Cyclosporin A as an Alternative Neuroimmune Strategy to Control Neurites and Recover Neuronal Tissues in Leprosy

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Leprosy, also known as Hansen’s disease, continues to have a substantial impact on infectious diseases throughout the world. Leprosy is a chronic granulomatous infection caused by Mycobacterium leprae and shows a wide clinical and immunopathological spectrum related to the immune response of the host. This disease affects the skin and other internal organs with a predilection to infect Schwann cells, which play an active role during axonal degeneration, affecting peripheral nerves and promoting neurological damage. This chronic inflammation influences immune function, leading to neuroimmune disorders. Leprosy is also associated with neuroimmune reactions, including type 1 (reverse) and type 2 (erythema nodosum leprosum) reactions, which are immune-mediated inflammatory complications that can occur during the disease and appear to worsen dramatically; these complications are the main concerns of patients. The reactions may induce neuritis and neuropathic pain that progressively worsen with irreversible deformity and disabilities responsible for the immunopathological damage and glial/neuronal death. However, the neuronal damage is not always associated with the reactional episode. Also, the efficacy in the treatment of reactions remains low because of the nonexistence of a specific treatment and missing informations about the immunopathogenesis of the reactional episode. There is increasing evidence that peripheral neuron dysfunction strongly depends on the activity of neurotrophins. The most important neurotrophin in leprosy is nerve growth factor (NGF), which is decreased in the course of leprosy, as well as the presence of autoantibodies against NGF in all clinical forms of leprosy and neuroimmune reactions. The levels of autoantibodies against NGF are decreased by the immunomodulatory activity of cyclosporin A, which mainly controls pain and improves motor function and sensitivity. Therefore, the suppression of anti-NGF and the regulation of NGF levels can be attractive targets for immunomodulatory treatment and for controlling the neuroimmune reactions of leprosy, although further studies are needed to clarify this point.

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Introduction

Leprosy is a chronic infectious disease caused by the bacteria *Mycobacterium leprae*, an obligatory intracellular pathogen which has predilection for Schwann cells and mononuclear phagocytes [1]. After infection, dissemination results in progressive skin lesions and damage of peripheral nerves. Sometimes human Schwann cells infection by *M. leprae* result in neurodermatologic aspects that remain not so easy to be diagnosed [2]. Leprosy includes a range of subtypes which vary from main diagnosis in clinical presentation or typical severity. Initially, as consequence of *M. leprae* infection, the leprosy skin early loss the temperature and cutaneous pain sensitivity, characterizing the indeterminate form of leprosy [3–5]. The indeterminate form of leprosy is capable to polarize for tuberculoid disease when a cellular immune response to *M. leprae* is strongly developed, maintaining rare the bacilli quantity into well-organized granulomas, characterizing the paucibacillar form of leprosy [3, 6, 7], while in lepromatous spectrum the cellular immune response is ineffective, contributing to high load of bacteria and inflammatory response with foamy histiocytes [6–8]. In many cases, between the polar forms of leprosy exist with common characteristics: borderline-tuberculoid, borderline-borderline, and borderline-lepromatous forms. In addition, exists the pure neural form of leprosy, which does not show cutaneous manifestations because the presence of *M. leprae* is restricted in the nerves, increasing the risk of developing nerve damage and disability [9].

The disease shows reactions, which are immunological episodes that lead loss of nerve function and can be aggravated with disabling sequelae if not quickly controlled. Type 1 leprosy reaction or reversal reaction is a late cellular hypersensitivity reaction type IV that occurs in borderline forms of disease, the cell-mediated immunity against mycobacteria increases, secreting TNF-α and IFNy in skin and nerves, inducing oedema and pain [1, 10–12]. Type 2 leprosy reaction or erythema nodosum leprosum occurs in lepromatous spectrum and borderline-lepromatous forms of disease, is associated with antibody-mediated immunity, and induces the formation of immune complexes, infiltration of Th2-cells, and high concentration of TNF-α, developing systemic inflammation resulting in acute neurite that may affect the nerves mobility [9, 13].

Many immunological aspects of leprosy reactions are unknown and the conventional treatment with corticosteroid is the main way to control the neuritis or inflamed skin. During the steroid treatment, the inflammation is slow controlled, but in some cases, the conventional 6 months of treatment can not be sufficient, especially in steroid-unresponsive patients [10, 11]. Others immunosuppressant drugs are often used to treat reactions, including the cyclosporin A that reduced not only the skin and nerve inflammation but also control the neural pain and decreased the anti-nerve growth factor (NGF) antibodies, which are related to neurites, inflammation, and loss of cutaneous nociception [10, 11, 13]. We have studied the neuronal role of cyclosporin A and unpublished data suggest that cyclosporin A induces neuronal differentiation that may be important to improve the sensory impairment, muscular force, and pain of leprosy patients. Therefore, in this review, we explore how cyclosporine contributes to control leprosy reactions.

Leprosy and the Immunological Host Response

Leprosy is a chronic granulomatous infection caused by *M. leprae* and shows a wide clinical and immunopathological spectrum of host immune responses to pathogens. The major transmission routes of leprosy are the skin and nasal mucosa [14]. The incubation period for leprosy is variable, from a few weeks to even 20–30 years. The average incubation period for the disease is 5–7 years [15].

*M. leprae* is a mandatory intracellular parasite that is mainly found inside macrophages and Schwann cells; these tropisms lead to damage to peripheral nerves, skin, and mucous membranes, causing a disease that can vary from a benign clinical form to a malignant, disseminated form, thus constituting a chronic inflammatory disease [16–18]. *M. leprae* infection leads to chronic inflammation in skin and peripheral nerves that can lead to sensory, motor, and autonomic impairments. Therefore, leprosy is a demyelinating disease that can be aggravated during the inflammation and their nerve fibres are damaged and degenerated [1, 5, 19]. Previous studies have shown that *M. leprae* enter and modify adult Schwann Cells by a controlled loss of stability that could reprogram itself to become “stem-like cells” that contributes for persistence in peripheral nerves without activates the immune system and promotes dissemination of infection” [20, 21].

At this point of infection, the immunological host response will determine the clinical course of the disease. It is clearly known that many aspects that compromise immune functions, including living conditions, immunodeficiency diseases such as HIV and host immunogenetics,
are important to determine susceptibility factors and increase leprosy risk [15, 22].

Most of the serious symptoms and complications of leprosy are due to so-called leprosy reactions, which occur due to the body’s immune response against antigenic constituents released by the bacillus [10, 23, 24]. These reactive episodes or leprosy reactions can lead to disabling sequelae and are the major cause of leprosy-associated irreversible neuropathy [10]. Reactions may induce permanent damage to nerves and cause severe neuropathy, but not all pain in leprosy is neuropathic [1, 8, 25]. In addition, there are patients with nociceptive pain and both types of pain may coexist [1, 19]. Also, there are patients with reaction without pain and also pain in non-reactional neural pure patients [1, 25]. These injuries lead to an immune response near the site of injury. Schwann cells, the glial cells of peripheral ganglia, are activated, and each single peripheral sensory neuron is intimately associated with numerous Schwann cells [26]. In addition, these interactions release various neural and immune mediators in the terminals of primary afferents, and this crosstalk leads to the sensitization of postsynaptic neurons and the activation of glia. The activated glia, in turn, release pro-inflammatory factors, further sensitizing the neurons. M. leprae-specific phenolic glycolipid 1 induces an increase in reactive nitrogen species by infecting macrophages, conferring a neurotoxic response for neural mitochondria and demyelination [27]. The peripheral nerve injuries and infection of Schwann cells, causing axon demyelination, induce the downregulation of a key factor in myelination, krox-20, and the upregulation of p75NTR (low-affinity NGF receptor), suggesting possible targets for the repair of peripheral nerves [28]. However, these mechanisms are complex, are not yet fully understood, and are difficult to treat effectively.

**Neurotrophins in Leprosy**

Many clinical aspects of leprosy, including hypoalgesia, less skin pigmentation and peripheral neurodegeneration, are related to the depletion of neurotrophins in human and experimental animal models [29, 30]. Analysis of NGF, brain-derived nerve factor and neurotrophin-3 (NF3) has shown that NGF is a key component in leprosy and plays an important role in the survival and function of peripheral nerves. Currently, NGF is known as a neurokine because it functions not only in the nervous system but also in other systems, including the immune system. NGF is produced by many cells of inflammatory processes, including mast cells, keratinocytes, macrophages, T cells, and B cells [31–36]. Low-affinity p75NTR and high-affinity trkA NGF receptors are up-regulated during the pathophysiological inflammatory process in skin, especially during chronic inflammation that develops into peripheral sensory neuropathy and thermal hyperalgesia with loss of neural fibres [37].

However, in previous work, it was shown that NGF was decreased in multibacillary leprosy patients compared with non-infected individuals [3]. An analysis of affected skin and clinically unaffected skin from leprosy patients compared with control skin (non-leprosy) found a decreased level of NGF in both clinically unaffected and affected skin from leprosy patients. In contrast, there was only a slight alteration of high-affinity NGF receptor (trkA) in affected skin. Indeed, trkA may mediate inflammation-induced hypersensitivity during the progression of disease, and the reduction in NGF occurs before sensory loss in clinically unaffected skin in leprosy, suggesting that NGF may be important in restoring pain sensation [3].

These results were related to low expression of the axonal markers PGP9, PGP5, NF-L (neurofilaments-L), and p75NTR, indicating a direct association between Schwann cells and axons [30]. The first evidence showing NGF depletion by the autoimmunization-induced loss of thermal sensitivity to noxious stimuli was observed in an animal experimental model [29]. Recently, it was shown that blocking NGF by anti-NGF in an animal experimental model improved chronic neuropathic pain [38].

Natural autoantibodies against NGF were first found in autoimmune diseases, such as systemic lupus erythematosus, autoimmune thyroiditis, and rheumatoid arthritis, but the biological activity and physiological relevance of these autoantibodies were not well defined [39]. Previous work has indicated that natural antibodies against NGF suppress and inhibit prostate cancer cell metastasis and are directly connected with neuropathic and inflammatory pain [40, 41]. Therefore, targeting anti-NGF may be a novel approach to control cancer pain and peripheral and central sensitization [41, 42].

There are also human studies that show autoantibodies against NGF detected in the serum of all clinical forms of leprosy, which may explain the depletion of NGF [13]. In that work, a comparison of leprosy patients with and without chronic neuritis showed slightly lower NGF autoantibody levels in patients without chronic neurites, reflecting an effector function of these autoantibodies in the loss of nociception. When leprosy patients with chronic neurites were treated with cyclosporin A, anti-NGF levels...
were similar to those in control subjects (non-leprosy). Interestingly, after the treatment, there was an improvement in neuropathic pain early in the first month of treatment as well as partially recovered motor signals [13]. Thus, anti-NGF in leprosy could be functioning as an endogenous analgesic, but it compromises neural integrity during M. leprae infection, which can be attenuated after treatment with cyclosporin A.

**Cyclosporin A and NGF in the Nervous System**

Cyclosporin A is well established as a potent immunosuppressive drug that blocks the transcription of cytokine genes in activated T cells by inhibiting the phosphatase activity of the calcineurin/nuclear factor of activated T cell (NFAT) pathway to downregulate the expression of cytokines. NFAT is a transcription factor that plays an important role in the control of gene expression during cell activation and differentiation [43–45]. Cyclosporin also blocks the activation of the JNK and p38 signalling pathways [44, 46, 47].

Due to its potent immunosuppressive properties, cyclosporin A is useful for the treatment of allograft rejection and graft-versus-host reaction, corneal allograft rejection, and other ocular pathologies, including conjunctivitis and keratoconjunctivitis sicca [48]. NFAT has 5 members (NFAT1 to NFAT5), but NFAT5 function differently in a calcineurin-independent pathway because of the absence of the calcineurin regulatory motif [49]. NFAT5 was initially described as a transcription factor involved in the maintenance of cellular homeostasis against hypertonic and hyperosmotic environments and was named tonicity-responsive enhancer-binding protein [50, 51]. NFAT5 is also associated with immune regulation and the development of pro-inflammatory immune responses in hyperosmotic stress, inducing cell survival, proliferation, migration, and angiogenesis, and is related to the development of autoimmune diseases [49, 52, 53].

NFAT5 is also present in the embryonic and adult cells of the central nervous system, including neurons, microglia, and astrocytes, to protect against ischaemic and inflammatory processes [54–56]. The biological activity is dependent on the phosphorylation sites of NFAT5 and nuclear translocation kinase-dependent pathways, such as p38, ATM, GSK3β, CDK5, and CK1 [57].

Interestingly, the human corneal epithelial cell line expresses NGF after treatment with cyclosporin A via activation of MAPK p38 [58]. NGF alone is known to regulate the activity of calcineurin-dependent NFAT signalling in neurons to stimulate the rapid outgrowth and cell survival of embryonic axons in sensory neurons, but little is known about the relationship between NFAT5 and NGF in the central nervous system and peripheral nervous system [36, 59, 60].

Data not from our group provide evidence that cyclosporin A induces neuronal but not glial differentiation via NGF- and TrkA-dependent pathways after downregulation of NFAT5 in glial cells. Figure 1 summarizes these results, which are consistent with the findings of the role...
of cyclosporin A in the neuroimmune axis and can explain the clinical improvement in leprosy patients treated with cyclosporin A [13], characterizing an alternative treatment for erythema nodosum leprosum that focuses on the neuroimmunomodulation of leprosy reactions [61].

Conclusion

Neuroimmune reactional episodes, which initially induce neuritis with severe neuropathic pain, represent the main concern of patients with leprosy. Many alternative strategies have been studied to identify the best way to control leprosy reactions before the neurodegeneration worsens, characterized by irreversible neuropathy and deformity because of the death of sensory and motor nerves. The use of an established drug such as cyclosporin A as an alternative treatment, not only to control the neuroimmune reactions but also to decrease the anti-NGF autoantibodies and local inflammation, to control neuronal pain and to increase motor force, helps injured tissues via the induction of NGF expression and its receptors p75NTR and trkA, which can favour neuronal and glial remodelling. Thus, we suggest that the use of cyclosporin A, with its neuroimmune effects in leprosy patients that help with the repair of injured peripheral nerves, is a promising strategy to control neuroimmune reactional episodes and treat them before they develop into an irreversible deformity.
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