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Revisión

Micronutrients influencing the immune response in leprosy

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Abstract

Leprosy is a chronic infectious disease caused by Mycobacterium leprae, an intracellular bacillus of airborne transmission. The disease affects the skin and peripheral nerves and can cause neurological sequelae. The bacillus multiplies slowly in the host and the disease probably occurs due to malfunctioning in host immune response. This review addresses the role of some specific micronutrients in the immune response, such as Vitamins A, D, E, C, Zinc and Selenium, detailing their mechanisms of actions in infectious diseases, and in leprosy. The immune response to pathogens releases harmful substances, which lead to tissue damage. This review discusses how a decreased level of antioxidants may contribute to an increased oxidative stress and complications of infectious diseases and leprosy. As the nutrients have a regulatory effect in the innate and adaptative immune responses, a perfect balance in their concentrations is important to improve the immune response against the pathogens.

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Key words: *Nutrition. Leprosy. Oxidative stress and antioxidants.*

MICRONUTRIENTES QUE INFLUYEN EN LA RESPUESTA IMMUNE EN LA LEPRA

Resumen

La lepra es una enfermedad infecciosa crónica causada por el *Mycobacterium leprae*, un bacilo intracelular de transmisión aérea. La enfermedad afecta la piel y los nervios periféricos y causa secuelas neurológicas. El bacilo se multiplica lentamente en el hospedador y posiblemente la enfermedad ocurre por el mal funcionamiento de la respuesta inmunitaria del hospedador. Esta revisión aborda el papel de algunos micronutrientes específicos en la respuesta inmunitaria, tales como las vitaminas A, D, E, C, el cinc y el selenio, detallando sus mecanismos de acción en las enfermedades infecciosas y en la lepra. La respuesta inmunitaria a los patógenos libera sustancias nocivas que producen lesión tisular. Esta revisión también aborda cómo una menor cantidad de antioxidantes puede contribuir a un aumento del estrés oxidativo y a complicaciones de las enfermedades infecciosas y la lepra. Puesto que los micronutrientes poseen un efecto regulador de la respuesta inmunitaria innata y adaptativa, es importante un equilibrio perfecto de sus concentraciones para mejorar la respuesta inmunitaria frente a los patógenos.

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Palabras clave: *Nutrición. Lepra. Estrés oxidativo y antioxidantes.*

Abbreviations

WHO: World Health Organization.
SINAN: Information System and Reporting of Health Problems.
IFN-γ: Interferon-γ.
TNF-α: Tumor Necrosis Factor α.
TL: Tuberculoid.
LL: Lepromatous Leprosy.
BB: Borderline.
BT: Borderline Tuberculoid.
BL: Borderline Lepromatous.
NO: Nitric Oxide.
ROS: Reactive Oxygen Species.
LPO: Lipid Peroxidation.
MDA: Malondialdehyde.
MT: Metallothionein.
SOD: Superoxide Dismutase.
HIV (+): Human Immunodeficiency Virus.
GP_{xs}: Glutathione Peroxides.
VDR: Vitamin D Receptors.
TLR: Toll-like receptors.

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Definition, epidemiology, clinical aspects and immunopathology of leprosy

Leprosy is an infectious disease caused by the intracellular alcohol acid resistant bacillus *Mycobacterium* *leprae (M. leprae).*[1] Its transmission occurs by upper airway through the contact of susceptible individuals with non-treated multibacillary leprosy patients. The disease, affects mainly the skin and peripheral nerves, the mucosa of the upper respiratory tract, and the eyes of the infected persons, provoking severe deformities that lead in many cases to mutilation and social stigma.[2]

According to the World Health Organization (WHO) the global number of cases of leprosy decreased from 2003 to 2009; however, the prevalence of the disease is still high in specific countries, such as India and Brazil.[3] Epidemiological data from WHO show that there were 213,036 new cases in 2009. In Brazil there was a reduction in new cases from 2006 to 2011; however, the country still has a rate of two or more cases per 10,000 inhabitants, still not reaching the goal of the WHO for leprosy control (1 case/10,000 inhabitants). The northeast Brazil was the third area with the highest incidence of leprosy cases. Among the northeast states of Brazil, Maranhão is the most affected by the disease (68.4/100,000 inhabitants), being the fourth Brazilian state most affected. According to data from Information system and reporting of health problems (SINAN 2010), the state of Sergipe presented a detection rate of 18.4/100,000 habitants in 2010.[4] The WHO states that the main intervention strategy is the identification of cases and multidrug treatment.[2] The global strategy by the World Health Organization (2011 to 2015) aims at reducing the rate of grade 2 of physical disability.[3]

In leprosy, a wide spectrum of clinical phenotypes is seen.[5] The Ridley-Jopling classification considers six clinical forms: indeterminate, tuberculoid, lepromatous and borderline forms (borderline tuberculoid, mid borderline, and borderline lepromatous borderline).

The indeterminate form (I) is determined by the presence of hypopigmented lesions with sensory disturbance, loss of hair and absence of horripilation. There is no involvement of the nerve trunks and the individuals are not contagious. The tuberculoid form (TL) is defined by skin lesions such as plaques delimited with erythematous-brownish and elevated borders. In this case there is activation of the Th1 response, producing interferon (IFN)-γ, and tumor necrosis factor (TNF)-α, cytokines that activate macrophages to kill the *M. leprae*. This clinical form is paucibacillary, but can be associated with the presence of peripheral neuropathy that may lead to physical debility.[6-8] In the lepromatous leprosy (LL), the skin injuries have imprecise limits. When there is a deep infiltration on the face, with natural grooves are accentuation, and the condition is called leonine facies ("lion face"). In this form there is activation of a Th2 response, producing interleukin IL-4, IL-3 and IL-10, which suppresses the Th1 response, facilitating the bacterial replication. This clinical form is multibacillary and is the most contagious of the disease, with continuous infiltration in the skin and nerves, leading also to neurological damage and physical disabilities.[5] In the mid borderline (BB) clinical form, patients present with well-defined plaques with areas of normal skin within the plaque, giving a "Swiss cheese" appearance. This clinical form also tends to be multibacillary and contagious. The peripheral nerves are frequently compromised and this involvement is intense and extensive. The peripheral neuropathy can continue for many years after the clinical cure of the disease resulting in physical disabilities. The borderline form can also present with lesions reminiscent of the tuberculoid form (borderline tuberculoid-BT), or the lepromatous form (borderline lepromatous-BL).[6]

M. leprae is a pathogen of low proliferation rate and pathogenicity, and in the course of infection it can be seen that the majority of the individuals do not develop the disease. However, the factors that lead the individual to present with disease are unknown. It is an imbalance between the cellular and humoral immune responses that causes the different clinical forms of leprosy. Individuals with the lepromatous clinical forms have a predominance of the Th2 response, which induces a humoral immune response, which is not efficient at destroying the bacillus. This Th2 response also produces IL-10, which suppresses the Th1 response (the cellular immune response), leading to a dissemination of the bacillus and to more severe multibacillary forms of the disease (LL, BL). It is still unclear if the IL-10 is produced only by Th2 cells, or if T regulatory cells are also involved.[9] A more recent study described the presence of higher numbers of T regulatory cells expressing FoxP3, CTLA-4 and IL-10 in LL patients than in TT leprosy patients.[10] Although some components present in the surface of *M. leprae* are known to induce IL-10, explaining the suppression of the Th1 immune response of the host, not all individuals develop severe clinical forms. In fact, some individuals present an effective cellular immune response and develop the paucibacillary clinical forms of the disease (I, TL and BT).

The course of the infection is dependent on individual factors that influence the host immunologic response. In its turn, the immune response can be influenced by genetic and environmental factors, including the patient s nutritional status.[7-8,11,12] Nutritional deficiencies are common in countries in which leprosy is endemic. It is possible that the clinical presentation of the disease is a result of nutritional deficiencies interacting with other environmental and genetic factors of the host. Nutrition is known to influence immune response in several aspects. Deficiency of trace elements and vitamins affect the innate and adaptive immune response, causing an unbalance of the host response to pathogens.[13]

This review of the literature was performed using the terms "leprosy", micronutrients", "immune response", "oxidative stress" "antioxidants". The variety of terms used allowed a significant coverage in order to conduct a comprehensive search on the topic. Proceeded to the query through the databases PubMed, SciELO and HighWire, covering the years 2000-2013, including also articles relevant to the topic, published previously cited in the articles previously selected. Were included especially intervention studies, randomized controlled trials, and studies with experimental animals, that were used predominantly for the development of concepts as well as the description of mechanisms of action.

The following topics will describe specific nutrients that influence the immune response, detailing their mechanisms of action, and specific effects in leprosy or in other intracellular infections.

Immunologic response to infections and the influence of nutrition

The human organism is an environment rich in nutrients, which are maintained with an elevated and uniform temperature and that are constantly renewed. Thus, we are an attractive hostel for many pathogens. Therefore all organisms are infected by some kind of pathogen, being responsible for diseases or not. The protection against infections is mediated by natural boundaries and by their immune response. The natural boundaries can be mechanical, chemical or biological. The mechanical barriers consist of epithelial and mucosa surfaces, skin loss and the rapid capacity of mucosa and skin regeneration, air flux, ciliary movement, peristaltic movement, and the mucus produced by the respiratory and digestive mucosas. Chemical barriers include fatty acids, (skin) bactericidal substances such as lysozyme (saliva, sweat and tears), proteolytic enzymes (in the stomach and intestines), acid pH, and anti-bacterial peptides (the skin and the intestines). The biological barriers consist of the normal bacterial flora, which compete for nutrients and for the epithelium receptors with the pathogenic agents and produce the microbiocidal substances.[14]

The immune response is activated when the pathogen overcomes these natural barriers. The infection or pathogen control will depend on the host s ability to produce a response against the infectious agent, and depending on the host, the disease can be exacerbated or controlled.[15] Nutritional deficiency can affect both natural barriers and lead to suppressed immune response, since certain micronutrients are important for the maintenance of the integrity of natural barriers, and the appropriate functioning of different components of the immune system, such as cellular response and antibody production.[16]

The clinical manifestations of an infectious disease can also be a result of intense tissue inflammation mediated by the immune response, as described in the highly sensitive reactions. Products such as TNF-α, nitric oxide (NO) and reactive oxygen species (ROS), although displaying important microbiocidal action, also induce tissue damage. Thus, antioxidant substances (endogenous or nutritional) and modulating cytokines have fundamental role for a balanced immune response, which controls the pathogen multiplication and protects the host tissue.[17]

In different infections the malnutrition effect is variable and difficult to measure. In diseases as measles and tuberculosis the nutritional deficiency presents a relation with the increase in susceptibility and worsens the disease prognosis.[18] One of the consequences of the infections from persistent pathogens is the generation of autoimmunity and inflammatory diseases. Although pathogens are the main trigger for the inflammatory response, the hypothesis that nutritional factors can have important contributions in the disease progress cannot be excluded.[19]

The risk of leprosy is significantly associated with poverty, poor education, dietetic inadequacy, related to total caloric intake and reduced intake of vegetables, fruits and fish.[20]

Studies conducted in India and Brazil demonstrated dietetic inadequacies among individuals with leprosy and their relatives, specially related to the lack of vegetables and fruits intake. The observed unbalanced diets were explained by inadequate feeding habits mainly associated with the lack of knowledge about the nutritional value of these foods. The economic status was not the main predictor of the diet quality.[21,22]

Both low body weight and overweight individuals are reported to have dietetic inadequacies regarded to the quality of the foods. Overweight individuals from Brazil and from other developing countries are reported to have a diet based on empty calories foods. In a study conducted in Brazil, 41.9% of individuals with leprosy were overweight or obese, and only 3.6% were underweight. The proportion of overweight or obesity and underweight was similar among individuals with leprosy reaction and no reaction.[23] Overweight and obesity among leprosy patients was also reported in other studies in Brazil.[22] Hipercaloric diets seem not to protect individuals against the disease; however, the low quality of the diet is associated with higher risk of leprosy regardless the weight status, mainly because the low intake of antioxidants substances is associated with impaired immunological defense against pathogens such as *M. leprae*.[1,21,23,24]

In a study of fifty-eight patients with leprosy conducted in India, it was observed nutritional deficiency in different forms of leprosy, but mainly in the lepromatous, the most aggressive clinical form. They described a decrease of serum levels of substances with antioxidant potential, such as retinol (vitamin A), tocopherol (vitamin E), ascorbic acid (vitamin C), zinc, magnesium and selenium.[25]

Endogen substances such as reactive species of nitrogen and oxygen are the main mechanism of destruction of intracellular agents.[26-27] In *M. leprae* infection macrophage activation is important for the control of this microorganism in which the main mechanism of destruction is mediated by ROS and NO. However these radicals have an oxidant activity and can contribute to tissue damage, together with other inflammatory substances produced by the immune system. Dietary substances with antioxidant action can

Tabla I
Main function of micronutrients and their role in the immune system

Nutrient	*Source food*	*Function*	*Role in the immune system*	*References*
Lipid	Vegetable oils, olive oil, almonds, walnuts, peanuts, coconut and avocado.	Assists in the transport and absorption of liposoluble vitamins and cell membrane component	↑ Production of cytokine IL-1β↑ and IL-6. ↑ Production TNF-α and inflammatory response.	Kim, 2011; Sreekumar, 2001; Demori, 2006; Krause, 2002.
Iron	Liver, seafood and lean meat.	Component of hemoglobin and myoglobin and important in oxygen transfer.	Involvement of Fenton reaction with production of free radicals with antimicrobial action.	Bogdan, 1999; Wanasen, 2007; Krause, 2002.
Selenium	Giblets, fish, seafood, wheatgerm and brazil nut.	Involved in the metabolism of fat and vitamin E.	Component of glutathione peroxides.	Fairweather-Tait, 2011; Krause, 2002.
Zinc	Meat, fish, poultry and dairy.	Acts on growth and cell replication.	Structural and catalytic component of the superoxide dismutase.	Koury, 2003; Krause, 2002.
Vitamin C	Citrus fruits, tomatoes, peppers and leafy vegetables.	Increases the absorption of nonheme iron.	Enzymatic cofactor with redox properties.	Murray, 2002; Krause, 2002.
Vitamin A	Liver, egg, milk and carrots.	Protective action on the skin and mucous and essential role in retinal function.	Inhibition the lipid peroxidation and the generation of hydroperoxides.	Sies, 1995; Krause, 2002.
Vitamin E	Vegetable oils, olive oil, almonds and avocados.	Protection of cell membrane unsaturated phospholipids.	Decreased lipid peroxidation.	Vijayaraghavhan, 2005; Vanuucchi, 1998; Krause, 2002.
Vitamin D	Fish (oil liver), egg yolk, butter, cheeses and meats.	Maintaining homeostasis of calcium and phosphorus.	Expression of antimicrobial peptides.	Santiago, 2008; Liu, 2006; Chocano-Bedoya, 2009; Krause,2002.

counterbalance these effects of the oxidative stress, and a reduction of the antioxidant species can contribute to complications in the treatment and associating to the progression of the disease.[28] The table I describes nutrients with oxidant and antioxidant actions, their food sources and effects in the immune system. However, no studies have investigated the role of iron and lipids in the protective response against infectious agents, although, it has been shown that lipids have a role in the inflammation, being the inducers of metabolic alteration found in obesity.[29]

Thus, the antioxidant substances protect the tissue and body lipids from the lesion caused by oxidant produced by normal metabolism or by response to the inflammation. Moreover, these substances reestablish the balance and allow the organism to tolerate the oxidative stress. In the presence of disease and/or malnutrition, the rupture of this balance occurs and consequently the severe stress, with alteration of the cellular metabolism, DNA lesion and lipidic peroxidation. These events contribute to the progression of systemic inflammation, culminating in cellular death and multiple organ dysfunction.[30]

The control of a variety of socioeconomic, environmental, and behavioral risk factors associated with the adequate implementation of multi-drug therapy would minimize the occurrence of leprosy in an endemic area.[20] The quality of the diet is a behavioral factor that. should be further explored to understand the role of micronutrients in the immune response against *M. leprae*. Nutrition education would be a suitable approach to improve the patients' nutritional status aiming to achieve better clinical outcomes related to treatment response, inflammatory manifestations as leprosy reactions and neurological disabilities.[31]

Vitamin A - The role in the immune response and studies associating vitamin A deficiency with leprosy

Vitamin A and its precursors, retinoic acid and β-carotene are important antioxidants in the body. These nutrients are capable of interacting with free radicals, such as peroxyl, inhibiting lipid peroxidation and the generation of hydroperoxides through the stabilization of the peroxyl radical.[32] Through their photoprotective action, the carotenoids quench the singlet forms of the oxygen generated in the cells, transforming them into less reactive species. Besides this action, through its double conjugated bond, carotenoids capture free radicals that could induce oxidative damage.[33] However, some factors in biological systems interfere in this antioxidant capacity, β-carotene acts as an antioxidant nutrient in low partial oxygen pressures and when there are high oxygen concentrations, vitamin E can complement this antioxidant action.[34] An increase in oxidant stress is documented in leprosy-affected individuals.[1]

Vitamin A also has an important role in the regulation of several components of the immune response, including both innate and acquired immunity (both cellular and humoral).[35,36,37] Regarding innate immunity, the deficiency of vitamin A is associated with a decrease in phagocytosis and oxidative burst activities of macrophages.[16] A decrease in NK cells was also reported under this condition.[38] In acquired immunity, studies evaluating the effects of vitamin A deficiency are controversial, describing a decrease in IFN-γ production, which represents the Th1 response and a deficiency in Th2 or humoral response.[13-39] In an Indonesian study conducted in children with vitamin A deficiency, a decrease in *ex-vivo* production of IFN-γ was detected.[13] Given the importance of IFN-γ for exerting critical functions in Th1 type immunity, this observation suggests the importance of vitamin A in control of infections by intracellular microorganisms, such as *M. leprae*. On the other hand, the addition of retinoic acid *in vitro* induced the production of IL-10 and an anti-inflammatory response through the inhibition of IL-12 and TNF-α production in mononuclear umbilical cord and monocyte lineage cells. Since IL-12 is important for the induction of Th1 diferenciation, the administration of this vitamin inhibits the Th1 response, described that vitamin A deficiency compromises also Th2 responses and decreases IgG1 and IgE antibody production.[13-39]

In leprosy, a previous study reported a decrease in serum concentrations of vitamin A, predominantly in lepromatous leprosy (LL) patients, where there is a depression of the Th1 immune response and replication of *M. leprae* in macrophages, and a predominance of the humoral response.[1] A reduction in serum concentrations of vitamin A was also seen in children with visceral leishmaniasis in northeastern Brazil, another intracellular microorganism.[40] These data support the findings of Wieringa and colleagues[13] that vitamin A deficiency has more detrimental effects on the Th1 immune response. A recent study shows that the induction of T regulatory cells by an antigen from *Schistosoma mansoni* eggs, called w1, is dependent on vitamin A. T regulatory cells can down modulate both Th1 and Th2 responses, and are important in the control of inflammatory and autoimmune diseases.[41] Additional studies to clarify the effects of vitamin A in the immune response to infectious agents, such as leprosy, appear merited. This is especially valid in countries such as Brazil, where general or specific nutritional shortages can be found in the context of many infectious diseases. In this review we report the data of a nutritional study in leprosy patients and observed that over 50% of the individuals with leprosy and controls living in the same house present with consumption below recommended levels for vitamin A, evaluated by the food consumption using food records and classifications of the DRI's adequacy, 2006.

Vitamin E - Protective role in the oxidative stress in leprosy

It was demonstrated that free radicals and lipid peroxidation suppress immune responses. Vitamin E most important lipid soluble antioxidant, as it is required to protect the lipid membranes against peroxidation. Additionally, it has been demonstrated in elderly rats that a diet rich in vitamin E increases the production of IL-2 and IFN-γ induced by infected lymphocytes in influenza. These observations suggest that increasing vitamin E ingestion above normal levels improves the immune response against infections.[42] α ±-tocopherol, the most active form of vitamin E, has affinity for phospholipids of the mitochondria, endoplasmatic reticulum and plasma membrane and constitutes the first line of defense against the peroxidation of polyunsaturated fatty acids contained in these phospholipid membranes. When interacting with the cellular membranes reactive oxygen species (ROS) break the polyunsaturated fatty acids that compose the membrane. An example of this is when the hydroxyl radical interacts with the fatty acid of the membrane, holding a hydrogen atom resulting in the generation of lipid radical (fig. 1). This lipid radical can be unpaired incorporates rapidly the oxygen molecules in the structure and transforms itself in a peroxide radical originating an hydroperoxide, forming a new free lipid radical; this chain reaction causes the loss of the membrane integrity.[43] The tocopherols can neutralize these reactions via the donation of phenol hydrogen to the peroxil free radical of the polyunsaturated fatty acid, resulting in neutralization, as depicted in figure 2.[34] The inhibition of the lipid peroxidation by neutralizing the peroxil radical by vitamin E can be aided by vitamin C, reduced gluthatione and NADPH. However, a perfect balance of the concentrations of these components is necessary, because the isolated elevated concentrations of vitamin E can have a pro-oxidant effect, inducing to classical alterations of the free radicals.[44]

In a case-control study, untreated leprosy patients presented with higher levels of lipid peroxidation (LPO) than healthy individuals, demonstrating the presence of oxidative stress in this disease. However, the levels of LPO were not reduced after the beginning of treatment, probably because of the increase of the production of free radicals during the immune-mediated killing of *M. leprae*. A subgroup of the leprosy patients received the conventional multidrug therapy (dapsone, rifampicin and clofazimine) and was simultaneously treated with 400 IU of vitamin E, daily, during 12 months. This additional vitamin E treatment reduced LPO levels close to the normal range.[45] This vitamin E deficiency and supplementation with nutrients during treatment was also described in tuberculosis.[46-47] Patients with tuberculosis showed reduced values for vitamin C and E before treatment, and also higher serum level of Malondialdehyde (MDA) when compared to healthy controls.[47] Interestingly, the

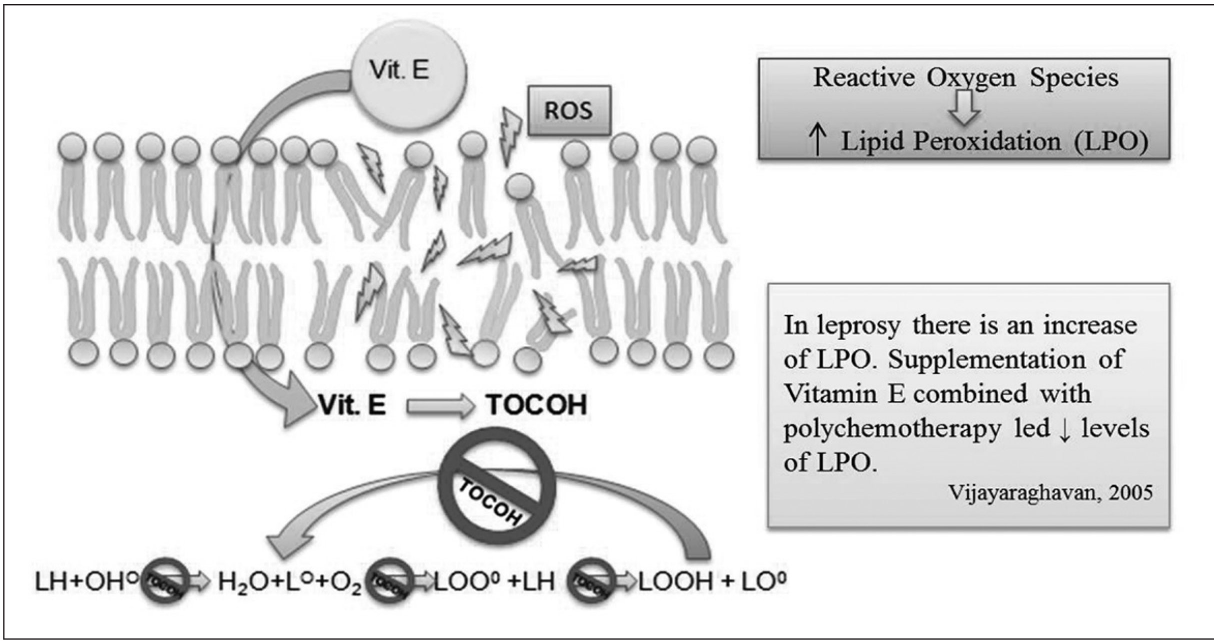

Fig. 1.—Reactive Oxygen Species and vitamin E precursor action in the cell membrane. The interaction of ROS with cellular membranes breaks polyunsaturated fatty acids by the successive generation of free radicals. When ROS interact with fatty acid of the membrane frees a lipid radical, that interact with oxygen, generating peroxide radical, which interacts with other molecules of fatty acid, originating a hydroperoxide, and once more, breaking the lipid structure of the cellular membrane. The active form of vitamin E, the α-tocopherol, neutralizes this chain reaction (represented by the block sign in red) because it donates hydrogen atoms to the free radicals generated in this process. In Leprosy, it was observed an increase in the lipid peroxidation (LPO), and the treatment with vitamin E associated with polychemotherapy reduced the LPO levels.

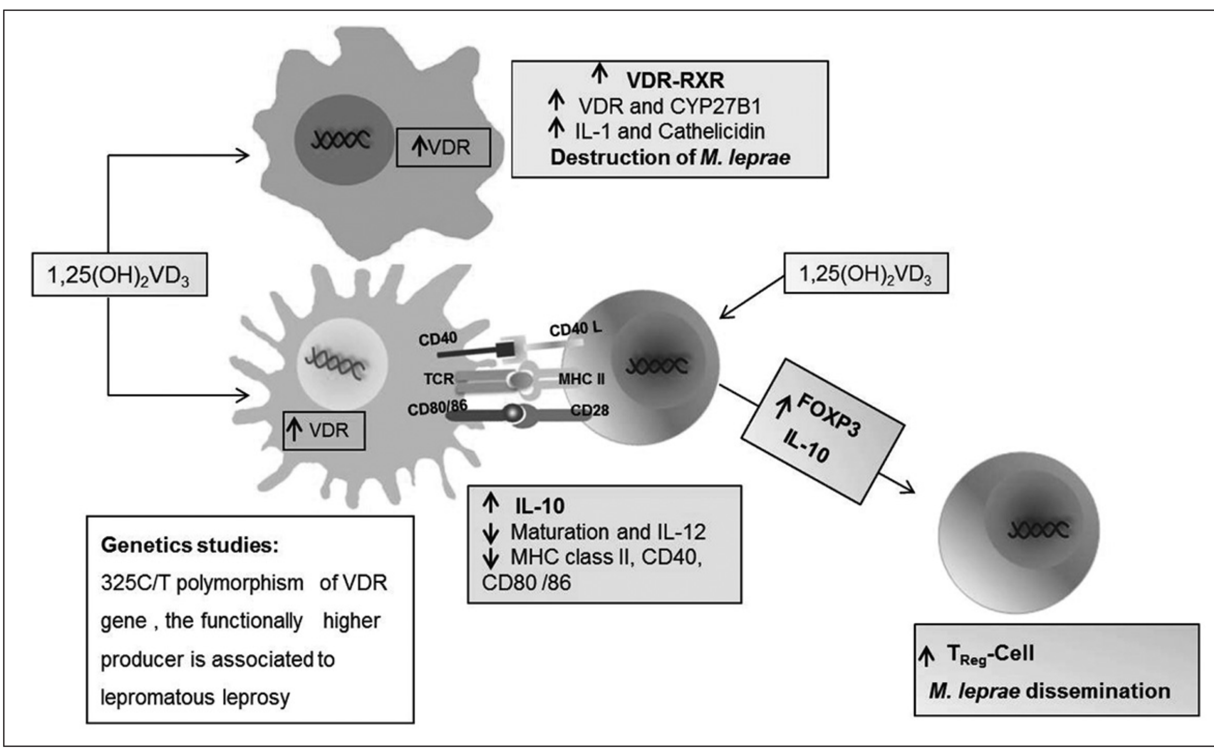

Fig. 2.—Participation of the active metabolite of Vitamin D (1,25(OH)2 VD3) in the immune response against M. leprae. The 1,25 (OH)2 VD3 increases the expression of VDR, IL-1 and the peptide cathelicidin in macrophages, contributing for the bacteria clearance mediated by the innate immune response. However, the dissemination of mycobacteria can be associated to the action of 1,25 (OH)2 VD3 in dendritic and T cells. The 1,25(OH)2 VD3 reduces the maturation of the dendritic cells by reducing the IL-12 expression, MHC class II, CD 40, CD80, CD60, and increases the IL-10 production and FoxP3 expression in T cells, favoring the generation of the regulatory T cell. Regulatory T cells suppresses the Th1 response and interferes in the microbicidal functions of macrophages, contributing to the persistence of the mycobacteria in the host.

supplementation of 140 mg of vitamin E and 200 μg of selenium for two months in patients with pulmonary TB treated with standard chemotherapy resulted in a decrease oxidative stress and improved antioxidant status compared to TB patients treated with standard chemotherapy but without receiving supplementation of nutrients.[46]

Vitamin C and zinc - Their role in the immune response to infectious diseases

Vitamin C and Zinc and are essential for maintaining our health and are important for resistance to infections. Deficiencies in vitamin C or zinc negatively impact the immune system.[48]

Vitamin C participates in several metabolic processes and acts as an enzymatic co-factor in the oxi-reduction processes, increasing the absorption of iron and inactivation of free radicals.[49,50] Vitamin C constitutes a reduction agent that reduces some molecular oxygen components. This oxi-reduction action occurs when vitamin C captures the oxygen present in the medium, through stable chemical reactions, being unavailable for the process of auto oxidation.[34]

Antioxidant substances that are not enzymatic, such as ascorbic acid (vitamin C), provide an important role in the control of the inflammation induced by ROS. It is known that ROS presents an essential role in the death of intracellular bacteria such as *M. leprae* and other intracellular pathogens; however the immune system can also be vulnerable to these oxidative attacks. The oxidative stress induced by high concentrations of ROS can decrease the integrity of the cell membrane, with resultant alterations in the membrane flow and communication between cells affecting the immune response.[51-52] Therefore, in clinical conditions such as infections that present with high ROS concentrations, vitamin C deficiency can exacerbate the clinical status of the patients.

Studies with healthy children and adults using supplementation of vitamin C of 20 mg/kg/day and 1-3 g/day, respectively, enhance the capacity of neutrophils and macrophages to eradicate microbes. Peritoneal macrophages from mice treated *in vivo* with antioxidant vitamins, including vitamin C, have an improved phagocytosis function.[48] Although no studies have formally evaluated the role of vitamin C in leprosy, it is likely that vitamin C deficiency adversely affects the disease, considering these negative effects in phagocytic cells.

A decreased in serum levels of ascorbic acid was described in patients with tuberculosis, when compared to healthy controls.[53-54] After 1 month of multidrug therapy, 15 patients were supplemented with 1 g of ascorbic acid and 600 mg of vitamin E, and these patients presented an increase in plasma antioxidant capacity.[55]

Studies in humans show that the zinc deficiency provokes a deficiency of Th1 response. Inflammatory cytokines, such as IFN-γ, IL-2 and TNF-α that are important for the control of intracellular pathogens, such as *M. leprae*, were reduced, while the production of IL-4, IL-6 and IL-10 were not affected.[56] Conversely, prolonged zinc supplementation increased the production of IL-2 and significantly decreased the incidence of respiratory infections.[56] IL-2 is a cytokine that induces proliferation of Th1 cells.

The possible antioxidant role of zinc can be associated with regulation of metallothionein (MT) expression, a protein with low molecular weight and rich in cysteine residue, which has antioxidant properties in many conditions such as radiation, drugs and heavy metal exposures.[57] Zinc is a structural and catalytic component of the superoxide dismutase (SOD), and is important for its activity. SOD is an antioxidant enzyme that reduces oxidant effect of the oxygen reactive species, transforming superoxide (O_2^-, $+O_2^-$, $+2H^+$) in hydrogen peroxide ($H_2O_2 + O_2$), a form that minimizes the chain reaction of the cellular injury. There are two types of SOD, a cytoplasmic form, which contains copper-zinc in its molecule (CuZnSOD), and a mitocondrial form, which contains manganese (MnSOD).[58] The loss of zinc in the cellular membrane can affect its function, flow, the sodium and calcium transport channels and the hydro and osmotic balance of the cell. Zinc still can stabilize the reduced form of sulfhydryl groups, protecting against the effects of lipid peroxidation of the cellular membranes.[59]

A study compared the levels of zinc in blood and scalp hair of males infected by the human immunodeficiency virus (HIV+) with healthy males controls, and showed that HIV+ patients had lower levels of zinc when compared to controls ($p < 0.001$). The zinc deficiency may contribute to the emergence of other secondary infections in HIV + patients, increased morbidity and mortality of these individuals.[60]

Selenium - The role of in the inflammatory response

Selenium is a micronutrient classified as an essential trace element that is strongly linked with complex enzymatic and metabolic functions. Selenium has several biological functions, the most important being its interaction with the glutathione peroxides (GP_{xs}). The glutathione catalyzes the reduction of hydrogen peroxide and organic hydroperoxides thus it is important to protect the lipids from the membrane and other cellular constituents against oxidative injuries.[61]

Selenium also affects the chemotactic and microbicidal activities of phagocytic cells, components of the innate immune response. Selenium modulates leukotriene synthesis and peroxide regulation in the microenvironment of immune competent cells.[61-62] However, a perfect balance of selenium must be maintained, because, while phagocytosis and lymphocyte activity can be stimulated in an adequate selenium supplementation, higher doses are inhibitory. Although clinical studies have demonstrated selenium deficiency in patients with tuberculosis, asthma and HIV, there are no reports regarding the selenium levels in leprosy patients.[63-64]

Although several studies show the oxidant and antioxidant effects of each specific nutrient, the supplementation of these nutrients must be carefully evaluated due to the interaction of their effects. It is observed that high doses of a single antioxidant nutrient might induce an opposite oxidant effect. Moreover, it is important to take into account that the biochemical, clinical and genetic individuality of the response to the nutrients, being difficult to establish their ideal doses and specific effects in the prevention and treatment of diseases.[65]

Vitamin D – Immunemodulatory functions in mycobacteria infections and specifically in leprosy

During many years it was defined that vitamin D presents an essential role on the development of bones mineralization, however other role for vitamin D has been suggested after the discovery of vitamin D receptors (VDR) in tissues that are not involved in calcium and phosphate metabolism. The VDR are also seen in many tissues and body cells, with the capacity to develop a great variety of biological response. The immunomodulatory action of vitamin D happens through direct action of T cell function and the cell presenting antigens.[50,66]

Mycobacterium tuberculosis (*M. tuberculosis*) and *M. leprae* are intracellular bacteria, so the defense mechanisms of host against the pathogens are similar. Interestingly, before discovering the etiological cause for tuberculosis, it was usual to use vitamin D from cod liver oil and exposure to sun radiation for its treatment.[67-68] In fact, recent studies associate vitamin D deficiency with the increase in development of tuberculosis.[37,69,70] The biological mechanisms through which vitamin D modulates the immune system to fight *Mycobacterium* are still under study, though some are already known. The vitamin D active metabolite, 1α, 25-dihidroxivitamin D3 (1α, $25\text{-}(OH)_2D_3$), present an *in vitro* antimicrobial activity in monocytes and macrophages (fig. 2). The $1\alpha,25\text{-}(OH)_2D_3$ acts by improving the phagosome and lysosome fusion in infected macrophages, reverting the ability of *Mycobaterium sp.* of preventing the fusion of phagosomes to lysosomes.[71] The $1\alpha,25\text{-}(OH)_2D_3$ also promotes the death of the *Mycobacterium sp.*, by inducing the production of antimicrobial peptides in the infected macrophages and neutrophils. These peptides have immunomodulatory activity in innate immunity. They are divided into two families: cathelicidins and defensins, both involved in immune response in several infectious diseases.[72] One of the studied peptides of the cathelicidin family is the LL-3, that is involved in the first line of defense against infections, recruiting monocytes, T-cells and neutrophils to the infection site. Additionally, the LL-37 activates macrophages by binding to Toll-like receptors (TLR), inducing the death of *M. tuberculosis*. These data are corroborated by an study which showed that serum samples from individuals from populations known to have a high susceptibility to develop tuberculosis present low concentration of 25-hydroxivitamin D and low efficiency of the cathelicidin peptide.[73] Matzner and colleagues, 2011 compared the levels of cathelicidin and 25OH-vitamin D3 in 29 leprosy patients and 19 healthy subjects, and showed that levels of cathelicidin in the leprosy patient were lower than the control's group ($p < 0.001$), although the levels 25OH-vitamin D3 did not differ between the groups.[74]

It is also known that IFN-γ, secreted by Th1 cells, which have a known protective role against intracellular agents, potentiates the effect of the 1α-hidroxilase enzyme. This enzyme converts the vitamin D from inactive to active form ($1\alpha,25\text{-}(OH)_2D_3$). Additionally, IFN-γ also inhibits the induction of an enzyme which participates in the $1\alpha,25\text{-}(OH)_2D_3$ inactivation.[75] On the other hand, $1\alpha,25\text{-}(OH)_2D_3$ can contribute to the dissemination of *M. leprae* by affecting dendritic cells and T-regulatory cells. In the dendritic cells, $1\alpha,25\text{-}(OH)_2D_3$ decreases maturation, inhibiting the MCH class II, CD40, CD80, CD86 expression, decreases the IL-12 and increases the IL-10 production. In T-cell, $1\alpha,25\text{-}(OH)_2D_3$ can promote FoxP3 expression and the IL-10 production, favoring the development of T regulatory cells[76] (fig. 2).

As infectious diseases are multifactorial it can also be influenced by other environmental and genetic factors. The genetic factors can also affect the susceptibility to infections and the levels and effects of nutrients, such as of vitamin D. A polymorphism in the vitamin D receptor gene (codon 352 C/T) was described, which is functional and leads to a decrease of the active metabolite of this vitamin and affects bone mineralization. However, this receptor also affects the Th1/Th2 immune response. Individuals who present tt homozygous gene alleles tend to develop a Th1 response, and those who present TT homozygous gene alleles tend to develop a Th2 response. It was shown, in a case-control study with 2015 Africans, that genotype tt was less frequent in tuberculosis patients.[77] A study done in India with 231 leprosy patients (107 tuberculoid and 124 lepromatous) from Calcuta, India, has evaluated this polymorphism in the vitamin D receptor gene (codon 352 C/T) and verified that genotype tt was associated with tuberculoid leprosy (Odds ratio [OR] = 3.22 [95% CI 1.47-7.13]), genotype TT was associated with lepramatous leprosy (OR 1.67 [95% CI 1.02-2.75]) and the resistance in developing the disease can be associated to the heterozygous genotype (Tt) (OR 0.58 [95% CI 0.38-0.89])[78].

The data above suggest that VDR and vitamin D can have great importance in the human infection by intracellular agents such as in tuberculosis and leprosy. Their stimulating actions to innate immune response and down modulatory actions to the adaptive immune response can be fundamental for the asymptomatic balance of the infection. Therefore, studies evaluating deficiency of this vitamin and the polymorphism of its receptor can contribute to predict the clinical evolution and its dietary reposition must be evaluated as adjuvant for the treatment of this disease.

Conclusions

Due to the complexity of clinical presentations, the multitude of factors involved in the control of *M. leprae*, and the complications that can occur, leprosy remains a huge challenge for clinicians and scientists. Immunologically, leprosy is a spectral disease model that involves components of both the innate and adaptive immune response. These contribute not only to protection but also to pathogenesis, with skin and neurological injuries that can ultimately culminate in permanent disability. Leprosy is still relatively understudied, particularly in relation to the impact of various nutritional factors, taking into account that the disease affects developing countries. Leprosy patients, with different clinical forms, but particularly in the lepromatous form, present a reduction in potential antioxidant substances. Several studies show familial aggregation and the influence of genetics in disease outcome, which open the possibility of an influence of a combination of genetic background with environmental factors. The importance of nutritional status, specially related to micronutrients should be investigated, mainly because the disease develops in long term and the nutritional balance might reduce the risk of acquiring the disease. It is also known that a reduction in the level of antioxidant and immune modulatory nutrients can contribute for an increase in the oxidative stress and complication in the disease and in its treatment, since the decrease of these nutrients can be one of the reasons for the increase of the skin and neurological injuries induced by immune response products against the pathogen. Thus, further studies considering the action of these antioxidant and immune modulatory nutrients in patients infected with *M. Leprae* should be designed to elucidate pathogenic mechanisms. This knowledge is of great importance to give support for dietary supplementation as an adjuvant for improvement of the leprosy treatment.

Conflict of interest

The authors declare there is no conflict of interest in the development of the study.

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Author contributions

CMPV: Wrote the manuscript. RSMN: Contributed to the revision of the manuscript. TRM: Assisted the preparation of figures and contributed to the revision of the manuscript. RPA: Contributed to the revision of the manuscript. MSD: Revised the manuscript. ARJ: Helped to write and review the manuscript.

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