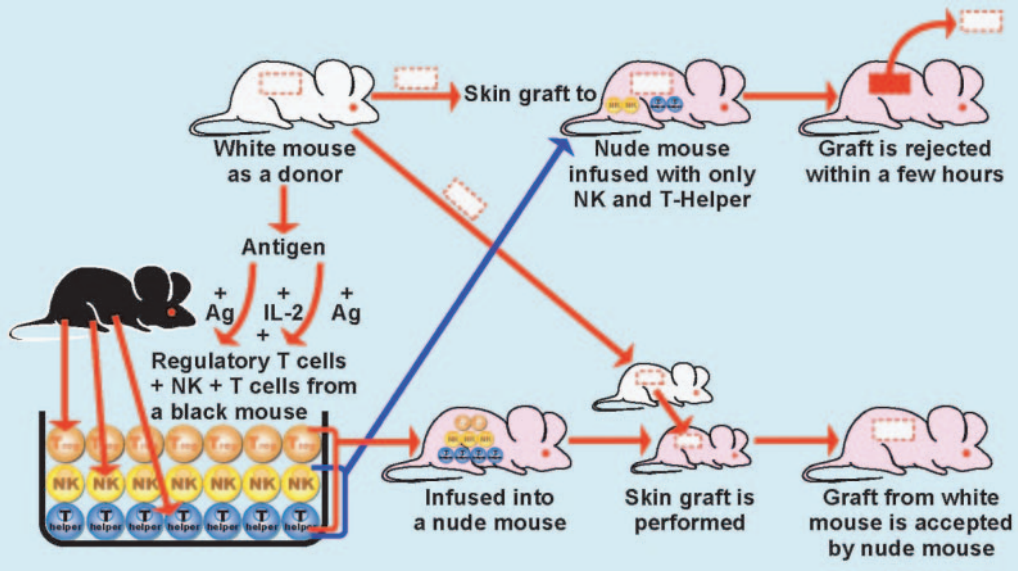

Figure 3. The importance of regulatory T cells in the prevention of organ rejection.

include IL-10 secretion and IL-2 receptor-mediated suppression of donor T cell expansion (66). Co-injection of donor BM and T_{reg} cells, stimulated with donor-type APC and high levels of IL-2 *ex vivo*, into mice, resulted in long-term alloantigen-specific protection of the BM graft (20).

In a similar fashion, T_{reg} cells promote conditions conducive to long-term acceptance of allografts (67). Activation of T_{reg} cells could result in the acceptance of donor organs as seen in Fig. 3. Culturing of T_{reg} cells with IL-2 and antigen from a donor mouse produced antigen-specific T_{reg} cells. These cells were infused into skin graft recipient mice, preventing skin graft rejection even though the mice were also infused with NK and T cells (68,69). Graft recipient mice, without introduction of antigen-specific T_{reg} cells, rejected the skin graft. The observed induction of specific tolerance occurred with just one injection of antigen-specific T_{reg} cells. This shows the possibility the generation of antigen-specific T_{reg} cells, followed by their infusion into a patient, prior to organ transplantation, may encourage success of organ or tissue transplants (70).

The aforementioned problems necessitate the discovery of new methods for immune tolerance, in order to maintain the life saving benefits of BM grafts without the potential complications (20).

Mechanisms of Self Preservation: Integral Functions of T_{reg} Cells for Maintenance of Immune Harmony

Innovative research of T_{reg} cell involvement in various pathologies related to autoimmune and alloimmune responses elucidates the mechanisms involved in disease pathogenesis and, subsequently, identifies plausible means for ameliorative therapies. It is apparent that the T_{reg} cell quantity and activation state are integral and equally important factors in the development and progression of autoimmunity, alloimmunity and cancer (71). Increased research is necessary in order to determine T_{reg} cell functioning in relation to individual disease states. This will afford CAM researchers insight into the appropriate means of approaching a variety of human disorders with respect to T_{reg} cells.

T_{reg} cell's essential role in the management of immune response to specific auto, allo and tumor antigens has been detailed. Harmony between regulatory and effector arms of the immune system is a necessity for good health (1,2). T_{reg} cell intricacy and specificity to individual antigens impels further research and highlights T_{reg} cell's overall importance to human health and CAM. The conceptual framework laid down provides a solid basis from which to explore the diversity of therapeutic methodologies possible for the direct manipulation of T_{reg} cells to attenuate hypersensitivity, cancer, infection, autoimmunity and alloimmunity.

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Conflict of Interest

Aristo Vodjani is co-owner of Immunosciences Lab, Inc. and Jonathan Erde is an employee of Immunosciences Lab Inc. They declare no conflict of interest.

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