

## Review

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## Regulatory T Cells, a Potent Immunoregulatory Target for CAM Researchers: Modulating Allergic and Infectious Disease Pathology (II)

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Regulatory T ( $T_{reg}$ ) cells maintain dominant control of immune responses to foreign materials and microbes. Appropriate  $T_{reg}$  cell suppression of immune responses is essential for the maintenance of efficacious defensive responses and the limitation of collateral tissue damage due to excess inflammation. Allergy and infection are well studied and frequent afflictions in which  $T_{reg}$  cells play an essential role. As such, they provide excellent models to communicate the significance and relevance of  $T_{reg}$  cells to complementary and alternative medicine (CAM).

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### $T_{reg}$ Ubiquity and Universality: Relations to Foreign Bodies

The major principles underlying the dynamic immune system, including constituents, interrelationships and feedback mechanisms have been established, with emphasis on regulatory T ( $T_{reg}$ ) cells (1). The goal of this article is (i) to detail  $T_{reg}$  cells, (ii) to delineate  $T_{reg}$  cell function in allergy and infection and (iii) to examine the pathological consequences of aberrant  $T_{reg}$  cell activity. Allergy and infection are well-studied and frequent afflictions, and as such, they provide excellent models to communicate the significance and relevance of  $T_{reg}$  cells to complementary and alternative medicine (CAM).

Considerable inflammation, resulting from allergic hypersensitivity or immune response to infection, has the potential to induce deleterious effects on an individual's tissues and overall well-being. Recent evidence has served to elucidate the mechanism of action and substantiate the usage of a veritable array of traditional herbs, folk medicines and other compounds found in nature, which have been employed to attenuate inflammatory complications. Of interest to

practitioners, researchers and patients of CAM modalities are those compounds that maintain powerful immunomodulatory capacity via direct or indirect action on  $T_{reg}$  cells (Table 1).

A cornucopia of herbal medicines has shown clinical effectiveness in the attenuation of allergic and infection-induced inflammatory pathology. Traditional therapeutics for allergic complications includes immunotherapy, antihistamines and glucocorticoids. A newly researched compound, termed anti-asthma herbal medicine intervention (ASHMI), which is an extract of three herbs, has shown effectiveness and benefit over traditional treatment options for asthma. ASHMI mainly downregulates  $T_{H2}$  cell responses, increases lung function through direct modulation of smooth muscle contraction and decreases peripheral blood eosinophils and serum IgE. ASHMI does not induce a state of general immunosuppression like steroids, such as prednisone, which suppress  $T_{H1}$  and  $T_{H2}$  cell responses.

Treatment of infection with antimicrobial drugs poses an array of complications, including resistance and physical ailments, e.g. diarrhea. *Allium sativum*, extracted from garlic, has the potential to bolster  $T_{H1}$  cell-mediated responses to pathogens, such as *Leishmania major*. The proposed mechanism includes enhancement of  $T_{H1}$  cytokine response, T lymphocyte proliferation and NK cell activity. This method of antimicrobial treatment offers benefits over pharmaceutical drugs; however, its efficacy and mechanism of action needs further research and elucidation.

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**Table 1.** CAM compounds with regulatory capability and utility in Allergy and Infection

Natural product	Activity	Reference
Brazilian green propolis	Modulate initial inflammation Lower parasitemia	(2,3)
<i>Ganoderma lucidum</i> (mycelia extract)	Activate $\kappa$ B DNA binding Increase TNF- $\alpha$ , IL-6, IFN- $\gamma$ Inhibit histamine release	(4,5)
<i>Phellinus linteus</i> (mycelia extract)	Lower TNF- $\alpha$ , IFN- $\gamma$ production Lower IgG levels	(6)
<i>Piper methysticum</i> (kava)	Inhibit TNF- $\alpha$ release Induce NF- $\kappa$ B transcription	(7)
<i>Allium sativum</i> (garlic)	Increase T <sub>H1</sub> cytokine response Enhance macrophage and NK cell activity Increase T cell proliferation	(8)
(-)-epigallocatechin gallate (green tea)	Decrease IL-2, IFN- $\gamma$ , TNF- $\alpha$ levels Decrease T cell proliferation	(9)
Probiotics	Induce T <sub>H1</sub> and T <sub>H3</sub> induction	(10–14)
<i>Lactobacillus casei</i>	Propogates oral tolerance	
BGN4 ( <i>Bifidobacterium bifidum</i> )	Decreases IgA and IgE production	
VSL #3	Dampens NF- $\kappa$ B proinflammatory signals Decreases levels of cell degranulation	
<i>Staphylococcal</i> superantigen B	T <sub>reg</sub> cell suppressor	(2,15)
Ergosterol peroxide ( <i>Tricholoma populinum</i> )	Decreases allergic symptoms	(5)
ASHMI	Decreases TH2 response and cytokines profile	(16,17)
<i>Ganoderma lucidum</i>	Lowers IL-5, IL-13 and IgE levels	
<i>Radix Sophora flavescens</i>		
<i>Radix Glycyrrhiza uralensis</i>		
KRN7000	Immunostimulation	(18)
<i>Agelus mauritanus</i> derivative	Increases IFN- $\gamma$ , IL-4, and IL-12 Enhances NK cell activity	

CAM benefits greatly from research done to determine a scientific basis for confident treatment decisions, as it lends credibility to CAM modalities and, most importantly, offers efficacious treatment options to a large segment of the population afflicted with allergic and infectious complications. Knowledge of the dynamic relationship between T<sub>reg</sub> cells and immune system responses to foreign antigens is essential in order to approach T<sub>reg</sub> cells as a clinical target for the alleviation of complications arising from allergy, asthma, dermatitis and infection.

## T<sub>reg</sub> Subsets: Three of a Kind

The family greatly responsible for immunomodulation consists of two key subsets: naturally arising and peripherally induced T<sub>reg</sub> cells. Although the ontogenic relationship between the two is not well understood, both subsets have been characterized by distinct differentiation patterns and functions (19).

### Naturally Occurring T<sub>reg</sub> Cells

Naturally occurring T<sub>reg</sub> (nT<sub>reg</sub>) cells compose 5–10% of peripheral T cells, maintain a distinct lineage and develop in the thymus. nT<sub>reg</sub> cells are directed to largely aid in tolerance to self-antigen in the periphery via suppressive actions on both T<sub>H1</sub> and T<sub>H2</sub> cell-mediated immune responses, with greater specificity for the former (20–24).

nT<sub>reg</sub> cells inhibitory action is contact dependent. Interaction of CTLA-4 and TGF- $\beta$ 1, expressed on the cell surface, with respective ligands and receptors on target cells, triggers the downregulation of effector cell IL-2R $\alpha$  receptors and a subsequent decrease in fitness (20,23,25). Alternate mechanisms proposed include the perforin-dependent induction of T cell apoptosis and the reduction of dendritic cell's ability to prime T cells, through direct suppression of cytokine secretion or tryptophan metabolism (2,15,26–28).

### Peripherally Induced T<sub>reg</sub> Cells

The adaptive T<sub>reg</sub> cell subset includes type 1 T<sub>reg</sub> (T<sub>r1</sub>) cells and T helper 3 (T<sub>H3</sub>) cells. T<sub>r1</sub> and T<sub>H3</sub> cells are implicated in immune responses to foreign antigens in the periphery, alongside nT<sub>reg</sub> cells (22).

While extrathymic generation of T<sub>reg</sub> cells is not as well understood as nT<sub>reg</sub> cell generation, T<sub>r1</sub> and T<sub>H3</sub> cells are derived from naïve T cells in an environment supporting suitable antigenic and cytokine stimulation (24,29). Located in peripheral lymphoid tissue, these cells produce a distinct cytokine profile upon TCR-mediated activation (22). T<sub>r1</sub> and T<sub>H3</sub> cells mediate immunosuppression through the secretion of IL-10 and TGF- $\beta$ 1 (21,23,30,31). T<sub>r1</sub> and T<sub>H3</sub> cells become significant in peripheral self-tolerance when the pool of self-antigen-specific nT<sub>reg</sub> cells is deficient (31).

### T<sub>reg</sub> Cell Singularity

For the purposes of this review and owing to the fact that definitive comparative research on the subsets is insufficient for precise differentiation, nT<sub>reg</sub>, T<sub>r1</sub> and T<sub>H3</sub> cells will be collectively referred to as T<sub>reg</sub> cells.

## At One with Nature: Modulating Responses of Allergic Challenge

Aberrant immune responses to environmental allergens are relatively common with wide variations in severity and manifestation. Depending on the allergen size and mode of exposure, an atopic individual may experience afflictions ranging from asthma to seasonal allergic rhinitis to atopic dermatitis.

Atopic allergic sensitization involves the overproduction of IgE against environmental allergens, e.g. grass, house dust mites, pollen and animal proteins (30,32). Allergen-specific IgE, on the surface of mast cells and basophils, upon binding to allergen, triggers the release of histamine and mediators resulting in immediate symptomology (33). Often, patients with allergic diseases have a deficient ability to suppress T cell responses to allergen by T<sub>reg</sub> cells (32,34).

T<sub>reg</sub> cell's role in the prevention of sensitization to allergens has recently been explored (32). Evidence of significant T<sub>reg</sub> cell suppressive action on T<sub>H2</sub> cell-mediated immune responses supports the notion that their depletion or functional dysregulation may be responsible for atopic pathology (32).

### Allergy

Seasonal allergic rhinitis, more commonly known as Hay Fever, involves the deposition of allergens on the nasal mucosa followed by an immediate hypersensitivity reaction. Allergens involved, like grass pollen, are typically too large to enter into the lower airways, rendering asthmatic complications unusual. T<sub>reg</sub> cells have the capability to reduce or prevent T<sub>H2</sub> cell-mediated allergic rhinitis and atopic sensitization disorders (32,34).

Allergen-specific T<sub>reg</sub> cells are also modulators of immune response to dietary allergens and intimately involved in the development of oral tolerance. Karlsson *et al.* illustrates T<sub>reg</sub> cell functionality in an experiment utilizing children with allergy to cow's milk (35). A majority of the allergic children developed oral tolerance to milk following a period of milk restriction. Development of  $\beta$ -lactalbumin-specific T<sub>reg</sub> cells, in those children who developed tolerance to cow's milk, was found to be responsible for the newfound tolerance. Induction of tolerance to allergen, via antigen-specific T<sub>reg</sub> cell generation, is noted in individuals encountering pollen, dust mites and other environmental antigens (2,32). This signifies the malleability of immune system response and balance afforded by antigen-specific T<sub>reg</sub> cells (35,36).

Taken altogether, the response of an individual to allergen encounter is dependent on many factors, including the quantity and activity of antigen-specific T<sub>reg</sub> and the T<sub>H2</sub> effector populations (36).

### Asthma

Asthma is a chronic airway inflammatory disease triggered by allergic exposure and hallmarked by airway inflammation, bronchial hyperreactivity, lung eosinophilia and excessive T<sub>H2</sub> cytokine production (28,34).

Inappropriate T<sub>H2</sub> cell response to inhaled allergens elicits airway inflammation. The complex biosignaling cascade resulting in asthma manifestation is mediated by the over production of IL-4, IL-5, IL-9 and IL-13, which serves to regulate IgE production and to sequester effector cells to the airway. Effector cells that are directed towards airway tissues enhance airway inflammation and hyperresponsiveness through the generation of additional proinflammatory cytokines and

autocoids (32,37). In many cases, asthma pathology is due to an excess of T<sub>H2</sub> cell quantity or activity, leading to a skewing towards a proinflammatory cytokine profile (28).

Mouse models of airway inflammation allow for the examination of allergen-specific T<sub>reg</sub> cells activity *in vivo*. The transfer of ovalbumin (OVA) peptide-specific T<sub>reg</sub> cells to OVA sensitized mice reduced T<sub>H2</sub> type cytokine expression in the lungs, airway hyperreactivity and effector cell recruitment. The ameliorative effects were dependent upon IL-10; however, T<sub>H2</sub> cells were the source of IL-10 secretion, rather than T<sub>reg</sub> cells (34). T<sub>reg</sub> cells may reduce inflammatory response through a contact-dependent manner, e.g. enhancing the secretion of IL-10 by T<sub>H2</sub> cells.

T<sub>reg</sub> cells aid in the suppression of inflammatory responses to inhaled antigen and are essential for the induction of allergic tolerance. Administration of inhaled allergen, with prior induction of allergen-specific T<sub>reg</sub> cells, prevents allergen sensitization and airway inflammation upon later exposure. Thus, immunotherapy offers a powerful function based on the modulation of allergen-specific T<sub>reg</sub> cell suppression of T<sub>H2</sub> responses (32).

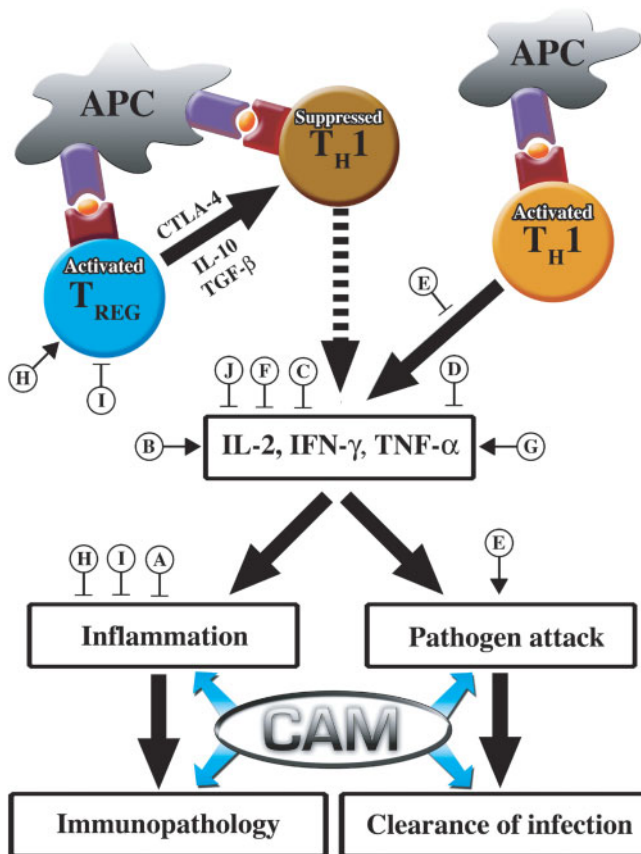
### Dermatitis

Atopic dermatitis is a chronic inflammatory skin disorder in which T<sub>H2</sub> effector cells migrate to the dermis, and under IL-12 conditions, become T<sub>H0</sub> or T<sub>H1</sub> cells. In atopic individuals, a dysregulation of effector T cells and an impairment of T<sub>reg</sub> cell suppression are involved in the development of inflammatory pathology (38). While various treatment options exist, including the use of steroids, antihistamines and aggregative factor elimination, an increasing number of patients are finding little relief and are requiring other therapeutic modalities, some of which may be derived from CAM (39).

### Throwing Water on the Inflammation: T<sub>reg</sub> Cell's Delicate Relations to Infectious Pathology

To deal with microorganisms, the body has evolved intricate defense mechanisms. The process of pathogen control involves the recruitment of immune system cells to the site of infection. Necessary components include inflammatory cells, cytotoxic T cells and NK cells. The generation of antigen-specific T<sub>reg</sub> cells is a crucial regulatory element in the immune response to infection by bacteria, parasites, fungi and viruses, as well as the fostering and maintenance of tolerance to non-pathogenic microbes (21,40). Dysregulation of T<sub>reg</sub> cell-mediated anti-inflammatory pathways poses potentially great risks to health (41).

Effective immune response to pathogen is often accompanied by a great deal of inflammation. In excess, this inflammation can cause collateral tissue damage and pathology that necessitates the activation and proliferation of pathogen-specific T<sub>reg</sub> cells (19,21,42–44; Fig. 1). Conversely, a decrease in defense responsiveness owing to underlying



**Figure 1.** Modulation of  $T_H1$  cell immune response by  $T_{reg}$  cells and CAM. Sites of action throughout the  $T_H1$  cell-mediated inflammatory pathway. A, Brazilian green propolis; B, *Ganoderma lucidum*; C, *P. linteus*; D, *Piper methysticum*; E, *Allium sativum*; F, (-)-epigallocatechin gallate; G, KRN7000; H, probiotics; I, *Staphylococcal* superantigen B; J, Ergosterol peroxide.

immunosuppression renders the host susceptible to pathogenic infection and subsequent harm. Depending on a number of variables, a reconstitution of immune system responses or  $T_{reg}$  cell populations may be necessary *in lieu* of appropriate antimicrobial or inflammatory therapy.

### Bacterial Infections

IL-10 secreting  $T_{reg}$  cells attenuate bacterial-induced abnormalities caused by massive inflammation. This immunosuppressive cytokine is imperative for dampening excessive inflammation, owing to  $T_H1$  responses and increase in TNF- $\alpha$  production, while it is potentially detrimental to pathogen attack, thus making the level of IL-10 in sites of inflammation extremely delicate and specific (44).

A variety of bacterial-induced inflammatory diseases ranging from peritonitis from *Escherichia coli*, to chronic gastritis from *Helicobacter pylori* and to chronic hepatitis from *Helicobacter hepaticus*, reinforce a correlation between IL-10 deficiencies and disease severity. To illustrate this, mice with a deficiency in IL-10, upon exposure to *Listeria monocytogenes*, have a greater severity of brain lesions,

because of increased proinflammatory cytokine production in the brain (44–46). In this instance, either specialized  $T_{reg}$  cells or their IL-10 secretions help to limit  $T_H1$  cell-mediated inflammation and damage during infection.

Recognition of hazardous microbes, allergens and toxins as pathogenic agents activates the gastrointestinal immune system. Antigen-specific  $T_{reg}$  cells, which mediate oral tolerance to commensal microbes, differentiate between harmless inhabitants of the gut and pathogens. A break in the development or maintenance of oral tolerance may result in an astounding array of detrimental inflammatory disorders, including inflammatory bowel disease (IBD) and colitis.

IBD and colitis are conditions in which the immune system of patients reacts excessively to indigenous intestinal bacteria.  $T_{reg}$  cell depletion in these disorders effectively breaches tolerance and allows for massive inflammation in the gut. *In vivo* transfer of  $T_{reg}$  cells suppresses disease development, through IL-10, TGF- $\beta$  and CTLA-4-dependent mechanisms (21).

Colonization of gastric and duodenal mucosa by *H. pylori* induces strong immune responses involving innate immune system cells as well as *H. pylori*-specific T and B cells (27).  $T_{reg}$  cells dampen the immune response to *H. pylori*, effectively limiting acute infection-induced pathology, at the cost of bacterial persistence and long-term pathology, i.e. chronic infection (27). Prevention of inflammation via IL-10 and TGF- $\beta$  may prove useful in the control of *H. pylori* infection (21).

Interestingly, varieties of bacteria including *Mycobacterium tuberculosis*, *Yersinia enterocolitica* and *Bordetella pertussis* induce production of IL-10 by macrophages. This cytokine manipulation efficiently triggers immunosuppression and attenuates bacterial attack, by means of inducing development of IL-10 secreting  $T_{reg}$  cells (46,47).

The appropriate balance between inflammation and bacterial destruction so that the body accrues minimal tissue damage while putting up adequate host defense as well as development of tolerance to commensals are essential roles of IL-10 secreting  $T_{reg}$  cells. Thus, they pose a promising target for the suppression of extreme inflammation and tissue damage during bacterial exposure or infection. It is important to note that different clinical outcomes may result from  $T_{reg}$  cell activation status.

### Fungal Infections

$T_{reg}$  cell activation limits inflammatory pathology induced by fungal infection but compromises fungal clearance. *Pneumocystis carinii* and *Candida albicans* are used in models to ascertain  $T_{reg}$  cell function. Infection of  $T_{reg}$  cell deficient mice with *P. carinii* yields fatal pulmonary inflammation, with damage owing to CD4<sup>+</sup> effector T cells.  $T_{reg}$  cell infusion prevents inflammation and disease development at the cost of increased pathogen load (19,21,48–50). Similarly,  $T_{reg}$  cell depletion renders adequate control of *C. albicans* infection



while allowing for large-scale gastrointestinal inflammation to ensue (21).

### Parasitic Infections

IL-10 or TGF- $\beta$  secreting T<sub>reg</sub> cells have great utility in the balance between parasite clearance and induced immunopathology, as seen in malarial, *Plasmodium chabaudi* and *L. major* infections.

The severity of malarial infection directly correlates to the ratio of TGF- $\beta$  and IL-10 levels to TNF- $\alpha$  levels, i.e. a greater proportion of suppressive cytokines attenuates severity of infection-induced inflammation. IL-10-deficient mice, infected with *P. chabaudi*, maintain severe infection and massive inflammatory responses resulting in significant host damage (44,51–53). In the case of *L. major* infection, removal of T<sub>reg</sub> cells results in the effective parasite clearance, however bad lesions and a robust T<sub>H2</sub> response ensue (19,21,27,54). This substantiates the importance of TGF- $\beta$  and IL-10 secreting T<sub>reg</sub> cells in controlling parasitic infection and pathology.

Various parasites that have adapted to T<sub>reg</sub> cell host-defense mechanisms have the ability to modulate T<sub>reg</sub> cell cytokine production and activation (55–57). Therefore, the role of T<sub>reg</sub> cells in inflammatory pathology and pathogen clearance may vary significantly between individual parasitic infectious agents.

### Viral Infections

T<sub>reg</sub> cells reduce the severity of immune-mediated inflammatory lesions in viral-induced diseases through the suppression of pathogenic CD4<sup>+</sup> T cell activity and the limitation of inflammatory cell sequestration.

Chronic hepatitis C virus infection results in massive hepatic inflammation and damage. In liver biopsies, there is an inverse correlation between peripheral T<sub>reg</sub> cells and histological inflammatory score (21). T<sub>reg</sub> cells, specifically those secreting IL-10, are essential for the attenuation of such organ detriment.

Theiler's virus induces murine encephalomyelitis, a T<sub>H1</sub> cell-mediated inflammatory disease of the central nervous system, and provides a model of human multiple sclerosis from which exploration of T<sub>reg</sub> cell functionality in autoimmune detriment is possible. B cell proliferation and autoantibody production, in some viral infections, plays a major role in the development of viral-induced autoimmunity (51,58). Infected mice may experience acute encephalomyelitis or chronic demyelinating disease depending on the strain. Virus-specific T<sub>reg</sub> cells sufficiently suppress this aberrant CD4<sup>+</sup> T<sub>H1</sub> cell-mediated response (59). T<sub>reg</sub> cell modulation of reactions to self, regardless of causation, holds promising implications for a broad spectrum of deleterious autoimmune diseases.

During chronic viral infections, T<sub>reg</sub> cells are beneficial to the host by maintaining a balance between efficient viral defense and inflammation, while preventing the induction of autoimmune disorders (56–63). Similar to other pathogens,

certain viruses, like HIV, have developed mechanisms that directly affect T<sub>reg</sub> cell function, resulting in reduced antiviral response and increased viral persistence (62–65).

### The Maginot Line: T<sub>reg</sub> Cell's Blockade of Allergic and Infectious Immune Responses

Innovative research and subsequent elucidation of T<sub>reg</sub> cell involvement in various atopic and infectious pathologies opens up numerous avenues for ameliorative therapies while describing mechanisms of disease pathogenesis. It is apparent that the T<sub>reg</sub> cell quantity and activation state are integral and equally important factors in the development and maintenance of inflammatory immunopathology (36).

T<sub>reg</sub> cell involvement in immune response to pathogens is delicate since T<sub>reg</sub> cells actively suppress immunopathology during infection, while concomitantly supporting persistence of infection during chronic disease. Aberrant modulation of immune responses by T<sub>reg</sub> cells may be owing to inappropriate quantity or functionality of T<sub>reg</sub> cells. Excessive immune suppression results in enhanced pathogen survival, through clearance inhibition, which may lead to long-term persistence, pathogen damage and increased potential for transmission (21). On the other hand, depressed T<sub>reg</sub> cell activity allows little control of inflammatory responses resulting in collateral tissue damage (21,41). A balance between effector T and T<sub>reg</sub> cell responses in sites of chronic infection may allow parasite survival in host while maintaining host immune memory and control of the pathogen (44).

Pathogens are evolving self-serving strategies to increase survival potential, through the establishment of favorable conditions for T<sub>reg</sub> cell priming, recruitment and survival (21). Certain pathogens and their products, e.g. *Staphylococcal* superantigen B, HTLV-1 and HIV, directly target and modulate T<sub>reg</sub> cell function (2). Evolved pathogen mechanisms for T<sub>reg</sub> cells manipulation underscores the power and utility that T<sub>reg</sub> cells hold and presents another means for medical treatment of related diseases, e.g. implementation of means to effectively antagonize pathways in which pathogens directly modulate T<sub>reg</sub> cell functioning.

Increased research is necessary in order to determine T<sub>reg</sub> cell functioning in relation to individual allergic and pathogen induced disease states. This will afford CAM researchers insight into the appropriate means of approaching a variety of human disorders with respect to T<sub>reg</sub> cells.

T<sub>reg</sub> cell's essential role in the management of allergy and infection has been detailed using specific allergens or pathogens as examples. Harmony between regulatory and effector arms of the immune system is a necessity for good health. T<sub>reg</sub> cell intricacy and specificity to individual allergens or pathogens impels further research and highlights T<sub>reg</sub> cells overall importance to human health and CAM. The conceptual framework laid down is consistent with various disease states, including autoimmunity and tumor pathogenesis, which will be a futile subject.

## Conflict of Interest

Aristo Vodjani is co-owner of Immunosciences Lab. Inc. He declares no conflict of interest.

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