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Regulatory T Cells in Leprosy: Implications for Immune Response and Pathogenesis

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Abstract: This study investigates the role of regulatory T cells (Tregs) in the inability to control pathogen propagation in severe chronic granulomatous diseases, focusing on leprosy. Study examined 28 newly diagnosed leprosy patients, including 16 with lepromatous leprosy and 12 with tuberculoid leprosy, along with six healthy individuals exposed to Mycobacterium leprae. Immunohistochemistry and flow cytometry were used to identify Tregs in peripheral blood mononuclear cells (PBMCs) stimulated with *M. leprae* antigenic preparation and phytohemagglutinin in vitro, as well as in skin lesions. Stimulated PBMCs expressed IL-10, TGF-β, and CTLA-4 and displayed lymphoproliferative, interleukin-10, and interferon-gamma responses in vitro. Study findings show that M. leprae antigens significantly reduced lymphoproliferative responses (LPR) in lepromatous patients and their contacts but significantly increased the likelihood of T cell development. No significant differences were observed in mitogen-induced Tregs and LPRs among the three patient groups. Higher levels of TGF-β and IL-10, correlating with a higher number of Tregs, were observed in lepromatous patients. In lepromatous lesions, histiocytes were vacuolized throughout, while Tregs were generally absent in tuberculoid lesions. The presence of numerous Tregs in patients with uncontrolled bacillary multiplication suggests a critical role of Tregs in the progression of lepromatous leprosy, contrasting with their absence in patients who limit *M. leprae* growth. This study highlights the importance of Tregs in the pathogenesis of leprosy and their potential impact on disease management.

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Introduction

Leprosy is a chronic infectious disease caused by Mycobacterium leprae, which manifests in a wide range of clinical, bacterial, and histological presentations due to varying immune responses among patients [1,2]. The disease spectrum includes tuberculoid leprosy, characterized by a robust immune response, and lepromatous leprosy, which demonstrates a weakened immune defense [3]. In tuberculoid leprosy, the immune response is marked by the infiltration of CD4+ T lymphocytes and the formation of compact granulomas, with histopathology revealing minimal bacterial presence [4]. Patients with this form of leprosy exhibit a Th-1 cytokine profile and strong lymphocyte proliferation in vitro, indicative of an effective immune response [5]. Conversely, lepromatous leprosy features a poor granulomatous response, with a low number of infiltrating CD4+ T cells and a high bacterial load [6]. This form was associated with a reduced Th-1 immune response and a shift towards Th-2 immunity, leading to inadequate lymphoproliferative responses when stimulated in vitro [7]. A critical subset of CD4+ T cells, known as regulatory T cells (Tregs), has been identified to exhibit suppressive functions. These cells are characterized by the expression of CD25, the interleukin-2 α-chain, and the transcription factor FoxP3 [8]. The mechanisms by which Tregs suppress effector functions of other T cells include direct interaction through costimulatory molecules like CTLA-4, which inhibits CD4+ T cell proliferation and IL-2 secretion [9]. Additionally, Tregs secrete anti-inflammatory cytokines such as IL-10 and transforming growth factor-β (TGF-β), further modulating the immune response. While the role of Tregs in autoimmune disorders and chronic granulomatous diseases, such as tuberculosis and leishmaniasis, is well-documented, their function in leprosy remains unclear. Evidence suggests that lower levels of Treg cells in peripheral blood are associated with autoimmune conditions, indicating their crucial role in immune regulation. Given their significance in controlling immune responses to microbial infections, understanding the involvement of Tregs in leprosy could provide insights into the pathogenesis and progression of the disease [10]. This study aims to elucidate the role of Tregs in leprosy, particularly in the context of severe chronic granulomatous disease. By examining the immune profiles of patients with lepromatous and tuberculoid leprosy, we seek to determine the impact of Tregs on pathogen propagation and immune suppression. This research may reveal critical mechanisms underlying leprosy's immune evasion and persistence, contributing to better therapeutic strategies for managing this complex disease.

Methodology

This study examines 28 consecutive new cases of leprosy. Among the participants, 16 patients were diagnosed with lepromatous leprosy, comprising 13 cases of borderline lepromatous leprosy (BL) and three cases of polar lepromatous leprosy (PL). All patients were HIV-negative and had no other infectious comorbidities prior to starting specific therapy. The remaining 12 patients included 10 with borderline leprosy (BT) and two with polar leprosy (RCL), with an average age of 45 ± 5 years; the group consisted of seven females and five males. Multibacillary disease was prevalent in the first group, while paucibacillary disease was more common in the second group. To represent the control group, six healthy individuals living in close contact with lepromatous leprosy patients were selected. Exposure to *Mycobacterium leprae* was confirmed through a lymphoproliferation assay, which was considered positive upon detecting a positive reaction to the *M. leprae* antigen. Immunohistochemistry and flow cytometry were utilized to identify regulatory T cells (Tregs) in peripheral blood mononuclear cells (PBMCs) stimulated with *M. leprae* antigenic preparation and phytohemagglutinin in vitro, as well as in skin lesions. In addition, the expression of IL-10, TGF-β, and CTLA-4 in stimulated PBMCs was examined, and lymphoproliferative, interleukin-10, and interferon-gamma responses were assessed in vitro. By analyzing these parameters, the study aims to elucidate the role of Tregs in leprosy and their impact on pathogen propagation and immune suppression, contributing to a deeper understanding of the disease's pathogenesis and potential therapeutic strategies.

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Statistical Analysis

Statistical analyses were conducted using Kruskal-Wallis test and Dunn's post-test for immunoassays, and Mann-Whitney tests for IHC.

Results

A study evaluated the effects of PHA and MLCwA on the expansion of Tregs in PBMC cultures of tuberculoid, lepromatous, and contact patients. Compared with controls and patients with tuberculoid leprosy, lepromatous leprosy patients had significantly more MLCwA-induced Tregs. Due to the similar frequency of Tregs induced by PHA from all three groups, it appears that Treg expansion capacity was not different among them. To determine whether the observed high Treg number in lepromatous patients was functionally relevant, two phenotypic markers were examined, namely, the production of high levels of IL-10 and the absence of proliferative responses to M leprae antigens. A lymphocyte from a tuberculoid patient or contact had a vigorous proliferative response to PHA and MLCwA, whereas a lymphocyte from a lepromatous patient could only proliferate to PHZ. A significantly higher response to MLCwA was observed in tuberculoid patients and contacts than in lepromatous patients. However, PHA response was not significantly different between tuberculoid and lepromatous patients. On the other hand, lepromatous patients and contacts released significantly more IL-10 when unstimulated and when stimulated with MLCwA, whereas those who were stimulated with PHA released no significant differences. In addition to measuring the IFN-g levels, we determined the protein concentration in these supernatants. Lepromatous patients and their contacts produced significantly lower levels of IFN-g when stimulated with MLCwA than tuberculoid patients and their contacts; PHA-stimulated cultures were similarly low. As a result, increased expression of Treg cells in lepromatous patients corresponded to low antigen specific INF-g and lymphocyte proliferation responses, but increased IL-10 production. In order to evaluate Tregs in leprosy patients further, their frequency was investigated in their lesions. The majority of patients with tuberculoid and lepromatous lesions had samples of their lesions available for biopsy. Tregs infiltrated the cutaneous lesions with a significantly greater frequency in lepromatous patients than in tuberculoid patients. As compared to lepromatous patients, tuberculoid patients showed few Tregs, often within the granulomas, There were also vacuolized hystiocytes mixed with Tregs throughout the lesion. It was not possible to find labeled cells outside of the zones of inflammation. The presence of Tregs in normal human skin is rare; of 10 normal skin biopsies, no immunostained cells were detected in eight biopsies, and only one immunostained cell was identified in both biopsies. Lesions of lepromatous patients exhibited higher levels of IL-10 than those of tuberculoid patients when IL-10 and TGF-b were analyzed. Additionally, CTLA-4 expression was higher in lepromatous lesions compared to tuberculoid lesions.

Discussion

It is a major characteristic of chronic granulomatous infectious diseases that T-cells become anergized to antigens [11]. It is still unclear what triggers this anergy. In addition to providing insight into the mechanisms, elucidating these issues may facilitate the development of new strategies to combat these often dangerous threats. As a result of the discovery of a subset of T cells with regulatory properties, disease-associated pathology is being prevented [12]. It is, however, important to remember that prevention of infectious diseases may come at a cost of reduced immunity against the pathogen. The Tregs play a role in contributing to host failure to control invading organisms, which results in persistent invading organisms and chronic inflammation as a result. [13] Based on the agent, localization, and clinical presentation, these cells play a variety of roles in immunopathology. Leishmaniasis is characterized by high levels of Tregs with immunosuppressive function during acute lesions, but these cells decline afterward and reappear during chronic lesions. However, there was a reduction in peripheral blood levels in comparison with healthy subjects [14]. There was no increase in Tregs among chronic cutaneous leishmaniasis patients, despite CTLA-4 overexpression, an inhibitory molecule, in this study. Their immunosuppressive

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effects were nonetheless manifested in skin lesions [15]. Visceral leishmaniasis patients do not have Tregs in their peripheral blood or spleen, which is paradoxical because Tregs do not play a role [16]. The same was found to be true in Sudanese patients with post-kala-azar dermal leishmaniasis, but not in those with post-kala-azar dermal leishmaniasis from India. As well, those with disseminated tuberculosis had elevated levels of Tregs containing suppressive functions in their peripheral blood, whereas there were no such increases in those with localized pulmonary tuberculosis. Furthermore, peripheral blood accumulated more of them at disease sites than at healthy locations. In most studies, Tregs are characterized by their expression of CD25 or Foxp3 but not both molecules, which may contribute to the variability. A couple of studies have attempted to clarify Tregs' role in leprosy [17], but they have not been conclusive. The results present here indicate increased Treg numbers and a potential pathogenic role for patients with lepromatous leprosy, but not for those with the ability to inhibit M. leprae growth. It was found in vitro that there was a higher number of CD25+ FoxP3+ T cells, as well as at sites where bacteria caused leprosy to stimulate PBMCs and disease sites. One study found more Tregs circulating in the peripheral blood of tuberculoid patients than in lepromatous patients, contrary to our findings. The differences may be attributed, at least in part, to the use of different cytometry strategies to determine Treg populations. Additionally, this study examined ex vivo cells, while our analysis was focused on in vitro-stimulated cells. This may explain the altered number of Treg cells within ex vivo PBMCs that may have been redirected from the peripheral blood to the disease site as a result of redistribution. FoxP3+ Tregs were discovered in leprosy biopsies previously. Since there were only a few patients within each category, the study found no statistical difference between Treg frequency between them. In addition, no in situ double staining or quantitative imaging analysis was performed. In addition, IL-10 and CTLA-4 were expressed higher in situ by Tregs but not by TGF-b, possibly indicating that these cells inhibit CD4+ T cell responses. It has been demonstrated several times that Tregs isolated from disease sites are immunosuppressive in vitro, but the mechanism through which they exert this ability is yet to be discovered. Patients with tuberculosis were shown to have elevated TGF-b and IL-10 expression, but these cells were not Tregs. There is a potential role for IL-10 in cutaneous leishmaniasis, with levels correlated with parasite load17, whereas other studies have demonstrated that IL-10 and TGF-b are released more often by Tregs derived from skin lesions, as well as CTLA-4 expression is higher [18]. There was an increase in parasite burden after Kala-azar dermal leishmaniasis when IL-10 expression increased, but not CTLA-4 expression. Unfortunately, we have not been able to analyze Tregs and other immunoregulatory molecules functionally to provide direct evidence that they play a role in leprosy immunopathology. In contrast to T cells, Treg cells exhibit local immune suppression instead of systemic immunity. Chronic HIV-infected patients showed, despite decreased peripheral blood Tregs, an increase in intestinal Tregs correlated with HIV replication status, demonstrating the importance of in situ studies. Our studies have found that lepromatous patients have higher Treg and IL-10 levels not only in blood, but also in situ. As a result of increased Treg expression, our lepromatous patients exhibited depressed antigen-specific proliferative responses and reduced IFN-g release, resulting in deficient Th-1 responses and bacillary load control. Lepromatous lesions in fact have a high number of cells expressing IL-2 compared to tuberculous lesions, which may explain our finding of a lower number of IL-2-producing cells in lepromatous lesions. Similar results were found by the same group in sequential IHC studies of lepromin reactions. Patients with lepromatous and tubercular diseases produced similar levels of IL-2-producing cells in the early phases of the lepromine reaction [19]. However, later phases of the reaction showed diminished numbers of these cells in lepromatous patients. Leprosy and other chronic granulomatous diseases are both characterized by Treg interactions [20], which are important to the outcome of the host–parasite interaction. It is imperative in the management of this still-challenging infectious disease that the Treg function is maintained at an

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appropriate level to achieve the goal of parasite control without triggering immunopathology.

Conclusion

This study highlights the crucial role of regulatory T cells (Tregs) in the pathogenesis of leprosy, particularly in lepromatous cases where uncontrolled bacillary multiplication occurs. Elevated levels of TGF-β and IL-10 in lepromatous patients correlate with higher Treg counts, suggesting that these cells contribute to the suppression of effective immune responses. In contrast, the absence of Tregs in tuberculoid lesions aligns with a more robust immune response and better control of Mycobacterium leprae growth. Understanding the immunoregulatory mechanisms in leprosy can inform more targeted therapeutic approaches and improve disease management.

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