Nutraceutical and Drug Measures for Expanding the T Regulatory Lymphocyte Pool

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Abstract

T regulatory cells, owing to their down-regulatory impact on inflammatory mechanisms, can aid control of autoimmunity, certain neurodegenerative disorders, atherosclerosis, and metabolic syndrome. Safe and practical measures for expanding the Treg pool may therefore have broad utility for providing protection from a host of common health disorders. Measures which appear likely to be useful in this regard include: subcutaneous or oral administration of heat shock proteins, or epitopes thereof; the cyanobacterial protein phycocyanin and its chromophore phycocyanobilin; vitamin D; PPARgamma agonists such as pioglitazone; GABA or GABAergic drugs; and possibly a vegan diet. Functional combinations of these measures which best promote Treg induction and activity may have considerable potential in prevention and therapy.

Treg Expansion May have Broad Clinical Utility

T regulatory (Treg) cells play a key role in the prevention and control of autoimmunity and chronic inflammatory syndromes, including certain neurodegenerative disorders.[1;2] Tregs also oppose atherogenesis and can stabilize plaque, likely decreasing risk for heart attack, stroke, and aneurysms.[3-11] Notably, Treg levels tend to be decreased in patients with unstable angina, as opposed to patients with stable angina or healthy controls. And Tregs in visceral adipose tissue, via production of IL-10, can dampen adipose inflammation and thereby help to preserve good systemic insulin sensitivity and prevent the metabolic syndrome.[12-20] Hence, practical strategies for expanding the body's pool of Tregs could confer a wide range of health benefits.

Vaccination with Heat Shock Proteins

Oral or subcutaneous vaccination with heat shock proteins, or epitopes derived therefrom, has been shown to promote Treg activity, likely because a significant proportion of thymic-derived nTregs recognize hsp-derived epitopes.[21-23] These Tregs may be induced to proliferate and activated when dendritic cells present them with these epitopes; they then may migrate to inflamed tissues, where they can tolerize dendritic cells presenting hsp epitopes, as well as interacting directly with T cells to suppress their pro-inflammatory activity. Oral administration of bacterial heat shock proteins – as with the lyophilized E.coli extract Subreum (OM-89) – has shown clinical utility in rheumatoid arthritis comparable to that of DMARD drugs.[24-29] Oral administration of hsp60 (or an epitope derived from it) to LDL receptor-deficient mice, markedly suppressed development of atherosclerosis while promoting Treg induction.[30] Parenterally administered hsp epitopes are effective in rodent models of autoimmunity,[21-23;31-34] and subcutaneous injection of hsp-enriched monocytes, heat-shocked *ex vivo* and then incubated at physiological temperature for a further 24-48 hours prior to injection, has been reported anecdotally to achieve clinical benefit in a wide range of autoimmune disorders (the MAM-14 strategy).[35]

Phycocyanobilin – A Bilirubin Mimetic

Bilirubin, as well as its homologue phycocyanobilin (PhyCB), a major component of cyanobacteria used as food such as spirulina, promote the conversion of CD4+ lymphocytes to foxp3+ Tregs, in vitro and in vivo.[36-40] This may explain why induction of heme oxygenase-1, which generates bilirubin's precursor biliverdin, has a similar effect.[36;41-43] Bilirubin is an endogenous activating ligand for the aryl hydrocarbon receptor (AhR),[44-47] which can promote the induction of T regulatory cells (both foxp3+, and foxp3- producting IL-10);[48-51] this likely explains the impact of bilirubin on Treg induction (Libor Vitek, personal communication). Activating ligands of AhR have shown therapeutic efficacy in rodent models of multiple sclerosis, colitis, type 1 diabêtes, autoimmune uveitis, peanut allergy, and graft rejection.[52-58] It is reasonable to predict that phycocyanorubin, produced within cells by bilirubin reductase-mediated reduction of PhyCB, is likewise an activating ligand for AhR. Oral administration of phycocyanin or its chromophore PhyCB may therefore be useful for Treg expansion, and oral phycocyanin is beneficial in rodent models of autoimmunity and chronic inflammation.[59-64] The antioxidant efficacy of these agents may also contribute to their anti-inflammatory impact.[65;66] The nutraceutical diindolylmethane likewise has agonist activity for AhR, and has potential for control of autoimmunity.[67-69] There is recent evidence that the kynurenine generated by indolamine 2,3-dioxygenase activity also serves as an endogenous ligand for AhR.[70;71]

Vitamin D

Low vitamin D status has been linked to increased risk for various autoimmune disorders, as well as metabolic syndrome and atherogenic disease.[72-75] Activated dendritic cells express vitamin D 1-α-hydroxylase activity, and hence can convert cholecalciferol to the active hormone calcitriol; the extent to which this occurs will be proportional to the circulating concentration of vitamin D.[76] Dendritic cells exposed to calcitriol develop a tolerogenic phenotype – expressing IL-10 and IDO – and can interact with naïve CD4+ lymphocytes to convert them to Tregs.[77;78] Calcitriol can also act directly on lymphocytes in the microenvironment of these dendritic cells; a vitamin D response element has been identified in the first intron of the Foxp3 gene, and promotes its transcription.[79] A physiologically significant dose of vitamin D – 140,000 IU monthly – has been reported to expand the pool of CD4+CD25+Foxp3+ lymphocytes in healthy humans.[80]

PAPRgamma Agonists

There is recent evidence that PPARgamma agonists, such as the drug pioglitazone, can act both on dendritic cells and naïve CD4+ lymphocytes to promote generation of iTregs.[81-87] A key effect of these agents is to boost the capacity of dendritic cells to synthesize retinoic acid, which promotes induction of iTregs; the maturation of dendritic cells is also opposed, rendering them more tolerogenic.[87;88] PPARgamma activity within lymphocytes boosts Foxp3 expression by modulating methylation of DNA in the Foxp3 promoter region.[85] PPARgamma is highly expressed in the Tregs that congregate in visceral adipose tissue, and likely contributes to the favorable impact of thiazolidinedione therapy on insulin sensitivity in diabetics.[15;16] Pioglitazone administration was found to be beneficial in rodent models of autoimmune arthritis and uveitis, and to stabilize plaque in atheroma-prone mice.[84;86;89] Moreover, a controlled clinical trial found that this drug was a useful adjuvant to methotrexate therapy in rheumatoid arthritis.[89]

GABA and GABAergic Agents

Oral administration of gamma-amino-butyric acid (GABA) is protective in a mouse models of type 1 diabetes (NOD) and rheumatoid arthritis, owing in part to anti-inflammatory effects mediated by GABAergic receptors on macrophages and T cells.[90-92] GABAergic drugs (topiramate, vigabatrin) ameliorate the course of experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis.[93] GABA administration (2 g /l in drinking water) likewise aids maintenance of insulin sensitivity in fat-fed mice; this latter study reported an increase in the CD4+Foxp3+ pool in these mice.[94] How GABA promotes Treg induction requires further clarification, but may reflect a tolerizing effect on dendritic cells.[93] Hence, ample oral doses of GABA, or treatment with GABAergic drugs, might have clinical potential for promoting Treg activity.[94] GABA per se has the advantage that it does not cross the blood-brain barrier, and hence would not produce central side effects; but from the standpoint of convenience, GABAergic drugs likely have better pharmacokinetics. It has credibly been proposed that oral GABA could be useful for prevention or treatment of atherosclerosis.[95]

Fasting and Vegan Diet

Fasting increases the Treg pool in mice, and this has been shown to reflect an acute reduction in circulating levels of leptin, which opposes Treg induction and exerts various other pro-inflammatory effects.[96] A fast of several days duration, followed by adoption of a vegan diet, has been found to be clinically beneficial in rheumatoid arthritis.[97-99] The relatively low risk for autoimmune disorders in sub-Sahara Africans and East Asians during the early to mid-twentieth century remains unexplained.[100-102] It has been speculated that the leanness and relatively low intake of essential amino acids associated with long-term consumption of vegan diets may promote Treg expression; this hypothesis has not yet been tested.[101]

Multifocal Protocols for Treg Induction

A protocol consisting of hsp vaccination, complemented with adjuvant measures that might include vitamin D, phycocyanin, pioglitazone, GABA or GABAergic drugs, and a vegan diet, may therefore have clinical potential for treating autoimmune disorders, neurodegenerative conditions driven in part by activated microglia, metabolic syndrome/diabetes, and for stabilizing atherosclerotic plaque (thereby lessening risk for vascular accidents). Subcutaneous hsp vaccination (as in MAM-14) could be used episodically, whereas oral administration of hsps could be employed in the intervals between such therapy. Rodent models of autoimmunity, neurodegeneration, metabolic syndrome, and atherosclerosis might be employed to determine what combinations of these options might have the most important impact on Treg expression and activity, and on the progression of these syndromes.

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