

Review

Regulatory T Cells, a Potent Immunoregulatory Target for CAM Researchers: The Ultimate Antagonist (I)

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Over the past decade, great interest has been given to regulatory T (T_{reg}) cells. A vast body of evidence has shown the existence and highlighted the importance of T_{reg} cells in the active suppression of immune system responses. This form of immunoregulation is the dominant means utilized by the immune system to reach a harmony between reciprocal response processes in order to ensure adequate host defense with minimal host detriment. Therapeutically targeting T_{reg} cells is a direct and powerful means to manipulate the immune system to achieve beneficial effects on various disease pathologies, including allergy, autoimmunity and cancer, as well as the facilitation of organ transplantation. This powerful target for immunoregulation is of much concern to practitioners and researchers of complementary and alternative medicine because it allows a great deal of control and certainty in dealing with the prevalence of debilitating immune system-related disorders for which there has been little remedy outside of Western Medicine.

Keywords: immune system modulation – regulatory T cells – T_{reg}

Setting the Stage: Establishing the Pertinence of T_{reg} Cells to CAM

This review will familiarize complementary and alternative medicine (CAM) researchers with regulatory T (T_{reg}) cells and their important role in maintaining homeostasis in immune system functioning. The goal of this work is 4-fold: (i) to give an overview of the complex human immune system, (ii) to assess the methodologies for T_{reg} cell manipulation, (iii) to present the implications of T_{reg} cell manipulation for therapeutic uses, and (iv) to emphasize the relevance T_{reg} cells have to CAM practitioners and researchers.

A variety of homeopathic remedies have been utilized for hundreds of years, without any scientific evidence of efficacy, for the treatment of immune system-related disorders (1). Modern technologies have afforded researchers the ability to identify a number of immunomodulatory compounds, isolated from plants and fungi, and to elucidate their mechanism of

action (2–6). A great deal of these compounds have been shown to stimulate or suppress the immune system via direct or indirect T_{reg} cell modulation.

The Brazilian folk medicine, *sucupira* seed, which has been used for attenuation of inflammation, has shown clinical and immunomodulatory effects in collagen II-induced arthritis in mice. Suppression of B cell and CD4 T cell activation, by *sucupira* seed extracts, lends evidence for its usage in chronic inflammatory diseases (7). Beta-sitosterol (BSS) and sitosterolin (beta-sitosterol glucoside, BSSG) are found in leaf extracts. The combination of the two proves to have profound anti-inflammatory effects and immunomodulatory actions. Various natural products, including Ginko and Cat's Claw, contain phytosterols; however, it was found that a standardized mixture, with a ratio of 100 : 1 of sterols to sterolins, is optimal in restoring appropriate immune system responses (1,8). *Sucupira* seed extracts and plant sterols and sterolins have been found to not only alleviate symptoms of inflammation, but more importantly, repair the underlying immune system abnormality.

Agaricus blazei Murill, an edible fungus used in traditional medicine, has stimulating immune system properties.

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Experimental results reveal that the hemicellulase-derived mycelia extract induces the expression of interleukin 12 (IL-12), a cytokine involved in the generation of T_H1 cells, and the enhancement of natural killer (NK) cell activity (9). An alpha-D-glucan polysaccharide from the medicinal plant *Tinospora cordifolia* stimulates the T_H1 pathway-associated cytokine profile. Stimulation of T helper cell differentiation into T_H1 cells gives an added boost to cell-mediated immunity (10). The stimulation of cell-mediated immunity by both of these natural products may be useful in the successful immune defense against infection.

The attenuation of inflammation and the enhancement of cell-mediated immunity presented above are just two of several potential therapeutic means to treat disease pathology using natural products that modulate the immune system (1,4,11). Myriad compounds from nature have recently been investigated as to their capabilities for immune stimulation or suppression, for usage in infection, autoimmunity, allergy and organ transplant facilitation (6) (Tables 1 and 2).

This review series will commence with an overview of the development, the actions and the interactions of the constitutive components responsible for the functioning of the human immune system.

A Multifaceted Cast: The Delicate Structure and Commanding Functions of Host Defense

The body is protected by a diverse army of cells and molecules that work in concert to ensure survival through the evolution of both innate and adaptive immune mechanisms. The ultimate target that can trigger all immune responses is an antigen, which is usually a foreign macromolecule. In the presence of antigens, different cells of the immune system initiate an elaborate biosignaling pathway which leads to the production and proliferation of appropriate T cells and the most efficacious host defense response (25,26).

Stimulated Mobilization: Initiation and Mediation of the Immune Response

Initial immune responses to any antigen require that the antigen be recognized by a T lymphocyte (T cell). The surface of T cells displays T cell receptors (TCRs), which bind to a complex displayed at the surface of an antigen-presenting cell (APC), which include dendritic cells (DCs) and macrophages. Two means exist for antigen recognition. For exogenous antigens, APCs engulf antigen by endocytosis or phagocytosis,

Table 1. Natural products primarily modulating immune system cells

Active ingredient	Source	Mechanism of action	References
Fish oil	Fish	Regulation of T _{reg} cells Production of IL-10, TNF- α Suppression of NF- κ B activation Suppression of COX-2, IL-2 and IL-6	(12,13)
Curcumin (diferuloylmethane)	Turmeric (<i>Curcuma longa</i>)	Activation and regulation of dendrite cells Inhibit production of IL-1- β , IL-6 and TNF- α Inhibition of NF- κ B activation Blocks IL-12 signaling Expression of Δ F508 cystic fibrosis transmembrane conductance regulator	(14,15,16)
Bromelain (other hydrolytic enzymes)	Pineapple stems	Blocks activation of extracellular regulatory kinase-2 in T cells Inhibition of IL-2, IFN- γ and IL-4 expression	(17,18)
Vitamin-C and vitamin-E	Citrus fruits	Generation of T _{reg} cells Inhibition of NF- κ B activation	(19)

Table 2. Natural products modulating cytokine production and activation

Active ingredient	Source	Mechanism of action	References
Resveratrol	Skin of red grapes	Inhibition of NF- κ B activation	(20)
Royal jelly	Secretion by worker bees	Inhibition of TNF- α and IL-6 production	(21)
(-)-Epigallocatechin gallate	Japanese green tea	Inhibition of TNF- α production Inhibition of DNA damage and lipid peroxidation	(22)
Boswellic acid	Gum resin of <i>Boswellia serrata</i> tree	Downregulation of TNF- α expression Inhibition of NF- κ B signaling	(23)
N-acetylcysteine	Synthetic	Inhibition of NF- κ B activation	(24)

degrade it into fragments and display the antigenic peptides within a transmembrane class II histocompatibility molecule, located on the cell surface. These antigens may be recognized by CD4⁺ T cells. Antigens that are generated within a cell, e.g. viral proteins, are degraded into fragments and displayed at the cell surface within a class I histocompatibility molecule. These may be recognized by cytotoxic CD8⁺ T cells, which have the ability to destroy the infected cell.

Chemokine Attraction: The Beacon for Recruitment and Organization

Recruitment of appropriate immune system cells to the site of action is aided by chemokines, which are cytokines that attract leukocytes. Chemokines bind to chemotactic cytokine receptors (CCRs) on the plasma membrane of leukocytes. Logically, cell-mediated immune cells and humoral immune cells express different CCRs. T_H1 cells and macrophages express CCR5, while T_H2 cells, eosinophils and basophils express CCR3.

Response Reinforcement: Bolstering and Amplifying Immune Response through T cell Proliferation

In order for the immune system to mount an adequate defense, it must produce more T cells, through a process of differentiation and proliferation. Monocytes in peripheral tissues, upon antigen encounter, secrete monocyte growth factor, which activates monocytes to develop into macrophages. In turn, these macrophages, in the presence of bacterial antigens such as lipopolysaccharides (LPS), trigger the maturation of DCs. Matured DCs migrate into the lymph nodes to spread the carried antigen, through interactions with lymph node resident DCs. Increased concentration of bacterial antigen and cytokines in the lymph nodes, over time, stimulate activated DCs to interact with naïve helper T cells (T_H0) (27–30). Depending on various factors related to the immune response required, the end result of this cascade will be the differentiation of T_H0 into T_H1, T_H2 or T_{reg} cells, followed by clonal proliferation of that T cell subtype (31).

T_H1 cells are responsible for cell-mediated or innate immunity, while T_H2 cells are responsible for humoral or adaptive immunity. Both T_H1 and T_H2 cells are under the regulatory influence of T_{reg} cells (Fig. 1).

Cell-Mediated Immunity: The Immutable Guard

T_H1 cells are T lymphocytes that belong to the CD4⁺ subset. They participate in cell-mediated immunity which is essential for controlling intracellular pathogens like viruses and certain bacteria. Antigen presentation by DCs to the TCR of premature CD4⁺ T_H cells coupled with secretion of IL-12, initiates T_H1 cell production. IL-12 stimulates T_H1 cells to secrete their own lymphokines, including tumor-necrosis factor-beta (TNF-β) and interferon-gamma (IFN-γ). These cytokines stimulate macrophages and the recruitment of leukocytes to the site, resulting in inflammation. T_H1 cell action together with NK cell and macrophage activity maintain a complex signaling

cascade whose vital goal is the effective destruction of target cells, especially virally infected or stressed host cells (32–35) (Fig. 1). In order to reinforce the T_H1 immune responses, the T_H2 responses are suppressed in two ways. First, IFN-γ and IL-12, secreted by T_H1 cells, inhibit the formation of T_H2 cells. Second, IFN-γ inhibits class-switching in B cells.

Humoral Immunity: The Plastic Line of Defense

The humoral immune system affords the ability to adequately protect the host from unanticipated pathogens. This system is made possible through the generation of a plethora of receptors which can recognize a wide array of antigens.

T_H2 cells are produced in a manner similar to T_H1 cells, but with different paracrine stimulants/cytokines. T_H2 cells provide help for B cells and are essential for antibody-mediated immunity, which is needed to control extracellular pathogens. B cells have specialized receptors which bind and engulf soluble antigens through receptor-mediated endocytosis. The antigen is digested into fragments which are then displayed at the cell surface within a class II histocompatibility molecule. T_H2 cells with complementary TCRs bind the B cell and secrete lymphokines that induce B cell differentiation into a clone of plasma cells that secrete identical antibodies. These antibodies recognize and bind to an epitope on an antigen and trigger a constructive response to the antigen (Fig. 1).

T_H2 cells secrete four major lymphokines. Interleukin 4 (IL-4) stimulates class-switching in B cells to promote synthesis of IgE antibodies and promotes premature T_H cells to enter the T_H2 pathway while simultaneously inhibiting the T_H1 differentiation pathway. Interleukin 5 (IL-5) recruits and activates eosinophils at the site of action. Interleukin 10 (IL-10) inhibits IL-12 production by DCs therefore adding another inhibitory mechanism to the T_H1 differentiation pathway. Like IL-4, interleukin 13 (IL-13) also promotes the synthesis of IgE antibodies (36–38).

Reciprocal Inhibition: Rudimentary Mechanisms of Regulation

A balance between reciprocal responses, T_H1 and T_H2, is necessary for the immune system to achieve homeostasis. Each division, humoral immunity and cell-mediated immunity, has the ability to suppress the other's functioning, through inhibitory pathways. Also, there is evidence that each response is capable of self-inhibition, through negative feedback, when their respective cytokine production is high. This constitutes a system of checks-and-balances which ensures that neither type of immune response is uncontrollable. However, T_{reg} cells have a much broader and more dominant influence over both kinds of immune defense.

Regulatory T Cells: Can a Single Cell Type Actualize Harmony?

T_{reg} cells, secreting IL-10 or TGF-β, are a prevailing mediator of immunological tolerance and are responsible for

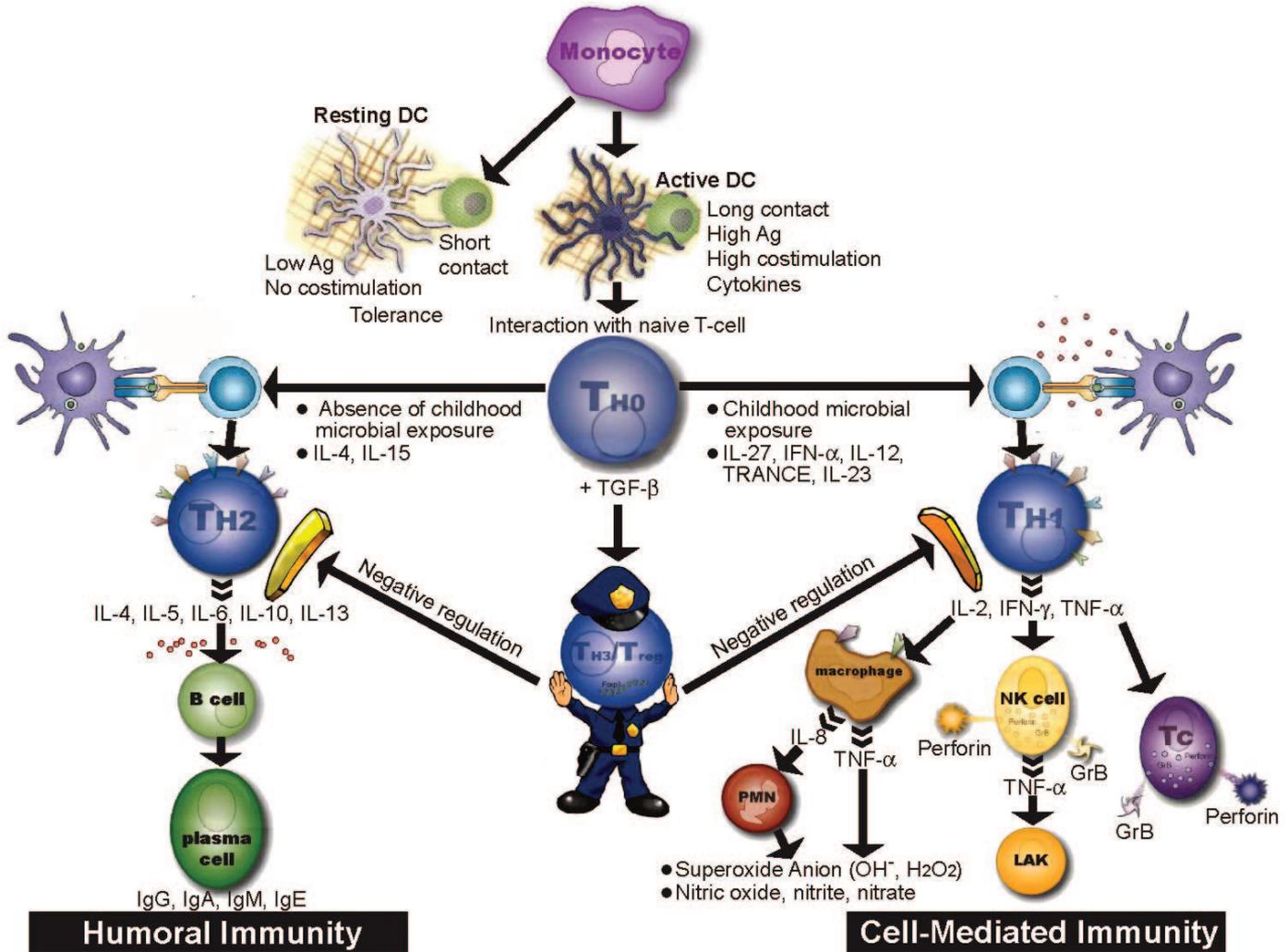


Figure 1. The mediators of immune system responses.

the appropriate control, via termination, of both T_{H1} and T_{H2} cell-mediated immune responses. They are the essential actor in the prevention of disease pathology related to the overaction of either response (39–44).

T_{reg} cells are generated from naïve T cells after antigenic stimulation and presentation by DCs. Various data suggests that T_{reg} cells represent a distinct lineage of T cell development (41). Characteristic expression of the interleukin 2 receptor-alpha (IL-2Ralpha) chain (CD25) and the FOXP3 transcription factor help to differentiate T_{reg} cells as a distinct subset of CD4⁺ T cells (45).

FOXP3 (Forkhead Family Transcription Factor) is essential to T_{reg} cell lineage commitment, development and function. In fact, transfer of FOXP3 into naïve T cells converts them into T_{reg} cells. The expression of FOXP3 has been found to correlate with T_{reg} cells ability to suppress immune responses, indicating a possible function of FOXP3 in the T_{reg} cell-mediated mechanism for the negative regulation of T cell

activation (42,45–51). These developmental and functional observations of FOXP3 indicate its importance in T_{reg} cell function and in regulation of the immune system.

If T_{reg} cells could balance the immune system completely, there would be no occurrence of immune system-related disorders. The prevalence of immune system-related disorders indicates that although T_{reg} cells have a great deal of control, there exist factors responsible for the inappropriate development or functioning of immune responses.

Immunological Development: The Hygienic Foundation of Response Strengthening

According to the hygiene theory, at birth, the human immune system is immature and favors T_{H2}-like cytokine production. A wide variety of biological stimuli, ranging from helminth to viral infections, contracted from siblings or peers, can induce T_{H1}-like cytokine production and effectively balance

T_H1- and T_H2-like cytokine responses and development. Children who have little contact with others or live in 'sterile' urban environments lack this T_H1-like cytokine production and consequently the T_H2-like cytokine response pattern persists unchecked. A bolstering of T_H2 cell development during childhood sacrifices T_H1 development and puts individuals at risk for allergy later in life. The logical progression of this idea is that once the immune system develops fully, individuals will have a far greater capacity for a T_H2 response to antigen, due to a lack of reciprocal suppression from T_H1 cytokine production. This imbalance can result in an increased risk of asthma and other atopic diseases. Suppressed T_H1 development and function may lead to allergic hypersensitivity, while suppressed T_H2 development may result in autoimmune disorders. Thus, great interest has been focused on the biological factors that influence and determine immune system development and differentiation.

Although this view seems sound, it does not take into account the observed rise in T_H1 cell-mediated autoimmune diseases in industrialized nations, nor does it address the inverse relationship between long-term helminth induced T_H2 cytokine response and allergy. Since T_{reg} cells may be implicated in these discrepancies, researchers now postulate that T_{reg} cells are the key to substantiate the Hygiene Theory, which underscores the immense role T_{reg} cells have in proper immune system functioning (52–59).

Given that immune responses may vary according to the environment in which individuals have been raised, the importance of T_{reg} cells in combating this imbalance becomes apparent. In many cases it seems that T_{reg} cell-mediated immunomodulation is not strong enough to overcome this deficiency in development, therefore methodologies to modulate T_{reg} activation and functioning may prove fruitful.

The Stage Has Been Set: T_{reg} Cell Essentiality to the Immune System and CAM

Harmony between the two branches of immunological host defense is imperative to human health. This review assessed the intricate tapestry of humoral and cell-mediated immune functioning, including constituents, interrelationships, feedback mechanisms, and reciprocal control. T_{reg} cells have been shown to maintain a crucial role in keeping the *status quo* through suppression of immune functioning. The wealth of cutting-edge knowledge of T_{reg} cell function in immune system-related disorders, coupled with substantial evidence of natural immune system modifiers, makes this cell an excellent target for investigation and application by CAM researchers. We now have a solid basis for delving into the specifics of T_{reg} cell action in the pathology of disease and exploring the use of T_{reg} cells as a therapeutic target in the treatment of hypersensitivity, cancer, infection, autoimmunity and organ transplant facilitation. Already at the molecular level Ventura (58) has proposed that 'CAM modalities may deeply affect both the signaling and transcriptional level of cellular

homeostasis. Such a perception holds promises for a new era in CAM, prompting reproducible documentation of biological responses to CAM-related strategies and compounds. To this end, functional genomics and proteomics and the comprehension of the cell signaling networks may substantially contribute to the development of a molecular evidence-based CAM'.

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