Immune Dysregulation in Childhood Leprosy

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Abstract

Leprosy is a chronic infection which is caused by *Mycobacterium leprae*. Leprosy has long been considered a complex disease, in which host and environmental characteristics are as important as the pathogen to determine disease outcome. Leprosy is an excellent model for examining the immunoregulatory functions. Children are believed to be the most vulnerable group to leprosy infection due to their immature immunity. This review is aimed to summarize the findings of previous studies about immune dysregulation in childhood leprosy. This study highlighted the plausible maternal-fetal connection which are related in immune dysregulation in childhood leprosy.

Key words: *Immune dysregulation, Childhood leprosy*

Introduction

Leprosy is a chronic infection which is caused by *Mycobacterium leprae*. Leprosy has long been considered a complex disease, in which host and environmental characteristics are as important as the pathogen to determine disease outcome.[1] World Health Organization (WHO) has proposed the leprosy elimination program that aimed to reduce the global prevalence of leprosy to less than one case per 10,000 population by the year 2000 back in year 1991.[2,3] Despite the efforts, leprosy remains endemic in some country, with relatively high burden in children and an increase in the new cases detection rate.[4,5]

Even though leprosy occurs at all ages, children are considered more susceptible. However, clinical leprosy is more commonly observed in adults. Paucibacillary disease is more common in children and the incidence of reactions is lower in this age group. Epidemiologically, childhood leprosy is an index of transmission of the disease in a population and helps in detection of the source of infection. In 2019, 177,175 registered cases and 202,185 new cases of leprosy were observed globally.[6]

The proportion of new leprosy in children is one indicator of failure on termination of leprosy chain transmission and reflects the number of undiagnosed cases in the community. WHO has a target to reduce disease transmission and grade II disability, especially in pediatric cases.[7] A study has reported that the children appear to be more prone to leprosy than other family members.[8] Children are believed to be the most vulnerable group to leprosy infection due to their immature immunity.[6]

One peculiarity of leprosy is that the majority of the people (about 90%) do not become ill due to one's own natural defense against *Mycobaterium leprae*, which is related to genetic influences.[7] Clinical manifestations

of leprosy rely on the interaction between innate and acquired immune reactions involving interactions between bacterial proteins and host immune components. These interactions can either prevent invasion and infection or encourage the growth and development of leprosy. The immune system has developed mainly to suppress infection, but in leprosy, the immune response is responsible for the broad clinical spectrum of the disease and, similar to autoimmune disease, tends to cause more complications such as nerve damage.[9]

Leprosy is an excellent model for examining the immunoregulatory function of innate immune molecules and their association with the nervous system, which can impair homeostasis and lead to the production of inflammatory episodes over the course of the disease.[10] While innate immunity affects the clinical forms of the disease, it is the components of the adaptive immune system that tend to be closely associated with the characteristic continuum of leprosy.[11] This review is aimed to summarize the findings of previous studies about immune dysregulation in childhood leprosy.

Leprosy in Children: Current Findings

Currently, the proportion of leprosy in children has not improved significantly.[7] Epidemiological data from 150 countries show there were around 16,979 cases of child leprosy in 2017. Delay in early diagnosis and difficulty in assessing sensory loss in children may contribute to the high leprosy rate in children.[6]

A study by Palit et al. indicates that childhood leprosy differs from adult disease in many ways; there is a gender preference for male leprosy; pauci-bacillary cases, mostly borderline tuberculoid (BT) disease, are more common and episodes of reactions and associated deformities are less common in this age group.[12] Familial and non-familial close contacts play a significant part in the epidemiology of childhood leprosy. The type of disease in the contact and proximity to the child (household or neighborhood) are essential determinants of transmission.[12]

An important source of infection is close contact with cases of leprosy at home, especially in children who have a poor immune response. It confirms the significance of screening of family members in a case of leprosy.[13] A skin patch is the first symptom of leprosy in most childhood leprosy cases. In a study by Darlong and Govindharaj, 90% had patches as the first symptom, and 97% were seen either by the children themselves or by the parents.[14]

Long diagnosis delays and a high proportion of MB signify a lack of early case finding.[15] Diagnosis can be delayed by the failure of the child to comply with sensory testing.

Presenting the test as a feather game is a helpful tool, and another is demonstrating on the mother's skin as the child watches, ask the child to imitate his/her mother. The differential diagnosis can be supported by biopsy (an unpleasant procedure in a very young child) in a study, 35% of questionable cases, may be confirmed by histology.[16]

Skin biopsies are more likely to detect multibacillary cases compared to those with smears as it covers more tissues, including cutaneous nerve twigs, with a bacillary load 1000 times greater than that of inflammatory infiltrates. A single hypopigmented patch on the face of children is at high risk of misdiagnosis, as there are multiple causes of hypopigmented patches in children and biopigmented patients.[17]

In a study in India, deformity occurred in 0-24% of cases. Most of the studies from India measured deformity according to the WHO disability grading. At the presentation, many children had noticeable deformity. Some children developed deformity after being released from therapy.[18]

In older children, around puberty, where exposure to bacillus is expected to be longer, other symptoms may appear more similar to those defined for polar types of the disease. The only symptom of the disease is sometimes the thickening of the superficial nerves, especially the muscles of the auricular, superciliary and ulnar muscles.[19]

Even though previous studies have stated the male dominance in childhood leprosy, a study by Sashidaranpillai et al. found surprising female predominance in relapse cases. The higher susceptibility of teenage females to leprosy relapse can be due to hormonal changes and eventual alterations in the immune system with menarche.[20]

Immune Dysregulation in Leprosy

The immune system of the host is a crucial factor in leprosy. A healthy immune system must be nurtured from the beginning of life by improving the perinatal health status of both mother and child, and by improving environmental conditions at an early stage, especially in the first 1000 days of life.[21] Some immune components known as having an important function in leprosy are Th1, Th2, Treg, and Th17. The dysregulation of these four components makes the host susceptible to leprosy.

The groups at high risk of leprosy are individuals who have poor living standards, poor nutrition, and poor environmental conditions[22]. These are thought to cause dysregulation of the immunity[23] which is very prominent in leprosy endemic areas, and makes leprosy transmission easier.[11]

The immune equilibrium model suggests that the immune system depends on a balance between these various forms of immune response: a balance that determines homeostasis. As a result, the inhibition of the other forms of reaction is triggered by a microorganism or injury that causes one form of response. Conversely, the loss of stimulation of one form of reaction contributes to exacerbation of the other forms of response, with potentially pathological implications.[24]

Immune system that does not function properly results in immune system deficiency, hypersensitivity, autoimmunity, and uncontrolled inflammation. Ivanova and Orekhov[25] state that T lymphocyte plasticity is an important mechanism that makes the immune system able to respond to environmental changes by changing its function during exposure to parasites and infectious agents. Several specific subsets of T cells are regulated by complex cytokine signaling and transcription factors, and disruption in these signaling can cause a variety of disorders.

Immune dysregulation will occur when individuals cannot prevent infection, malnutrition, exposure to pollution, prevent exposure to smoke and cigarettes in children, provide an ideal physical and psychological environment for children, and the early usage of antibiotics in children.[26]

Polarized T cell response (Th1/Th2 bias) to *M. leprae* is known to be a crucial factor of leprosy pathogenesis and its numerous clinical forms. Generations of Th1 effector cells mainly developing interferon-gamma cytokines (IFN- γ) vs. Th2 interleukin-producing cells-4 (IL-4) are considered mainly accountable for the polarized state of immunity.[11]

The clinicopathological features of leprosy have distinct immunological implications. Immunologically, lepromatous leprosy is characterized by a Th2 T-cell immune response (interleukin-4 [IL-4] and IL-10), antibody complex formation, the absence of granulomas, and failure to restrain *M. leprae* growth. Tuberculoid leprosy features a Th1 T-cell cytokine response (gamma interferon [IFN-γ] and IL-2), vigorous T-cell responses to *M. leprae* antigen, and containment of the infection in well-formed granulomas.[27]

Lepromatous leprosy lesions are characterized by a lack of $CD4^+$ T cells, numerous $CD8^+$ T cells, and foamy macrophages, whereas tuberculoid leprosy lesions have a predominance of $CD4^+$ T cells and well-formed granulomas. In lepromatous leprosy, robust antibody formation occurs but is not protective, and cell-mediated immunity is conspicuously absent. In contrast, in tuberculoid leprosy, cell-mediated immunity is relatively preserved, and there is little evidence of *M. leprae*-specific humoral immunity. However, as noted above, the majority of patients are not found at the poles of the leprosy spectrum but in the intermediate categories of BL, BB, and BT disease, which are clinically 'unstable'. The immunology of these borderline states is poorly understood.[27]

Innate immunity responds to leprosy infection: macrophages and complementary natural killer (NK) cells; and adaptive immunity: lymphocytes and dendritic cells.[28,29] *M. leprae* entering through the skin will meet the dendritic cell (DC) as the first host to respond. DC in the epidermis is known as the Langerhans cells and in the dermis as the dermal DCs. Langerhans cells express CD1a and langerin in leprosy skin lesions. These cells efficiently display antigens to T-cells as part of the host response to leprosy infection.[10,30]

CD1a+ cells are associated with the results of leprosy reactions. CD1a is expressed in CD123+ cells located in the dermis in both lepromatous and reversal

patients. Quantitative study has indicated a significant predominance of dendritic cells in tuberculoid leprosy. In tuberculoid leprosy lesions, dendritic cells have been associated with matrix metalloproteinase (MMP)-12 and lead to the growth of granuloma.[10]

At first, *M. leprae* entering the host is recognized by toll-like receptors (TLRs), which then cause NF-Kb activation and increase pro-inflammatory cytokines (GM-CSF, IL-1B, TNF-α, IP-α, IP-10, IL-12) and chemokines, such as macrophages, which trigger the migration and activation of antigen-presenting cells. This antigen-presenting cells (ACP) then introduces *M. leprae* to lymphoid T-naive cells.[6,31]

Depending on its co-stimulators, inhibitors or other cytokines, naive T cells can develop into Th1, Th2, Treg, and Th17. Several previous studies have demonstrated differences in host response in PB and MB types[6] where PB type leprosy is more dominated by Th1-mediated immune responses. Th1-dominated immune responses are mediated by protective IFN-gamma and IL-2 with microbicidal properties. IFN-gamma induces macrophage activation resulting in induced synthase of nitric oxide (iNOS) and NO destroying *M. leprae*. Additionally, this immune response also produces IL-1b, IL-6, TGF-β, and IL-23; as later discovered, these cytokines are also involved in Th17 induction.[32]

A study stated that these cytokines could be used to predict protective factors against *M. leprae*. Th1 cells are also largely associated with leprosy reactions, in addition to being associated with PB types. Immune dysregulation causes low levels of Th1-produced cytokines that cause low clearance by activation of APCs, macrophages, and natural killer cells (NKCs). Unlike leprosy of the PB type, MB type leprosy has predominant immune response mediated by Th2. Th2 is mediated by IL-4, which was investigated to have the effect of suppressing macrophage microbicidal response, diminishing Th1 response, and promoting *M. leprae* survival.[23]

The role of regulatory T (Treg) cells in maintaining self-tolerance and balancing immune reactions in autoimmune diseases and chronic infections is well known. Regulatory mechanisms, however, can also lead to an extended survival of pathogens in chronic infections such as leprosy and tuberculosis (TB). Treg plays a role in suppressing the immune system by inhibiting the proliferation of T cells and reducing cytokine production. Treg are CD4+CD25+ cells that express CXCR4 and CCR5 on their surface, and have FOXP3+ transcription factors that play a role in inflammatory response regulation. Treg can identify autoantigens that are derived from damaged tissues and thus induce and maintain self-tolerance. Treg regulatory function is performed by inhibiting the activation of effector T cells such as Th1 and Th17; and by activating, proliferating and recruiting other Treg cells at the injury site through intermediaries of inflammatory and chemokine mediators.[23]

In leprosy, Treg has a role to play in keeping the balance of Th1 and Th2 responses. Immune dysregulation causes accumulation of Tregs found in MB leprosy which suppresses the immune response and causes the host to experience irresponsiveness to *M. leprae* infections.[33,34] Tregs, which are high in leprosy patients, are responsible for host immune suppression by producing IL-10 and TGF-β like cytokines. The first study describing the conversion of Tregs into Th1-like and Th17-like cells using in vitro cytokine therapy in leprosy patients was a study by Tarique et al.[35] Mononuclear peripheral blood cells were isolated from leprosy patients, and stimulated with MLCwA, rIL-12 and rIL-23 for 48 h. FoxP3+ expression in CD4+ CD25+ Tregs, intracellular cytokines IFN-γ around, TGF-β around, IL-10 and IL-17 in Tregs cells was evaluated after stimulation by flow cytometry (FACS). Treatment with rIL-12 increases pStat4 levels in Tregs and IFN-γ production. PStat3+ and IL-17A+ cells increase in the presence of the rIL-23. rIL-12 and r-IL-23 treatment down controlled Tregs development of FoxP3+, IL-10 and TGF-β and enhanced expression of co-stimulating molecules (CD80, CD86). rIL-12 converts Tregs through STAT4 signaling into IFN-γ producing cells while rIL-23 converts Tregs into IL-17 generating cells through STAT-3 signaling in patients with leprosy.[35] Th17 (CD4+ Th17) is one of the more recently identified effector T cells in contrast to Th1, Th2, and Treg. The presence of IL-23, IL-6, and TGF-β influences the differentiation of the naive T cells to Th17. Th17, like Th1, is pro-inflammatory, developing IL-17A, IL-17C, IL-17D, IL-17E, and IL-17F.[36]

Immune dysregulation in leprosy and associated cytokines

TNF is a multifunctional proinflammatory cytokine that is essential for the control of mycobacterial and other infectious diseases and is produced by monocytes and macrophages. Treatment with TNF inhibitors may be associated with the development of leprosy, and the withdrawal of TNF inhibitor treatment in leprosy patients may also enhance the formation of a type I reversal reaction. There is also evidence that TNF inhibitors may be effective for the treatment of recurrent erythema nodosum leprosum.[27]

Th17 has a major role in host immunity to extracellular pathogens and fungal infections, as well as involving autoimmunity, allergies, tumor development, and responses to organ transplants. Th17 is thought to have protective properties in leprosy and is found more frequently in PB type leprosy than MB[6]. Another study stated that Th17 is more common in patients with BT/TT leprosy than BL/LL, proven by the presence of high levels of IL-17 and IL-1. IL-17 also plays a part in producing inducible Nitric Oxide Synthase (iNOS), to help kill *M. leprae* mediated by reactive oxygen species.[23]

Evaluation of the cell-mediated immune response based on IFN-γ release assays has been used to classify *M. leprae*-specific antigens capable of discriminating against asymptomatic illness and/or early stages of infection in endemic areas. It's been seen that *M. leprae* peptides better discriminate contacts and patients against healthy controls. However, they induce low levels of IFN-γ compared to proteins, particularly when evaluated by whole blood assays. Peptides that provide specific reactions in patients with leprosy from an endemic setting could theoretically be developed into a rapid diagnostic test for early detection of infection and epidemiological studies of the prevalence of leprosy, of which little is known. One of the hindrances to T-cell-based diagnostic tests is cross-reaction of *M. leprae* antigens at the T-cell level with antigens found in other mycobacteria, such as MTB or *M. bovis* BCG.[37]

Immune Dysregulation in Childhood Leprosy

The dysregulation of the immune system early in life is crucial for the occurrence of dysregulation in older age, as explained in the concept of the immune regulation. Dysregulation will occur in the event of infection, stress, exposure to pollution, malnutrition and obesity, exposure to cigarette smoke, alcohol or drugs, unavailability of ideal physical and psychological environment for pregnant women and children and also to encourage vaginal delivery, as well as prevent infection, exposure to pollution, stress, malnutrition, and early antibiotics in children.[26]

Maternal-fetal connection in immune dysregulation

Previous findings indicate that the condition of increased Treg levels and decreased Th17 levels in healthy mothers will be continued in the fetus. There is evidence from previous studies showing that host-pathogen interactions are predominantly influenced by the genetic composition of the host. These findings are summarized in a brief review of genetic susceptibility in leprosy by Cambri and Mira[38]. An early study showed that the predisposition for infectious disease incidence was hereditary, in contrast to non-genetic factor-dependent cancers. The innate predisposition to infection appears to be crucial for leprosy, it is estimated that only 5-12% of individuals exposed to M. leprae are infected and symptomatic.[38]

Regulatory T cells (Tregs) and IL-17-producing Th17 cells are functionally and developmentally reciprocal to each other. Naive T cells develop into Tregs in the presence of transforming growth factor (TGF)-b, while the combination of TGF-b and IL-6/IL-21 introduce naive T cells into Th17. Tregs with tolerance or immunosuppression functions are the primary mediators maintaining peripheral tolerance and are essential for the prevention of autoimmune diseases and chronic inflammatory diseases. However, Tregs may also suppress the appropriate host immune responses against infections. IL-17-producing Th17 cells with immunity/inflammation functions are a recently discovered and characterized subset of effector T helper cells, which have a reciprocal relationship with Tregs in subsets of developmental programs.[39,40]

Sadhu and Mitra[11] stated that in leprosy patients there was an inversion of Treg and Th17 levels. Therefore, under conditions of increasing T-reg levels, Th17 levels will decrease. A study by da Motta-Passos

et al.[41] concluded that low Th17 expression in leprosy patients is a genetic feature of the patient. In addition, the study also showed that patients with leprosy had lower Th17 expression than in healthy controls.

According to Arsyad et al.[42] because the immune response takes some time to form, it can be assumed that household contacts of leprosy have more antigen load (*M. leprae*) in their bodies. This study conducted PCR examination of *M. leprae* on nasal swabs and anti-PGL-1 antibodies using ELISA in household contacts and non-contacts of leprosy. This study found that contact with leprosy patients in the same household can be the cause of the accumulation of antigens and high production of specific antibodies. In this study, healthy mothers in endemic areas had elevated Treg levels.

Previous evidence suggests that maternal cytokine levels influence the cytokine profile of children early in life. Longitudinal studies conducted on pairs of mothers and children in Indonesia that examined the relationship between maternal and child cytokine levels showed that the production of IL-10 in infants is influenced by maternal cytokine levels and environmental factors during pregnancy and breastfeeding that affect the mother.[43]

There has been a growing number of studies looking at immune responses in early life and their regulation by environmental conditions, which could predispose individuals to such diseases in later life.[43]

A study by Djuardi et al. suggest a strong association between maternal and infant cellular immune responses even after taking into account several environmental effects that could directly or indirectly influence the infant's response through the uterine microenvironment.[44]

A study in Indonesia found that most of the mother–child cytokine relationships became weaker over time (IL-10, IFN-γ and TNF-α). These findings indicate that the strong association between cytokine responses of a pregnant mother and her child is not directly due to genetic factors but could likely result from similar immune conditioning during gestational period extending into early childhood. Furthermore, fetal Th cell responses may be biased by the maternal immune system.[43,44]

There are anecdotal reports of leprosy in infants probably indicating intrauterine transmission of leprosy in humans. The proof of suspected intrauterine infection lay in the continued presence of IgA and IgM type of anti-*M. leprae* antibodies in the cord sera associated with an early and significant increase in these antibodies after birth. A decrease in serum IgG anti-*M. leprae* antibody was demonstrated in one of the babies after complete response to treatment.[6]

A previous study has highlighted that human breast milk cells contain a limited number of viable bacteria and bacterial DNA that might have been transported from the mother's intestine to the mammary gland through an endogenous cellular route. An animal study suggests that this process begins in late pregnancy. The results suggest a novel form of mother-infant connection. Elevated translocation of bacteria or their components in the mother should have an effect on her immune status and may explain the physiological activation of innate immunity that occurs during pregnancy.[45] This could potentially be a new mechanism of immune regulation in healthy individuals.

Immune dysregulation and child development

Activation of late fetal or neonatal T cells by foreign antigen results in a response biased to Th2 immunity, which is enhanced by neonatal dendritic cells and epigenetic features. Thus, tolerogenic reactivity, decreased allo-antigen recognition and weak responses to foreign antigens characterize rather early-life adaptive T-cell immunity.[46]

The immune repertoire is also formed by intercurrent infections and vaccines as the child grows. Pathogenic infections can be documented by symptomatic illnesses suffered

by the child or adult, but for many viruses, such as influenza, infection may be subclinical, but still sufficient to stimulate or boost immune responses.[46]

The transmission of protective antibody protection from a mother to her child is hugely important, especially in environments where 15%or more infants and children die of infection. Paradoxically, a mother who avoided a dangerous childhood infection, through herd immunity, may actually put her child at risk by being unable to

transfer specific protective antibodies.[46]

Previous studies show an age-associated increase of IFN-γ and TNF-α release, which is also consistent with the findings from flow-cytometry studies. The importance of the age-interval of investigated populations is further supported by a publication in Indonesian children showing that the greatest age-associated increase for IFN-γ and TNF-α was seen after the first year of life.[43,47]

It has been stated that the higher susceptibility of teenage females to leprosy relapse can be due to hormonal changes and eventual alterations in the immune system with menarche.[20] This can be explained by the correlation of cytokines and menstrual cycle. Factors that are extrinsic to the immune system also contribute to the heightened proinflammatory environment. For example, both estrogen and testosterone decrease IL-6 production in vitro and in vivo and age-associated decreases in estrogen or androgen production are associated with increases in basal proinflammatory cytokine levels.[48] A study also found that IL-4 is positively correlated with estrogen while TNF-alpha is positively correlated with progesterone. Females were found to have significantly higher concentrations of TNF-alpha across all phases of the menstrual cycle, compared to males across similar time points.[49] This shows a plausible mechanism of leprosy incidence and leprosy reactions in females, especially those who are born from mothers who had leprosy.

Conclusion

The proportion of new leprosy in children is one indicator of failure on termination of leprosy chain transmission and reflects the number of undiagnosed cases in the community. The immune system of the host is a crucial factor in leprosy. It is important to understand immune dysregulation in childhood leprosy. This study highlighted the plausible maternal-fetal connection which are related in immune dysregulation in childhood leprosy. The study provided promising approaches and targets for further researches in immune dysregulation, especially in childhood leprosy.

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