

# Vaccination with Heat-Shocked Mononuclear Cells as a Strategy for Treating Neurodegenerative Disorders Driven by Microglial Inflammation

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## Abstract

Naturally occurring T regulatory cells targeting epitopes derived from various heat shock proteins escape thymic negative selection and can be activated by vaccination with heat shock proteins; hence, vaccination with such proteins has exerted favorable effects on rodent models of autoimmune disorders. A more elegant way to achieve such vaccination, first evaluated clinically by Al-Harbi in the early 1990s, is to subject mononuclear cells to survivable heat shock *ex vivo*, incubate them at physiological temperature for a further 24-48 hours, and then inject them subcutaneously; anecdotally, beneficial effects were observed with this strategy in a wide range of autoimmune and inflammatory conditions. There is growing evidence that M1-activated microglia play a primary or secondary role in the pathogenesis of numerous neurodegenerative diseases, as well as in major depression. T regulatory cells, by polarizing microglial toward a reparative M2 phenotype, have the potential to aid control of such disorders. It would be appropriate to test the heat-shocked mononuclear cell vaccination strategy in animal models of neurodegeneration and major depression, and to evaluate this approach clinically if such studies yield encouraging results.

## Heat Shock Proteins can Induce T Regulatory Cell Activity

Rodents and humans naturally express a set of T regulatory cells that recognize epitopes derived from a range of autogenous heat shock proteins. When appropriately stimulated by dendritic cells presenting these epitopes, these Tregs appear capable of interacting with macrophages, microglia, and dendritic cells in inflamed tissues, conferring on them a reparative M2 phenotype via mediators such as IL-10 and TGF-beta that dampens inflammation.<sup>1-3</sup> Hence, vaccination with various heat shock proteins has been shown to have an ameliorative impact in several rodent models of autoimmunity and inflammation.<sup>2, 4-13</sup> Presumably, some special mechanism protects these autoreactive CD4+ cells from deletion during their evolution in the thymus; this suggests that they may function physiologically to quell excessive inflammation.

Vaccination with specific heat shock proteins, by activating these hsp-targeted Tregs, has the potential to provide therapeutic benefit in autoimmune diseases and other inflammatory disorders driven or exacerbated by pro-inflammatory M1-polarized macrophages and microglia. The favorable impact of whole-body hyperthermia on autoimmunity reported in some studies might be mediated, in part, by increased endogenous synthesis of heat shock proteins.<sup>14-16</sup> But a more elegant approach, first implemented clinically by Saleh Al-Harbi and David Haines in the early 1990s in Kuwait, would be to vaccinate patients with human mononuclear cells which have been subjected to heat shock and other stressors *ex vivo*, such that they then express ample levels of the entire range of heat shock proteins [Al-Harbi S, Haines DD, personal communication]. Al-Harbi's basic strategy was to incubate autogenous or allogeneic mononuclear cells at 42°C for 30 minutes, and then incubate them for a further 24-48 hours at 37°C, during which time synthesis of heat shock proteins should be optimized; these cells are then

injected subcutaneously into the patient. This therapeutic procedure could be repeated at intervals of several weeks or months, as desired. Anecdotally, this treatment – dubbed “MAM-14” by Al-Harbi in honor of the prophet Mohammad and the holy family – was found to be therapeutically beneficial in the large majority autoimmune patients treated; in particular, positive results were noted in rheumatoid arthritis, multiple sclerosis, anterior uveitis, and incipient type 1 diabetes. These pilot studies were implemented in the chaotic Kuwaiti environment following the first Gulf War, at which time no institutional review board was available to provide approval; as a result, the observations could not be formally reported in medical journals. (An IRB-approved effort to study the MAM-14 strategy in patients with anterior uveitis at New York Eye and Ear Institute in 2006 was blocked by the U.S. F.D.A., owing to a lack of pertinent animal data.)

### **M1-Activated Microglia Promote Neurodegeneration and Depression**

M1-activated microglia, via production of nitroxidative stress and inflammatory cytokines, are believed to drive or exacerbate the progression of various neurodegenerative disorders, including ALS, multiple sclerosis, and Parkinson’s disease; they also seem likely to play at least a secondary role in the progression of Alzheimer’s and Lewy body dementia.<sup>17-27</sup> A vicious cycle mechanism, in which neuronal death or dysfunction promotes microglial activation, which in turn further traumatizes neurons, may be a common motif in neurodegenerative disorders. Direct toxic effects of microglial products on neurons, as well as effects on astrocytes which compromise glutamate clearance and hence promote excitotoxicity, may contribute to neuronal damage.

Very recently, a dramatic reduction of Treg (CD4+CD25+Foxp3+) levels has been reported in patients with major depressive disorder;<sup>28, 29</sup> it is tempting to speculate that this reduction is both a cause and a consequence of depression, in light of suggestive evidence that microglial inflammation may be a mediator of the neuronal dysfunction that characterizes this disorder.<sup>30, 31</sup> Moreover, administration of IL-10, a key mediator of Treg immunomodulatory activity, prevents the depression-like syndrome evoked by restraint stress in mice, and T-bet knockout mice, which lack effective Th1 activity (a driver of microglial activation), are protected from this syndrome.<sup>32, 33</sup> Hence, there is reason to suspect that major depressive disorder may be amenable to control by measures which promote M2-polarization in microglia.

### **T Regulatory Cells Oppose Neurodegeneration by Modulating Microglial Function**

Several recent reviews have proposed that stimulation of regulatory T cell activity may have potential for clinical management of neurodegenerative disorders in which activated microglia play a pathogenic role, as suggested by favorable results observed with activated Treg cells in rodent models of ALS and Parkinson’s.<sup>3, 34-36</sup> Conversely, Treg activity has been found to be depressed in patients with ALS, and correlates inversely with the rate of progression of the syndrome.<sup>37, 38</sup> In experimental autoimmune encephalomyelitis (EAE), a murine model for multiple sclerosis, pre-induction of heat shock protein expression via whole-body hyperthermia, as well as vaccination with a heat shock protein-derived epitope, and oral administration of a lactobacillus bioengineered to produce and secrete Hsp65, have been reported to prevent or ameliorate this syndrome.<sup>13, 39-41</sup> The lactobacillus study is of particular interest, as a marked induction of Tregs (both foxp3 and latency-associated peptide expressing) was noted in the mice receiving this therapy.<sup>41</sup>

These favorable effects of heat shock protein induction or vaccination on EAE are paralleled by Al-Harbi's claim that heat-shocked mononuclear cell injection (MAM-14) was therapeutically beneficial in multiple sclerosis. Moreover, with remarkable insight, he used this treatment in several patients with ALS, Parkinson's disease, and major depression, and claims to have observed apparent benefit. Evidently, controlled studies of sufficient size would be required to validate these anecdotal observations – from clinical efforts initiated over 20 years ago! - but these observations are at least credible in light of the potential of hsp-evoked Treg activity for controlling microglial inflammation.

Evidently, rodent studies evaluating the MAM-14 strategy in models of ALS, Parkinson's, multiple sclerosis, and major depression would be warranted. Moreover, in light of the excellent tolerability of this therapy in humans reported by Al-Harbi and Haines (the injected cells can be killed prior to administration, so as to avoid any minor risk of graft-vs-host reaction), as well as its simplicity and low cost, further pilot clinical evaluations of this protocol in various CNS neurodegenerative disorders (as well as systemic autoimmune diseases such as rheumatoid arthritis) should be encouraged.

### **Phycocyanobilin as a Nutraceutical Adjuvant for Treg Induction**

As a nutraceutical adjuvant to MAM-14 or other strategies intended to activate hsp-targeted Tregs, oral administration of phycocyanobilin (PhyCB) – the chromophore bound to phycocyanin in cyanobacteria such as spirulina – may have potential. Biliverdin/bilirubin derived from heme oxygenase activation appear to play a physiological role in Treg induction, an effect associated with increased synthesis of IL-10.<sup>42-46</sup> There is reason to suspect that PhyCB, a biliverdin derivative, may be able to mimic this effect.<sup>47, 48</sup> Moreover, PhyCB shares bilirubin's ability to inhibit certain NADPH oxidase complexes, which are mediators of microglial activation and cytotoxicity.<sup>19, 49, 50</sup> Notably, oral administration of phycocyanin or of whole spirulina is reported to provide protection in rodent models of Parkinson's, multiple sclerosis, and depression in mice, and inhibition of NADPH oxidase activity by other means was recently reported to prevent restraint stress-induced depression in mice.<sup>47, 51-55</sup>

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