Editorial

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Prospects for a peptide vaccine for human lupus

"...histone peptide epitopes might have unique and as yet uncharacterized mechanisms for their potent immunoregulatory effects, probably because they are derived from apoptotic cells, which are counter-inflammatory in the normal host."

Keywords: antinuclear autoantibodies • autoimmunity • histone peptide epitopes • human lupus T cells • nucleosomes • peptide vaccine • regulatory T cells • SLE • systemic lupus erythematosus • tolerance • Tregs

Global immunosuppression can control active lupus disease, but the drugs used are toxic and fail to achieve true immunological remission. Targeted downregulation of pathogenic autoimmune cells in lupus should follow such therapies to prevent recurrence and smoldering damage. Lupus patients' immune systems manifest many abnormalities [1] that lead to a pathogenic response to nuclear autoantigens derived from apoptotic cells produced routinely in the body. Normal humans and mice have regulatory mechanisms including Tregs that prevent such an abnormal response [2–4]. A prophylactic vaccine could re-establish the deficiency of Tregs associated with lupus. Even apparently healthy subjects and family members of lupus patients, who might be at risk of developing lupus (as predicted by genetic and other biomarkers), would benefit from such a tolerance vaccine to boost their regulatory mechanisms, which would suppress lupus development in the future. In this very brief editorial, we discuss requisite features of a lupus vaccine and challenges in developing one, using a vaccine candidate as an example with which we have first-hand experience.

Desirable qualities of a peptide vaccine for lupus

The requirements for a peptide vaccine have been listed in the context of other autoimmune diseases [5]. We have found that certain endogenous peptide epitopes in histones from nucleosomes can induce regulatory mechanisms that inhibit major components of pathogenic autoimmune response in established disease in lupus prone mice *in vivo*, and in lupus patients' cells *in vitro* [3–4,6–9]. Extensive studies in mouse models and recent work with lupus patients' cells have shown that the histone peptide epitopes have the requirements for a vaccine, as follows:

- The nucleosomal histone peptides are nontoxic, as they are endogenous, derived from body's own proteins which are used to 'educate' the immune system during development. The epitopes are naturally processed and unaltered peptide ligands (UPLs) derived from nucleosomes of apoptotic cells that are expressed in the thymus and bone marrow during ontogeny of the immune system [6,10–12] and, therefore, unlike artificially altered peptide ligands (APLs), the histone peptides are not associated with anaphylactic/allergic reactions [7–9]. Indeed recognition of histone peptide epitope in the thymus generates Tregs even in lupus-prone mice, indicating that the default response to the peptide epitopes is regulatory [11];

- The epitopes are effective *in vivo* at very low doses, and by subcutaneous or intranasal administration in suppressing pathogenic autoantibody production and renal inflammation in animal models of lupus. Only 1 μg (0.34 nM) of the histone peptide epitope/s is effective in low-dose tolerance therapy of mice with lupus, which would be equivalent to 0.2–2 mg range in lupus patients. Such low doses

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would lessen the chance of stimulating autoimmune effector T cells;

- The epitopes are highly soluble as they contain many charged residues, and they are rapidly absorbed systemically after subcutaneous injection [8,9]. By rendering plasmacytoid dendritic cells tolerogenic as soon as the peptides reach the lymphoid organs, the peptides generate long-lasting, antigen-specific Tregs that suppress disease pathology. Therefore, short half life or decay of the epitopes is not a concern as they are taken up by tolerogenic antigen-presenting cell (APC). Moreover, the stable, autoantigen-specific Tregs generated *in vivo* by the peptide therapy can also block accelarated lupus disease upon adoptive transfer [8];
- The histone peptide epitopes induce cross-reactive, 'tolerance spreading' or linked tolerance to other pathogenic T- and B-cell autoepitopes, thus suppressing the broad spectrum of autoimmune response in lupus, but they do not suppress responses to exogenous antigens;
- The peptides have both MHC class II and nested class I determinants so that they could genenrate both CD4 and CD8 Tregs [8], similar to the potent Tregs generated after stem cell transplantion that keep lupus patients in long-term immunologic remission [3];
- The peptides are recognized directly by antinuclear autoantibodies so that they could tolerize autoimmune B cells of lupus as well [12];
- Importantly, the epitopes are recognized by autoimmune T and B cells of all lupus patients tested irrespective of their HLA type [3–4,6–7,12–13];
- The peptide epitopes are effective even when the autoimmune disease and nephritis are already established [4,7–9];
- The peptides generate Tregs in lupus patients' cells even in the presence of commonly used drugs, hydroxychloroquine or steroids, or lupus serum containing type I interferon (IFN)-inducing immune complexes [4];
- The peptides also have direct effect on autoimmune B cells and dendritic cells in lupus, in addition to generation of Tregs [7,9,12], as the peptides could suppress autoantibody production by patients' cells to baseline levels irrespective of the degree of Treg induction and suppressed type I IFN gene signature [4];
- Even healthy people can have their regulatory mechanisms against nuclear autoantigens boosted by the histone peptides [3,4] suggesting the possibility of vaccinating apparently healthy people at risk for lupus with these peptides;
- Histone peptide therapy would be cheaper than many of the biologics.

Challenges in developing the peptide vaccine therapy

Regulatory immunity in the face of inflammation

With progression of disease lupus B cells become autonomous after developing high-affinity somatically mutated receptors with T-cell help. The histone peptide epitope therapy might optimally restore immunoregulation and maintain lupus patients in remission after systemic inflammation is suppressed and the load of pathogenic B cells diminished by more toxic or global immunosuppressive agents.

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Nevertheless, in animal models, the peptides, without help from any Treg-enhancing agents, are quite effective by themselves in ameliorating established lupus disease and inflammation in kidneys [7–9]. Since the peptides induce Tregs *in vivo*, such Tregs are more stable in the face of inflammation than *in vitro*-induced Tregs. Moreover, lupus patients' sera, which contain, TLR-activating immune complexes, could not inhibit Treg induction by the peptides, and in peripheral blood cells from patients with active lupus, dexamethasone or hydroxychloroquine actually augmented Treg induction by the peptides [4], indicating that anti-inflammatory measures to diminish hyperactivity of IRF5 and TLR pathways in lupus do not interfere with regulatory effects of the peptides to take hold. The peptides also have direct effects on autoimmune B cells and dendritic cells in lupus, in addition to generation of Tregs [7,9,12], and the peptides could suppress type I IFN signature and autoantibody production to baseline levels in lupus patients' peripheral blood mononuclear cells even without increased Treg induction [4].

Need for IL-2

IL-2 is necessary for Treg expansion and IL-2 production by CD4 T cells is impaired in lupus [1,14]. However, the histone peptide epitopes administered

by themselves, without exogenous IL-2, can induce expansion of potent Tregs *in vivo*, blocking lupus disease in various mouse models of spontaneous systemic lupus erythematosus [8–9,15–17]. An initial transient activation of autoreactive T cells by low-dose peptide could provide IL-2 for the Treg expansion. Later on, the *in vivo*-induced Tregs may depend on other signals such as inducible T-cell costimulator [18].

Route of administration

In lupus-prone mice the histone peptide epitopes are effective in blocking lupus upon administration through intravenous, intraperitoneal or intranasal route in high doses. In low doses the peptides are effective subcutaneously. Local inflammation at the site of injection has to be avoided to prevent any concurrent immune reactivity to the peptides. In humans many proteins, such as insulin, other hormones, biologics, intravenous immunoglobulin and so on, are administered subcutaneous or intradermaly (patches) without causing inflammatory response. Newer approaches for intradermal delivery of vaccines using nanotechnology are being developed.

Dosage & frequency of administration

Tregs require continued antigen-specific stimulation delivered by dendritic cells. Repeat injections of low-dose peptide epitopes at appropriate intervals may be necessary to maintain immune regulation in patients with systemic lupus erythematosus. In animal models, regulatory immunity induced by the peptide epitopes is detectable at 6 weeks after subcutaneous injection. In human lupus studies *in vitro*, cocktails of the peptide epitopes in low doses rather than a single peptide, were more effective in suppressing pathogenic autoantibody production and type I IFN signature [4].

Overall, the fact that even healthy people can have regulatory mechanisms against nuclear autoantigens boosted by the histone peptides [3,4] suggests the possibility of vaccinating apparently healthy people at risk for lupus with these peptides. Relatively higher doses of a native or altered versions of another category of peptides, related to variable regions of anti-DNA autoantibodies, also induce Tregs in mice *in vivo* and in lupus patients' cells *in vitro*, but not in healthy controls [2,19]. Thus, the histone peptide epitopes might have unique and as yet uncharacterized mechanisms [11,17] for their potent immunoregulatory effects, probably because they are derived from apoptotic cells, which are counter-inflammatory in the normal host [20].

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