Thymulin: An Emerging Anti-Inflammatory Molecule

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Abstract: Thymulin is a neuroendocrine hormone with immunoregulatory actions. Originally known as ‘serum thymic factor’ (FTS), thymulin binds to a carrier protein and zinc (Zn2+) to exert its biologic properties. Thymulin, albeit an essential hormone for the T lymphocyte differentiation and the normalization of the ratio of T-helper cells to suppressor cells, accumulating evidence suggests its involvement in inflammations of various etiologies. Recently, thymulin has been shown to have anti-nociceptive effects in hyperalgesia and in pain of neurogenic origin, ostensibly through action on sensory afferents and the release of anti-inflammatory mediators. Given its anti-inflammatory potential, thymulin downregulates the release of inflammatory mediators, such as cytokines and chemokines, upregulates anti-inflammatory factors, such as interleukin (IL)-10, and exerts molecular control via the regulation of transcription factors and mediators. Recent evidence tends to indicate that thymulin can be a therapeutic agent in many inflammatory diseases and in pathological conditions affecting the peripheral and/or the central nervous system. This review discusses current concepts in the anti-inflammatory actions of thymulin and correlates this activity with an emerging theme for therapeutic treatment.

Keywords: Anti-inflammatory, cytokines, IL-10, immune-therapy, inflammation, neurodegeneration, transcription factors, thymulin.

1. INTRODUCTION AND BACKGROUND

Thymulin provides an element of the interface in the discrete network established between the neuroendocrine and immune systems [14,41,73]. The biological activity of thymulin is dependent on equimolar interaction with zinc (Zn2+). The bio-availability of this ion affects cellular immune effectiveness under physiological and pathophysiological conditions [12,13,51]. It has been shown that thymulin is capable of modulating pro-inflammatory cytokines in disease [58,63], thereby providing evidence for a novel anti-inflammatory potential. In addition, thymulin participates in signaling pathways that mediate genetically-controlled cell fate and apoptosis [14].

The underlying mechanisms of thymulin-mediated immunoregulation are being elucidated.

It has been reported that the effects of thymulin in down-regulating an inflammatory signal are mediated, at least in part, by modulating intracellular cyclic nucleotides [44,53]. In addition to the potent anti-inflammatory properties of thymulin, Zn2+ can synergistically down-regulate a pro-inflammatory signal by reducing the release of inflammatory mediators [15,51,60] and by acting as a cytoprotective anti-oxidant in the face of oxidative challenge and stress [22,27]. Furthermore, the anti-inflammatory effect of thymulin is ostensibly mediated via the up-regulation of IL-10 in vitro and in vivo [68].

Thymulin represents an emerging theme in anti-inflammatory therapy, for that particular reason we are discussing current concepts pertaining to the role of this molecule in physiologic and pathophysiologic conditions, with particular emphasis on its anti-inflammatory and antihyperalgesic effects.

2. THYMULIN IN INFLAMMATION AND DISEASES

2.1. Role in Neuro-Immune-Endocrine Interactions

Thymic peptides, a heterogeneous family of polypeptidic hormones synthesized within the thymus, not only exert important regulatory effects, within both the immune and neuroendocrine systems, but are also themselves subject to control by hormones derived from the hypothalamic-pituitary-adrenal axis (HPA) and other endocrine glands [14,57,64,72,74]. Thymulin, a nonapeptide member of this family originally known as serum thymic factor (FTS) [11,50], is a critical modulator of immunity and a mediator of neuro-endocrine interactions [24,64], in addition to regulating immune-related diseases and inflammation [25].

Thymic endocrine function and its fundamental role in regulating neuroendocrine human diseases have captured a backlog of interest over the past few decades given the prowess influence on the immune system. Retrospectively, an initial report to correlate immunity with thymic peptides emerged with the role of a circulating thymic factor in self-recognition and self-tolerance [5]. Moreover, in thymecutomized mice, FTS restored colony-forming unit by entry into DNA synthesis after T-dependent antigen treatment [31,36]. Restoration of the failing thymic secretion did influence auto-antibody production, in a manner depending primarily on the auto-antigen eliciting the autoimmune response [6,9,82]. In addition, administration of sodium diethyldithiocarbamate (a chelating agent used primarily in the analytical determination of copper, arsenic, nickel and other
metals) gave evidence for a role of the thymus, and its hormones, in the control and regulation of factors inducing thymocyte differentiation [52,74].

2.2. Role in Disease Modulation

The role of thymulin in disease modulation emanated from its effect on natural and induced allergy, autoimmunity and related syndromes. For instance, thymulin exhibited therapeutic actions in conditions such as ageing [21], Alzheimer’s disease (AD) [37], chronic graft-versus-host disease [4], immune deficiency [7,28], subacute sclerosing panencephalitis [26], myasthenia gravis [39], cancer [25,32,80], rheumatoid arthritis (RA) [1,17], Down’s syndrome [19,38], diabetes [84], viral [42,46] and parasitic [34] infections, drug-induced toxicity [78] and transplantation [8,30,76]. Moreover, the in vivo treatment with synthetic thymulinameliorated acute experimental allergic encephalomyelitis (EAE) with specific restoration of lymphocyte populations [35]. This was corroborated with thymulin ability to modulate inflammatory responses in patients afflicted with multiple sclerosis (MS) [33,45,54].

Perturbations in T cells and T cell subsets of peripheral blood lymphocytes were also reported in patients with RA and systemic lupus erythematosus (SLE). For instance, T cell subsets alterations responsible for abnormal values of the CD4+/CD8+ immunoregulatory ratio were improved by in vitro incubation of the lymphocytes with synthetic thymulin in RA patients [17,18]. Nonetheless, no significant modification occurred for SLE patients’ lymphocytes, thus supporting the possible beneficial role of thymulin in the treatment of RA [1]. Moreover, the loss and dysfunction of human post-thymic precursor cells in SLE were partially corrected with thymulin administration [49,83]. Several clinical trials for thymulin-mediated anti-arthritis and anti-SLE have been undertaken [10,18,75]. The outcome of these studies needs further investigations.

2.3. Thymulin and Inflammatory Mediators

The thymus gland is a central lymphoid organ where bone marrow-derived T cell precursors undergo maturation, eventually leading to the migration of positively selected thymocytes to T-dependent areas. This process follows under the influence of the thymic microenvironment, by means of secretory polypeptides and cell-cell contacts [71]. The thymic microenvironment is a cellular network composed of epithelial cells, macrophages (monocytes), dendritic cells, fibroblasts and extracellular matrix elements, which regulate key elements in the neuro-immune-endocrine axes.

Cytokines, or the then named ‘biological response modifiers’ [3,29], hold promising avenue for the anti-inflammatory therapeutic approach of thymulin [21,23]. The first association emerged between IL-2 and thymulin, which synergistically enabled autologous rosette-forming T lymphocytes to generate helper and cytotoxic functions in normal [77] and nude [49] mice. Additionally, thymulin promoted IL-2 production in intact and thymus-deprived mice [81].

Interestingly, it has been reported that thymulin could modulate cytokine release by peripheral blood mononuclear cells, with implications to SLE [58]. Moreover, interferon (IFN) production and NK cell activity were enhanced in the thymulin-treated mice, suggesting that IFN might play an important role in the induced resistance to pseudorabies virus (PRV) infection in mice [47]. The in vivo administration of thymulin prevented D-variant of encephalomyocarditis (EMC-D) virus-induced diabetes and myocarditis in BALB/cAJcl mice [43]. This was accompanied by mild degeneration of the islets of Langerhans and myocardia, and the conspicuous suppression of inflammation and inflammatory mediators. Furthermore, thymulin reduced bleomycin-induced cytokine and chemokine production and inhibited pulmonary fibrosis in mice [86].

Similar results were obtained when NK cell cultures were incubated in vitro with thymulin prior to assessing cytotoxicity [40]. Thymulin enhanced the cytolytic activity for NK cells for several chicken strains and increased the responsiveness to NK cells to IFN? stimulation. This is in concert with the observation that thymulin could suppress macrophage responsiveness to IFN? [48]. Of note, a suppressor mechanism of thymulin on TNF-a-induced apoptosis in the mouse pancreatic β-cell line, via a negative feedback mechanism involving the inhibition of inducible nitric oxide synthase (iNOS) induction, clearly indicated an association between the anti-inflammatory and anti-apoptotic actions of this peptide [85]. Recently, thymulin was shown to reduce the hyperalgesia and cytokine up-regulation induced by cutaneous leishmaniasis in mice [34].

3. ANTI-INFLAMMATORY ACTIONS OF THYMULIN

3.1. Animal Models for Autoimmune Diseases

A conventional example was reported with EAE which was induced in Hartley guinea pigs and Lewis rats that were treated with synthetic FTS [35]. Intermittently, clinical symptoms of acute EAE were suppressed. Furthermore, histopathological evaluation showed that the severity of EAE in FTS-treated guinea pigs was less than in untreated animals. Of note, immuno-histochemical examination showed that the numbers of CD8+, W3/25+, W3/13+ and CD19+ cells in FTS-treated rats were less than in untreated rats and that the number of CD8+ cells in FTS-treated rats was greater than in untreated rats, suggesting that FTS induces CD8+ cells in inflammatory lesions and suppresses inflammation in acute EAE [35]. On the contrary, in a study aimed at restoring decreased T-cell functions and reduced susceptibility to proteolipid apoprotein (PLP) induced-EAE in old mice with thymic hormones, thymosin fraction 5 (TF-5) and FTS had no significant in vitro and in vivo effect on proliferative responses to PLP and concanavalin A (Con A), and on EAE induction in young and old mice [16]. These results suggested that decreased T-cell functions cannot be restored by these thymic hormones, at least in this model of inflammation being invested.
3.2. Animal Models for Rheumatoid Arthritis

Thymulin plays a major role in modulating natural and experimentally-induced arthritis and systemic lupus. For instance, mice from three different strains (NZB, B/W and Swan), which spontaneously develop a lupus-like disease and show a premature decline of their secretion of FTS, were treated repeatedly with FTS and followed for the evolution of their autoimmune disease [6]. The autoimmune sialoadenitis (Sjogren’s syndrome) appearing in NZB and B/W mice was completely prevented or even cured by FTS treatment. Furthermore, the increase in anti-erythrocyte autoantibody production was transiently delayed in aged NZB mice. Conversely, anti-DNA antibody production either remained unaffected or was accelerated (in B/W mice) by FTS treatment, demonstrating that the restoration of the failing thymic secretion does influence autoantibody production, in a manner depending primarily on the auto-antigen eliciting the autoimmune response [6].

In another experimental setup, in collagen induced arthritis (CIA), rats were immunized with bovine type II collagen plus Freund’s incomplete adjuvant, and then thymulin was administered intraperitoneally on the first day of the first immunization [2]. Serum thymulin levels in CIA rats were reported significantly lower than in untreated rats. In addition, thymulin diminished hind paw swelling (edema) and onset of arthritis compared with control rats. The serum anti-type II collagen antibody level was also reduced by thymulin. Of note, histopathological examination showed inhibition of cell degranulation and new bone formation after injection of thymulin [2]. These results suggest that thymulin plays a significant role in the onset and development of CIA, indicating that this peptide may be therapeutically effective in preventing the development of RA.

3.3. Animal Models for Localized Peripheral/Central Inflammation and Hyperalgesia

Growing evidence unequivocally support the anti-inflammatory, analgesic effects of thymulin. This effect is often associated with the exhibition of anti-inflammatory properties [64]. For example, in a model of peripheral localized inflammation, induced by intraplantar endotoxin (lipopolysaccharide; LPS), thymulin reduced inflammatory pain, as measured with mechanical and thermal hyperalgesia [59,64]. A recently synthesized peptide analogue of thymulin (PAT) was also reported to have antihyperalgesic and anti-inflammatory effects [69]. The effect of PAT has extended to implicate inhibitory effects on pain of neurogenic origin [56].

On the mechanisms mediating the suppression of hyperalgesia and associated inflammation, it has been observed that thymulin reversed LPS-induced hyperalgesia by down-regulating gelatinase activity, a degrading proteinase associated with certain pathological conditions [79]. In addition, PAT’s anti-inflammatory ability was attributed, at least in part, to the down-regulation of pro-inflammatory mediators [69].

Thymulin treatment, moreover, reversed inflammatory hyperalgesia and modulated the increased concentration of pro-inflammatory cytokines induced by intra-cerebroventricular LPS injection, in vivo, thereby providing behavioral and immunocochemical characterization of a rat model for intracerebral inflammation and indicates a neuroprotective role for thymulin in the central nervous system (CNS) [70].

In some models, thymulin anti-inflammatory ability is exhibited at the level of inducing the release of anti-inflammatory mediators. Recently, Haddad et al. [22] demonstrated an immunomodulatory potential of thymulin in the lung perinatal epithelium. In an in vitro model of fetal alveolar type II epithelial cells, thymulin selectively ameliorated LPS-induced release of IL-1β, but showed no inhibitory effect on IL-6 and TNF-α. Furthermore, Zn2+, purported as an anti-inflammatory antioxidant, which is required for the biological activity of thymulin, reduced the secretion of IL-1β, TNF-α and, to a lesser extent, IL-6 [22]. Of note, this cation amplified the effect of thymulin on IL-1β and TNF-α, but not on IL-6. Analysis of whether thymulin is up-regulating a counterpart anti-inflammatory signaling loop revealed the involvement of an IL-10-sensitive pathway, suggesting that thymulin acts as a novel dual immunoregulator by enhancing an anti-inflammatory cytoprotective response and depressing an inflammatory signal, an effect synergistically amplified, in part, by cationic Zn2+ [22].

Recently, we have described a mechanism involving nuclear factor-κB (NF-κB), an inflammatory transcription factor [22], by which thymulin may exert its anti-inflammatory effect. In a rat model of localized and central hyperalgesia, thymulin reduced NF-κB DNA-binding activity, ostensibly via the up-regulation of the cytosolic inhibitor IκB-α by down-regulating its phosphorylation in vivo [67]. Furthermore, evidence in vitro in dorsal root ganglia neurons (DRGs) indicated that thymulin antagonizes LPS-induced secretion of IL-6 and TNF-α, and, interestingly, up-regulates the release of IL-10 [68]. Moreover, in a rat model of septic shock, the level of thymulin in serum was increased; however, it has abolished activity as determined by the Rosette assay. Assessment of the possible mechanisms revealed that LPS depletes Zn2+, thus explaining the inactive thymulin despite its high concentration. Adding extracellular Zn2+ restores the activity of thymulin. On the other hand, ablation of capsaicin sensitive primary afferents prevented the increase of thymulin level by LPS, which suggests the involvement of neuroendocrine loop mediating the effects of septic shock on thymulin [65].

Despite the conspicuous anti-inflammatory, analgesic effects of thymulin, it is noteworthy to mention that this peptide, at low concentrations, can intriguingly induce opposite effects. For instance, intraperitoneal injection of thymulin to rats caused a significant reduction in both mechanical (paw pressure test) and thermal (hot plate and tail flick tests) nociceptive thresholds and increased IL-1β level in the liver [61,62]. Moreover, intraplantar injection of low doses of thymulin induced hyperalgesia via the up-regulation of IL-1β, nerve growth factor (NGF) and PGE2 in the skin of the injected paw [66]. Of particular interest, ablation of capsaicin-sensitive primary afferents (CSPA) could alter or abolish thymulin-induced hyperalgesia [55]. These antagonistic effects of thymulin were primarily purported to be physiologic rather than inflammatory per se [64].

The possible neuro-immune-endocrine mechanisms underlying the anti-inflammatory and the anti-hyperalgesic effects of thymulin are schematized in Fig. 1.
Fig. (1). A schematic diagram of the role of thymulin in regulating neuro-immune endocrine actions. The anti-inflammatory role of thymulin involves several messenger overlaps between the nervous, immune and endocrine systems.

4. SUMMARY AND CONCLUSION

The nervous, immune and endocrine systems communicate through multiple common messengers. Over evolutionary time, integrated defense systems have developed to coordinate these communications for specific contexts, including the stress response, acute-phase response, nonspecific immune response, immune response to antigens, immune tolerance, time-dependent sensitization, neurogenic switching and traumatic dissociation. Thymulin, a tri-dimensional communicator (neuro-immune-endocrine decipher), is an essential corner stone in the evolution of an integral immune system and a functional immune response. With its emerging role as an antiinflammatory decoder, thymulin may well serve the grounds of establishing an intervention therapy, especially for diseases affiliated with neurogenic inflammation. Research continues apace for better fathoming the interesting, yet intriguing, burgeoning role of thymulin in anti-inflammatory therapy.

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LIST OF ABBREVIATIONS

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<tr>
<td>ConA</td>
<td>Concanavalin A</td>
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<tr>
<td>EMC-D</td>
<td>D-variant of encephalomyocarditis</td>
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<td>EAE</td>
<td>Experimental allergic encephalomyelitis</td>
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<td>GH</td>
<td>Growth hormone</td>
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<td>iNOS</td>
<td>Inducible nitric oxide synthase</td>
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<td>IGF</td>
<td>Insulin-like growth factor</td>
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<td>Interleukin</td>
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<td>LPS</td>
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<td>MT</td>
<td>Metallothionein</td>
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<td>NK</td>
<td>Natural killer</td>
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<td>PAT</td>
<td>Peptide analogue of thymulin</td>
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<td>PRL</td>
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<td>PLP</td>
<td>Proteolipid apoprotein</td>
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<td>PRV</td>
<td>Pseudorabies virus</td>
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<td>RA</td>
<td>Rheumatoid arthritis</td>
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<td>FTS</td>
<td>Serum thymic factor</td>
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<td>SLE</td>
<td>Systemic lupus erythematosus</td>
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<td>TF-5</td>
<td>Thymosin fraction 5</td>
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<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor</td>
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REFERENCES


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