

Helminth-Related Tuftsin-Phosphorylcholine Compound and its Interplay with Autoimmune Diseases

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ABSTRACT: The hygiene theory represents one of the environmental facets that modulate the risk for developing autoimmune diseases. There is a reverse correlation between the presence of helminthes and flares of autoimmune diseases, which explains the rise in incidence of certain autoimmune diseases in developed countries. The protective properties of certain helminthes are attributed to their secretory compounds which immunomodulate the host immune network in order to survive. Thus, the helminthes use an array of mechanisms. One of the major mechanisms enabling manipulation of the host-helminth interaction is by targeting the pattern recognition receptors (PRRs)-dependent and -independent mechanisms, which include toll-like receptors, C-type lectin receptors, and the inflammasome. The current review provides a glimpse of numerous helminth secreted products which have a role in the immunomodulation of the host immune network, focusing on bifunctional tuftsin-phosphorylcholine (TPC). TPC is a natural compound based on phosphorylcholine of helminth origin that was used in the past to cover stents and tuftsin, a self-peptide derived from the spleen. TPC was proven to be efficient in three murine experimental models (lupus, colitis, and arthritis) and ex vivo in giant cell arteritis.

IMAJ 2019; 21: 158–162

KEY WORDS: animal models, autoimmunity, helminth, immunomodulation, tuftsin-phosphorylcholine (TPC)

Geoepidemiological studies show a reverse correlation between less developed countries and the incidence of autoimmune diseases [1]. There are more than 80 autoimmune diseases whose occurrence increased dramatically in the last few decades [1]. One of the major hypotheses to explain this trend is the hygiene hypothesis [3]. According to this theory, early and repeated exposure to infections enrich the innate and adaptive immune network, whereas improved sanitation increases autoimmunity (for example, there is no lupus in malaria-infected areas) [3,4]. A low parasite burden leads to a high-inflammatory condition (e.g., activation of Th1, Th2, and Th17), fibrosis and chronic pathology, while a high parasite burden results in a low-immune pathology (inhibition of Th1

and Th17 and modified Th2) [5]. Long-lived parasites such as helminthes have the ability to immunomodulate the host immune network. Therefore, treatment with helminthes or their ova ameliorate murine experimental models and patients with autoimmune diseases. The aim of the helminth is to survive inside the host environment by protecting itself from eradication by the host immune system [4-6]. This review will shed light on helminth secretory immunomodulatory small molecules, focusing on tuftsin-phosphorylcholine (TPC).

HELMINTH TREATMENT IN PATIENTS AND MURINE EXPERIMENTAL MODEL

Diving deep into the parasite world, Cox [7] revealed an interesting view of its history. This was evident already in the period of ancient Egypt, from 3000 to 400 BC, particularly in the Ebers papyrus, an Egyptian medical tome of herbal knowledge discovered at Thebes [8]. Some worm-related diseases emerged, as described in the Old Testament when the Israelites crossed the Red Sea on their exodus from Egypt in 1250 to 1200 BC [9], and in Hippocrates' writings [10]. The helminthes and their products, being pathogenic or protective, raised researchers' curiosity in ancient times as well as today.

Helminthes (parasitic worms) in certain geographic areas exist in reverse correlation with the flare of autoimmune diseases and inflammatory conditions. During recent decades, accumulating evidence showed the effectivity of therapy with helminthes and their ova in reducing inflammation and in the clinical score of autoimmune diseases, such as multiple sclerosis (MS), rheumatoid arthritis (RA), type I diabetes mellitus (T1DM), and inflammatory bowel diseases (IBD) [6,11-13]. Patients with active ulcerative colitis and Crohn's disease were treated with an ingestion of live helminth eggs, *Trichuris suis*, and their disease remitted [14-16]. The helminth therapy was not associated with side effects or serious complications attributable to the therapeutic agent [17].

Since MS, an inflammatory and demyelinating disease affecting the central nervous system, is mediated via a Th1 predominant immune mechanism, researchers assumed that MS patients could benefit from helminthes by inducing a Th2 shift [18,19]. A prospective double-cohort study was performed in 12 MS patients who presented with eosinophilia and parasitic

infection with *Hymenolepis nana*, *Trichuris trichiura*, *Ascaris lumbricoides*, *Strongyloides stercoralis*, or *Enterobius vermicularis*. Infected patients showed a significant improvement in clinical MS manifestations, as well as an increased myelin basic protein-specific T cell secretion of anti-inflammatory cytokines [interleukin 10 (IL-10) and transforming growth factor-beta (TGF β)], and reduction in inflammatory cytokines [IL-12 and interferon-gamma (IFN γ)]. Likewise, the number of T regulatory cells were increased [18]. In another study, 16 MS patients with relapsing-remitting multiple sclerosis (RRMS) were assessed for safety and brain magnetic resonance imaging (MRI) activity during oral administration of ova from the porcine whipworm, *Trichuris suis* (TSO). The study was performed during 5 months of screening observation, 10 months of treatment, and 4 months of post-treatment surveillance [19]. No serious symptoms or adverse events occurred during treatment. A trend was consistent, with a 35% diminution in active lesions when observation MRIs were compared with MRIs of the treated subjects; at the level of individuals, 12 of 16 subjects improved during TSO treatment associated with expansion of T regulatory cells during TSO treatment [19].

Employing experimental autoimmune models, amelioration of disease activity was achieved by administration of helminthes or their ova. Studies with non-obese diabetic (NOD) mice showed that inoculation with *Trichinella spiralis*, *Heligmosomoides polygyrus*, or *Schistosoma mansoni* using egg antigen or the worm antigen markedly reduced the rate of experimental type I diabetes mellitus (T1DM) and suppressed lymphoid infiltration in pancreas islets [16,19-22]. Amelioration of experimental autoimmune encephalomyelitis (EAE) was achieved following helminth treatment [23]. Schistosome worm infections prevented colitis, shifting the immune response towards Th2 phenotype [24,25]. *Syphacia obvelata*-infected rats developed less severe arthritis than uninfected rats. Extracts of the nematode *Ascaris suum*, *Schistosoma mansoni* and *Acanthocheilonema viteae* were also found to reduce the severity of collagen-induced arthritis (CIA) in mice [26-28].

Ingestion of parasitic worms ameliorated experimental autoimmune diseases via several mechanisms, mostly by induction of T regulatory cell (Tregs) expansion, stimulation of anti-inflammatory cytokines such as IL-4, IL-10, TGF β , and inhibition of circulating pro-inflammatory cytokines IL-1b, IFN γ , tumor necrosis factor-alpha (TNF α) and IL-17 [29].

HELMINTH SECRETORY IMMUNOMODULATORY SMALL MOLECULES

A number of animal models along with some human pilot studies evaluated the effects of live helminthes and their ova on diverse autoimmune diseases. Weinstock et al. in 2016 [30] proposed that the helminth *Heligmosomoides polygyrus bakeri* prevents colitis in mice via induction of regulatory dendritic cells (DCs). These tolerogenic DCs were associated with decreased expression of the intracellular signaling

pathway spleen tyrosine kinase (Syk) in intestinal DCs from *H. polygyrus bakeri*-infected mice. DCs sense gut flora and damaged epithelium via expression of C-type lectin receptors. Focusing on a C-type lectin (CLEC) 7A, which encodes for the dectin-1 receptor on DCs and drives Th1/Th17 development, the authors provided evidence that soluble worm products can block CLEC7A and Syk mRNA expression in gut DCs from uninfected mice after a brief in vitro exposure. Inhibition of Syk expression and phosphorylation in intestinal DCs may be one mechanism through which helminthes induce regulatory DCs that down-modulate colitis [30].

Another example of helminth secretory products that modulate the host immune response was introduced by another group [31-33]. They described a glycoprotein, ES62, secreted by the parasitic worm *Acanthocheilonema viteae* in which phosphorylcholine is presented as a moiety by glycans on the protein. This secretory molecule exerts anti-inflammatory activity in murine models of chronic asthma, lupus and arthritis affecting T cells, B cells, macrophages, dendritic cells (DCs), and mast cells [31]. This research group has produced a synthetic analog for ES62. This synthetic small molecule manipulates the pro-inflammatory scenario by inhibiting TLR2, 4, and 9 expression via the MYD88 pathway, inhibiting NF- κ B, which occurs as a result of inhibition of pro-inflammatory cytokine production [32,33].

In parallel, Donnelly et al. [34,35] found a 68-mer peptide, helminth defense molecule FhHDM-1, secreted by the helminth *Fasciola hepatica*, which potently modulates the host immune response. This peptide ameliorated disease in two autoimmune murine models, type 1 diabetes, and relapsing-remitting immune-mediated demyelination. In this case the secretory peptide had no effect on T cell response in the context of cytokine production and specific T cell response, but it reduced the clinical score. The FhHDM-1 peptide affects the host immune network via interaction with macrophages, reducing their capacity to secrete pro-inflammatory cytokines such as TNF α and IL-6 [34,35]. FhHDM-1 is a cathelicidin-like peptide that binds to macrophages, internalizes into endolysosomes, inhibits its acidification, and diminishes the activation of the NLRP3 inflammasome, resulting in reduction of IL-1 β secretion by macrophages but not the synthesis of pro-IL-1b [36].

HELMINTH-BASED BI-FUNCTIONAL IMMUNOMODULATORY SELF-MOLECULE TPC

Phosphorylcholine (PC) is a small molecule moiety presented by excretory/secretory products of many helminthes and cell wall of most serotypes of *Pneumococcus*. In the past, PC was used to cover stents in order to prevent platelet aggregation [37]. Tuftsin, having the sequence Thr-Lys-Pro-Arg, naturally occurs in human blood. This peptide is a fragment of the heavy chain Fc (289-292) of immunoglobulin G (IgG).

Tuftsins is an endogenous immunomodulator of a wide spectrum of biological activities, such as enhanced phagocytosis, polymorphonuclear cell chemotaxis, pinocytosis, and antimicrobial [38,39]. We conjugated phosphorylcholine to tuftsins and named this small molecule (~1 KD) TPC.

• **TPC in murine models of autoimmune diseases**

Successful treatment with TPC was conducted in three murine autoimmune models: lupus nephritis, dextran sulfate-sodium-salt (DSS)-induced colitis, and collagen-induced arthritis (CIA) [40-46] [Figure 1]. When administered prophylactically to NZBXW/F1 female lupus mice, TPC significantly inhibited the development of proteinuria and nephritis, as illustrated by PAS staining of the kidneys, up-regulated the expression of IL-10 and TGFβ, and inhibited inflammatory cytokines INFγ, TNFα, and IL-17, associated with enhanced T regulatory cell expansion [40] [Figure 1]. Microbiome analyses following TPC treatment in lupus mice resulted in maintenance of normal microbiota (e.g., decreased abundance of *Akkermansia* and increased abundance of several genera including *Turicibacter*, *Bifidobacterium*, Unclassified *Mogibacteriaceae*, Unclassified *Clostridiaceae*, *Adlercreutzia*, *Allobaculum* and *Anaeroplasma*) [41]. Likewise, TPC treatment was as effective as treatment with methylprednisolone in reducing nephritis

in established lupus mice. IL-1β and IL-6 were reduced and anti-inflammatory IL-10 was up-regulated [42].

In mice with dextran sulfate-sodium-salt (DSS)-induced colitis receiving TPC orally, the daily activity index (DAI) was inhibited, the colon length was normal, and the architecture of the colonic cells was similar to that of the healthy mice [43]. Observing the cytokine profile in gut lysates revealed enhanced expression of the anti-inflammatory cytokine IL-10, while the inflammatory cytokines were reduced [Figure 1].

TPC was given to collagen-induced arthritis (CIA) mice, which mimics human arthritis, subcutaneously or oral, prophylactic or established [44-46] [Figure 1]. The results show prevention of joint inflammation, inhibition of the clinical score, reduction of inflammatory cytokines (INFγ, IL-1β, IL-6 and TNFα), and elevated secretion of IL-10 anti-inflammatory cytokine associated with enhanced numbers of T regulatory cells (CD4+CD25+FOXP3+) and B regulatory cells (CD19+CD10+CD5+TIM+CD1d+). All maintained normal gut microbiota profile as compared to treatment with the TPC vehicle [46].

• **TPC mode of activity**

The common denominator in the studied murine autoimmune models of lupus DSS-induced colitis and CIA is reduction in

Figure 1. TPC effect on four murine experimental models and ex vivo giant cell arteritis

The murine autoimmune models include: systemic lupus erythematosus, collagen-induced arthritis, DSS-induced colitis, and experimental autoimmune encephalomyelitis. The studies in humans show the TPC effect on PBMCs and biopsies ex vivo from patients with giant cell arteritis

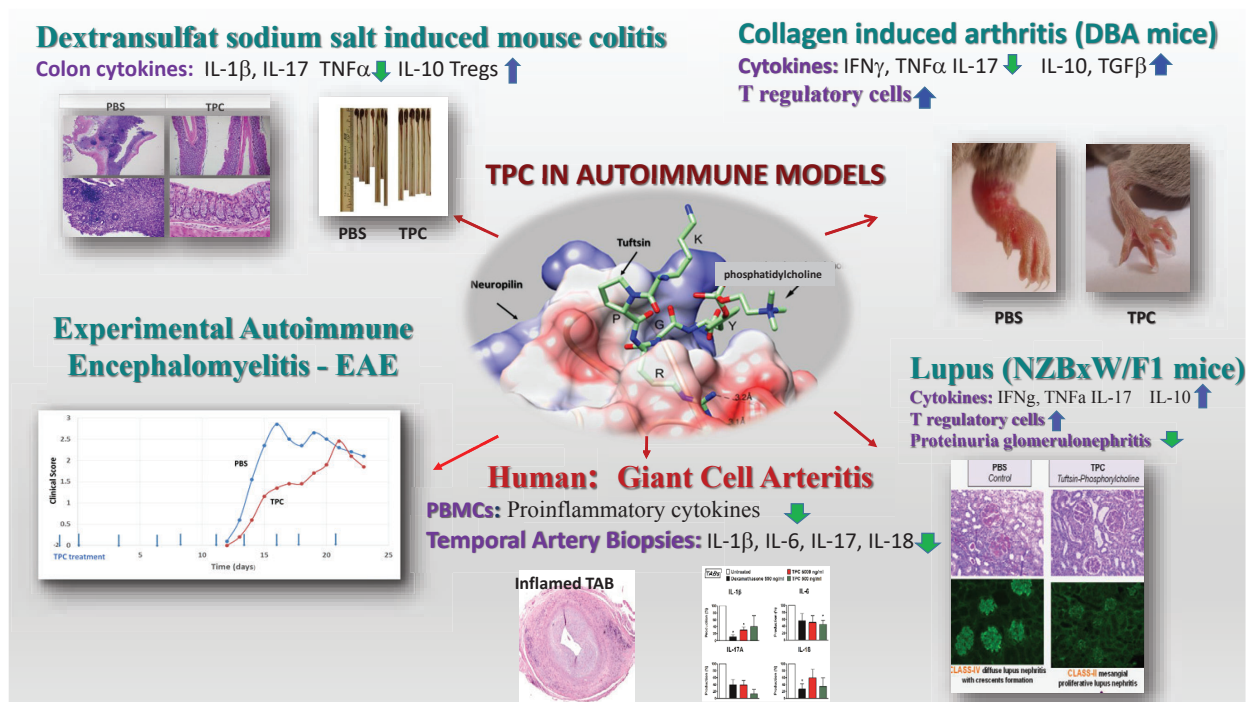
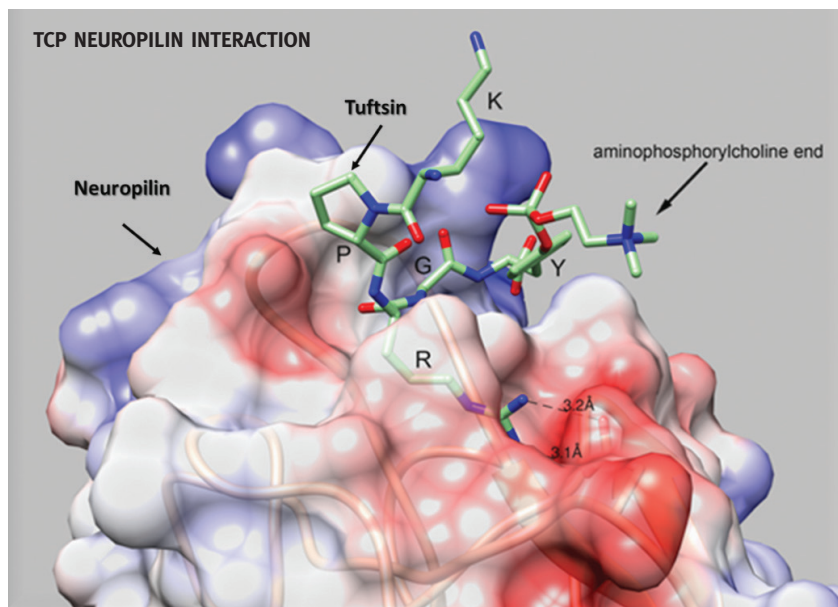


Figure 2. Model structure of the TCP neuropilin complex

Tuftsins are shown as a stick diagram with carbon, nitrogen and oxygen atoms in green, blue and red, respectively. The surface of neuropilin is shown, colored by the electrostatic potential: blue for positive, red for negative, and white for neutral. The surface was made transparent to show the binding of TPC residue R in a deep cavity and the bifurcated hydrogen bond between R and neuropilin residue D320



Blank M, Bashi T, Lachnish J, et al. Helminths-based bi-functional molecule, tuftsins-phosphorylcholine (TPC) ameliorates an established murine arthritis. *PLoS One* 2018; 13 (8): eo200615

clinical score and inflammatory cytokines (IFN γ , IL-1 β , IL-6, IL-17 and TNF α). Likewise, up-regulation of anti-inflammatory cytokine IL-10 expression was associated with enhanced T regulatory cell expansion.

TPC comprises two immunomodulatory molecules, phosphorylcholine and tuftsins, each of which has a definite activity and work synergistically. At this stage of research, it has been proven that TPC has a bi-functional activity: it inhibits NF κ B expression via inhibition of TLR4 activity by the phosphorylcholine edge of TPC, using a commercial inhibitor and HEK cells expressing just TLR4. Likewise, TPC shifts the macrophages from M1 inflammatory to anti-inflammatory M2, secreting IL-10 by the tuftsins part of the molecule targeting the neuropilin-1 [Figure 2] on macrophages and T regulatory cells [Figure 2] [45].

The fact that IL-1 β and TLR4 are inhibited by TPC paved the road for the idea that one of the TPC mechanisms of activity is via the NLRP3 inflammasome.

• TPC ex vivo in specimens from giant cell arteritis

Croci et al. [47] studied the effect of TPC ex vivo on both peripheral blood mononuclear cells (PBMCs) and temporal artery biopsies (TABs) obtained from patients with giant cell arteritis (GCA) and age-matched disease controls. GCA is an immune-mediated disease affecting large vessels. Croci's group activated the PBMCs ex vivo by CD3/CD28 beads and tested inflammatory cytokine secretion and IL-10 anti-inflammatory cytokine. Treatment ex vivo with TPC decreased the production of IL-1 β , IL-2, IL-5, IL-6, IL-9, IL-12(p70), IL-13, IL-17A, IL-18, IL-21, IL-22, IL-23, IFN γ , TNF α , and GM-CSF by activated PBMCs, whereas it negligibly affected cell viability. It

reduced Th1 and Th17 differentiation in PBMCs stimulated by phorbol 12-myristate 13-acetate plus ionomycin. In inflamed TABs, treatment with TPC down-regulated the production of IL-1 β , IL-6, IL-13, IL-17A, and CD68 gene expression. The effects of TPC were comparable to the effects of dexamethasone, included as the standard of care, with the exception of a greater reduction of IL-2, IL-18, and IFN γ in CD3/CD28-activated PBMCs, and CD68 gene in inflamed TABs.

CONCLUSION

Currently, available treatment options for patients with autoimmune diseases are mostly not curative and unfortunately are associated with significant adverse effects that contribute to morbidity and mortality. There is a need for small molecules with minimal side effects. Imitating the helminth immunomodulatory products may offer a rainbow of opportunities to develop such drugs for patients with autoimmune diseases.

The novel TPC molecule, comprising phosphorylcholine and tuftsins, which is based on a helminth product, demonstrated significant efficacy in several murine models of autoimmunity and GCA ex vivo. Hence, TPC and other described helminth products may prove to be potential future drugs to combat human autoimmune conditions.

Conflict of interest

Y.S. and M.B. have shares in TPCera

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“The cost of liberty is less than the price of repression”

W.E.B. Du Bois (1868–1963), American sociologist, historian, civil rights activist, and author