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Leprosy is an ancient disease that remains endemic and continues to be a major public health problem in some tropical countries, where it has been internationally recognized as being linked to the underdevelopment conditions. The natural course of the disease covers a wide variety of clinical conditions with systemic involvement. In this paper, we review the findings obtained in studies of the pathological mechanisms of leprosy, including a survey of the literature and of our own work. The understanding and control of the wide variety of clinical conditions should help improve patient care and thus prevent the onset of physical impairment and the stigma of the disease.

Key words: leprosy - immunopathology - visceral manifestation - coinfection - neuropathy

Leprosy is an ancient disease considered to be a neglected tropical disease that remains endemic and is still a major public health problem in some tropical countries, where it has been internationally recognized as being linked to the underdevelopment conditions. It still persists in nonendemic areas because of the great mobility of people around the world and the increasing rates of immigration from countries where leprosy is endemic (Ooi & Moschella 2001, Gill et al. 2005, Walker & Lockwood 2006). The natural course of the disease covers a wide variety of clinical conditions with systemic involvement. In this paper, we review the findings obtained in studies of the pathological mechanisms of leprosy, including a survey of the literature and of our own work.

Leprosy is a chronic infectious disease caused by the intracellular pathogen Mycobacterium leprae, which presents a long period of incubation, providing an extraordinary opportunity to investigate the human immune regulation of infection, because it presents a spectrum of clinical manifestations that correlate with the immune response of the host against the pathogen (Ridley & Jopling 1966). The disease affects the peripheral nerves, skin and multiple internal organs, with a consequent difficulty in its clinical recognition. Thus, knowledge about this disease is important for a differential and conclusive diagnosis.

Immunopathological mechanisms in leprosy - The spectrum of clinical manifestations is correlated with the level of cell mediated immunity (CMI). Tuberculoid leprosy (TT) is at one pole of this spectrum, characterized by restricted growth of the pathogen and high CMI. At the opposite pole is lepromatous leprosy (LL), in which the CMI is strikingly absent, affecting minimally resistant hosts with a predominantly humoral immune response in a T helper (Th)2 pattern and a widespread dissemination of the bacilli. Between these two poles, there is the borderline group, which can be divided into three subgroups: borderline tuberculoid, borderline-borderline and borderline lepromatous (Arnoldi et al. 1990).

During the natural course of the disease, reactional episodes may occur, during treatment and even after treatment, involving two types of reactions. The reversal reaction (RR) seems to be associated with a sudden increase in CMI against M. leprae antigens and is characterized by a predominantly type-1 cytokine profile consisting of interleukin (IL)-1β, tumour necrosis factor-α (TNF-α), IL-2 and interferon-γ (IFN-γ), more frequent in borderline patients. The erythema nodosum leprosum (ENL) type of reaction, which occurs in multibacillary (MB) leprosy patients (LPs), is a more systemic reaction than the RR and is also immunopathologically more complex (Naafs 1994). In this reaction, it has been shown that there is a selective increase in IL-6, IL-8 and IL-10 levels, whereas the levels of IL-4 and IL-5 remain unchanged (Yamamura et al. 1992).

Taking into account the clinical characteristics and the host’s immunological reactivity to the spectral manifestations of leprosy, we carried out investigations to understand whether the mediators could be related to these manifestations. First of all, we observed that there is a potent production of anti-phenolic glycolipid-I (PGL-I) antibody in LL, but not in TT, with the humoral response, therefore, considered not to play a protective role (Callera et al. 1993). The serological levels of anti-PGL-I antibody can be reduced with multidrug therapy (MDT) (Zenha et al. 2009). We found that the anti-PGL-I antibody can be detected in saliva and serum and its determination may be useful for the evaluation of antigen exposure and response to MDT and for distinguishing MB from paucibacillary (PB) patients (Bonfitto et al. 2009). Another application of the detection of this antibody was studied in the evaluation of household contacts (HHC) of LPs, where we observed that the serum anti-PGL-I IgM...
antibody may be useful for evaluating antigen exposure and as a tool for an early leprosy diagnosis in HHC (Bazzan-Furini et al. 2011). In addition, elevated TNF-α levels were detected in supernatant cultures of macrophages from TT patients compared to those from LL patients (Silva & Foss 1989a), reflecting the impairment of macrophages from LL patients. We also observed that a glycolipid fraction from M. leprae is able to induce an inflammatory response (Silva & Foss 1989b) and the PGL-I antigen can suppress the cytokine release of human monocytes, suggesting that the antigens from M. leprae could regulate the immunological response in leprosy (Silva et al. 1993). Moreover, a correlation was observed between serum TNF-α detection, increased plasma C-reactive protein (CRP) and in vitro suppression of the T lymphocyte response to a mitogen (Con-A) during ENL, indicating the serum CRP levels are a clinical parameter that can be used to evaluate the intensity of the inflammatory response in ENL (Foss et al. 1993).

Despite the evidence of the inability of LL macrophages to destroy the bacillus, the mechanisms involved in regulating the activity of these cells have not been elucidated. Thus, we studied transforming growth factor-β1 (TGF-β1), a cytokine with macrophage-suppressing activity present in diseases caused by intracellular parasites (Silva et al. 1991, Barral-Neto et al. 1992, Bermudez et al. 1993, Lotz & Seth 1993). Initially, the detection of TGF-β1 in dermal lesions of different clinical forms of leprosy was evaluated (Goulart et al. 1996) and marked immunohistochemical staining for TGF-β1 was detected in macrophages from lesions of LL patients, which contained large amounts of bacilli, demonstrating that the inability of macrophages to process M. leprae was probably associated, at least in part, with the action of TGF-β1 inhibiting the microbicidal activity.

TGF-β1, a cytokine produced by activated macrophages and other cells, is a potent pro-inflammatory and immunosuppressive molecule with a plethora of immunoregulatory effects which are described as bifunctional (Wahl et al. 1989), in addition to effects on cell growth and differentiation (Wakefield et al. 1988). The immunosuppressive effect of TGF-β1 on leprosy lesions is more evident by the inhibition of the production of intermediate oxygen-reactive and nitrogen-reactive factors (Tsunawaki et al. 1988, Ding et al. 1990), leading to the progression of infection.

The role of circulating monocytes directed against M. leprae and its products and their relationship with the production of TGF-β1 by macrophages resident in this microenvironment was investigated in blood monocytes from patients with different clinical forms of leprosy and healthy individuals. TGF-β1 produced in vitro by cultures of adherent cells from peripheral blood mononuclear cells (PBMC) was spontaneously released in all clinical forms of leprosy and in healthy individuals, but patients with ENL showed markedly higher TGF-β1 concentrations than all other patients and controls. This led to the conclusion that TGF-β1 appears to play different roles in leprosy: it presents a pro-inflammatory effect on the inflammatory reaction, especially in ENL, stimulating the Th2 response and an immunosuppressive effect in the presence of PGL-I or other M. leprae antigens (Goulart et al. 2000).

Although the immunological aspects of leprosy have been extensively investigated, the exact mechanism of the systemic involvement of the disease remains unclear (Britton & Lockwood 2004). Leprosy is a pleiotropic disease that can resemble many dermatologic and neurological diseases and affects multiple organs, making its recognition challenging. In particular, the neurological and endocrine manifestations caused by leprosy have been long recognized but underestimated, even by specialists.

**Endocrine axis in leprosy** - The pathogenic correlation between the patterns of inflammation in the poles of the leprosy spectrum and the hormonal disarrangements has been investigated. The action of the hypothalamic-pituitary-adrenal axis on the involvement of the adrenal glands has been recorded in about 30% of leprosy cases in autopsy series (Powell & Swan 1955, Desikan & Job 1968, Bernard & Vazquez 1973), in which granulomas bacilli and amyloidal infiltration were documented in the adrenal cortex of LL cases.

The evaluation of cortisol and adrenal androgen secretion in a series of untreated PB and MB patients without clinically manifested adrenocortical hypofunction at baseline showed that corticotrophin-releasing hormone-stimulated adrenocorticotropic hormone and cortisol secretion did not differ from control. However, the levels of the adrenal androgen dehydroepiandrosterone sulphate (DHEA-S) were significantly lower in LPs compared to sex-matched control subjects. Additionally, a significant inverse correlation of both IL-6 and TNF-α with plasma DHEA-S levels was observed, suggesting the influence of inflammatory cytokines on adrenal androgen secretion in leprosy (Leal et al. 2003). This study was the first to investigate the relationship between adrenal hormones and inflammatory cytokine patterns in leprosy. A link between DHEA-S and the pathogenesis of systemic inflammatory processes in humans has been poorly explored.

The role played by local cortisol-cortisone metabolism in the skin has been investigated in leprosy and it has been hypothesized that inflammatory cytokines could locally affect the concentration of cortisol in leprosy skin lesions and the development of leprosy reactions (Rook & Baker 1999, Andersson et al. 2007). This regulatory mechanism of effective cortisol concentration extended to other tissues such as the peripheral nervous system could be adjusted by the administration of exogenous cortisol in preventing the progression of neuropathy (Jardim et al. 2007).

The gonadal involvement in leprosy, restricted almost exclusively to the testis and its manifestations have long been recognized and addressed clinically and in histopathologic studies, semen analysis and hormonal assays (Baptista 1937). There is wide variability in the reported frequency of testicular involvement in leprosy, ranging from 8-80%. In random surveys, about 50% of the screened LL patients showed hormonal changes suggestive of unsuspected gonadal involvement (Ree et al. 1981, Levis et al. 1989). The occurrence of hypogonad-
ism secondary to leprosy and its important consequences such as infertility and osteoporosis may be underestimated by specialists in leprosy.

Studies of ovarian function assessment in female LPs are rare and in those in which histopathology was performed, the ovaries were not involved (Grabstald & Swan 1952, Bernard & Vazquez 1973). Neena et al. (2003) detected menstrual irregularity in 30% of women of reproductive age with MB leprosy, as well as significantly high levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH).

The hormone profile of the male gonadal axis in leprosy has been extensively studied, showing low basal levels of testosterone and high basal LH and FSH levels secondary to the loss of testosterone-induced negative feedback in 30-80% of LL patients investigated (Ree et al. 1981, Rea 1988, Levis et al. 1989, Saporta & Yuksel 1994). The data regarding plasma prolactin levels in leprosy are controversial (Rolston et al. 1981, Kannan & Vijaya 1984, Saporta & Yuksel 1994, Leal et al. 2006) and the high prolactin levels observed have been attributed to estrogen stimulation (Kannan & Vijaya 1984). High plasma estrogen levels were demonstrated in MB patients and were attributed to the peripheral conversion of androgens and to impaired hepatic metabolism (Shilo et al. 1981, Kannan & Vijaya 1984, Garg et al. 1989, Saporta & Yuksel 1994).

The clinical manifestations of testicular damage in leprosy include gynecomastia, erectile dysfunction, infertility and changes in secondary sexual characteristics (female distribution of body hair and reduced testicular volume). Gynecomastia is the most obvious manifestation of hormone dysfunction in leprosy, reported in up to 85% of LL cases (Baptista 1937, Grabstald & Swan 1952, Shilo et al. 1981, Abraham et al. 1990, Saporta & Yuksel 1994) and has been attributed to the hormonal imbalance between estrogen and testosterone (Leal & Foss 2009).

In an investigation of the pituitary-gonadal hormones and the interleukin pattern in a series of random selected PB and MB LPs, LH and FSH were positively correlated with IL-1β, IL-6 and TNF-α and testosterone was inversely correlated with IL-6 and TNF-α, suggesting that these cytokines may have a direct effect at the testicular level and may be of pathogenic significance in leprosy (Leal et al. 2006). This favours the hypothesis that the inflammatory cytokines seem to play a fundamental role in the local control mechanisms of testosterone synthesis in Leydig cells and indicates the treatment of hypogonadism in leprosy since testosterone therapy is associated with the improvement of clinical and inflammatory markers (Leal & Foss 2009). Additionally, Rodrigues et al. (2011) reported that inflammatory response to *M. leprae* is associated with alterations in the insulin-like growth factor-1 (IGF-1) signalling circulating IGF-1/IGF BP-3 levels as possible predictive biomarkers for ENL in LL patients at diagnosis.

Regarding calcium and bone metabolic disease, hypo and hypercalcaemia have been described in leprosy (Hoffman & Korzeniowski 1986, Rao & Saha 1986, Ryzen et al. 1988, Couri et al. 2004). The most common metabolic bone disease, osteoporosis, is of particular interest in leprosy because of the risk of bone fracture in patients who already have neural lesions and both specific and nonspecific bone changes that result in deformities and disabilities (Paterson & Rad 1961, Thappa et al. 1992, Illarramendi et al. 2001).

Hypercalcaemia and abnormal 1,25 dihydroxy vitamin D, parathyroid hormone (PTH) and PTH-related protein concentrations have been reported in only a few patients with borderline and LL (Couri et al. 2004). On the other hand, hypercalcaemia was observed in two unusual cases of chronic granulomatous diseases presenting normal levels of vitamin D in light of low levels of PTH (Sharayyef et al. 2011). It was reported that bone mass loss is an early event in LPs and is frequently already present at diagnosis (Ribeiro et al. 2007). The development of bone metabolism alterations in leprosy have been investigated and it was observed that *M. leprae* inhibits the expression of phosphate-regulating gene with homologies to endopeptidase on the X chromosome in Schwann cells and osteoblasts, and that it can lead to extensive bone disease (Silva et al. 2010).

There are few data regarding endocrine dysfunction in leprosy, which is usually neglected, even by specialists. Nevertheless, this condition contributes to the burden of the disease, especially the frequent involvement of the testis and secondary complications, such as infertility and osteoporosis (Leal & Foss 2009). Besides the activity on bone metabolism, the involvement of vitamin D in the steps of intracellular antimicrobial mechanisms has been reported (Matzner et al. 2011) and most recently it was demonstrated that microRNA-21 targets the vitamin D-dependent antimicrobial pathway in leprosy (Liu et al. 2012).

**Reational episodes** - Leprosy reactional episodes can be responsible for much of the permanent nerve damage, leading to disability and deformities, and there is evidence that these episodes may be associated with an infectious process. We investigated the role of coexisting factors in the patient’s condition that could be related to the exacerbation of *M. leprae* infection. First of all, we determined the frequency of leprosy reactional episodes and coinfections in LPs in order to evaluate whether there was a relationship between these conditions. We found that ENL was the most prevalent reaction type, especially in MB patients presenting any coinfection. Additionally, we verified that oral infections, including periodontal diseases and dental abscesses, were the most prevalent coinfection, followed by urinary tract infection, sinusopathy and viral hepatitis (ACF Motta et al., unpublished observations). These findings suggest that the presence of coinfections may be involved in the development and maintenance of leprosy reactional episodes. Thus, it is necessary to screen LPs for chronic systemic and local infections because treatment of coinfections may improve their care and help prevent disability caused by leprosy reactions.

Additionally, we evaluated the expression of proinflammatory serum biomarkers in LPs presenting oral infections (periodontal diseases, irreversible pulpitis, pulpal necrosis, or inflammatory periapical lesions) and
we observed higher CRP, IL-10, IL-1 and IL-6 levels in the presence of oral infections, suggesting that oral infections can act as a maintenance factor of the pro-inflammatory state (Motta et al. 2010, 2011). Thus, the coexistence of chronic oral infections with leprosy can stimulate the inflammatory reaction by elevating the expression of intracellular inflammatory markers.

Considering that the measurement of biomarkers in serum, saliva and plasma cannot show the real participation of peripheral blood cells and the amount of cytokines produced by each cell population, impairing the understanding of the inflammatory response, we determined the intracellular profile of pro-inflammatory cytokines such as IL-2, IL-4, IL-10 and IFN-γ in PBMCs from LPs stratified according to the presence of chronic oral infections (periodontal and/or dental infections) to determine whether these coinfections could be associated with pro-inflammatory activity in leprosy. We verified low percentages of CD3+ lymphocytes bearing IL-2, IL-10 and IFN-γ in LPs with oral infections and reduced percentages of IL-4 and IFN-γ in LPs without oral infections. However, LPs with and without oral infection presented high percentages of CD3+ cells bearing IL-4 (unpublished observations). We concluded that the occurrence of oral infections favours the expression of intracellular cytokines and probably the inflammatory reaction, acting as a stimulatory signal triggering the re-convalescent episodes in LPs.

Based on these studies, where we evaluated the relationship between leprosy reactions and coinfections, we can suggest that coinfection treatment may improve the care of LPs in order to prevent reconvalescent episodes. These results may help for the treatment of LPs as a tool for public health professionals, especially physicians, for the follow-up and treatment of leprosy.

Neurological abnormalities in leprosy - In addition to the systemic involvement consequent to the development of the infectious process induced by M. leprae, neurological abnormalities have been investigated in LPs. Initially the best technical approach to the detection of peripheral nerve involvement in leprosy neuropathy was extensively investigated. It was observed that the sensory action potentials are better evaluated by the near-nerve potential technique that records potentials from nerve fibres as small as 4-6 RM in diameter (Marques et al. 2003).

This result show that electrophysiology (EPG) is a sensitive resource for the detection of the early stages of peripheral nerve involvement in leprosy, even regarding the initial forms of the diseases, registering the earliest alterations of sensory fibres. However, EPG is an invasive procedure and the implementation of electromyography for the diagnosis of nerve involvement is limited, because it requires a high ability on the part of the neurologist, facts that limit the application of these procedures to an endemic disease such as leprosy. Thus, considering the limitation of the EPG, a new noninvasive technique was sought to detect peripheral nerve abnormalities in LPs. The option was to apply ultrasound to evaluate the peripheral nerves in leprosy.

We initially investigated the potential of ultrasound to detect neurological abnormalities in patients with the polar forms of leprosy looking for the role of ulnar nerve sonography in leprosy neuropathy with electrophysiological correlation; we observed that nerve sonography and EPG were complementary for identifying ulnar nerve neuropathy in patients with leprosy with clinical symptoms. This reinforces the role of sonography in the investigation of leprosy ulnar neuropathy (Elias Junior et al. 2009). The ulnar nerve was chosen because it is the most commonly affected nerve in leprosy and is easily accessible for clinical evaluation.

We are currently investigating the cross-sectional area of peripheral nerves in patients presenting the spectrum of clinical manifestations of leprosy and have observed that the ratio between the cross-sectional area of the pre-tunnel and the tunnel of the ulnar nerve can be helpful in the diagnosis of neuropathy caused by M. leprae (unpublished observations).

On the other hand, we also observed that patients with neuritis related to reactional episodes have been treated with corticotherapy for long time and this therapy causes an immunosuppressive state favouring the manifestation of opportunistic infections (Wambier et al. 2011). Taken as a whole, these findings could contribute to the understanding and control of these conditions and should help improve patient care and thus prevent the onset of physical impairment and the stigma of the disease.

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