Title: The role of vitamin D in the control of *Leishmania* infection

Ramos-Martínez Espiridión[a*], Gutierrez-Kobeh Laila[a], Villaseñor-Cardoso Mónica Irais[a].

[a]Unidad de Investigación en Medicina Experimental, Facultad de Medicina, Universidad Nacional Autónoma de México, México, D.F., México.

[*]**Corresponding author**

Espiridión Ramos Martínez. Unidad de Investigación en Medicina Experimental. Facultad de Medicina. Universidad Nacional Autónoma de México. Dr. Balmis 148 Colonia Doctores México D.F. 06720, México.
Tel.: +52 55 56232664; fax: +52 55 57610249.
E-mail address: espiri77mx@yahoo.com

Abstract

Vitamin D has been described as an essential element for maintaining the homeostasis of mineral content in the body and bone architecture. However, our view of the physiological functions of this micronutrient has radically changed due to a vast number of properties, not calcium-related, mediated by its nuclear receptor. This receptor has been found in a variety of cells including the immune cells, where many of the functions performed by vitamin D are related to inflammation. Although the effect of vitamin D has been widely studied in many diseases caused by viruses or bacteria, very little is known about its role in parasitic diseases, such as leishmaniasis, a vector-borne disease caused by different species of the intracellular parasite *Leishmania*. This disease occurs as a spectrum of different clinical syndromes, all of them characterized by a large amount of tissue damage, sometimes leading to necrosis. Due to the involvement of vitamin D in inflammation and wound healing, its role in leishmaniasis must be relevant and could be used as an adjuvant for the control of this parasitic disease, opening a possibility for a therapeutic application.

Key words: 1,25 (OH)2D3, leishmaniasis, inflammation, cytokines, immunoregulation, wound healing.

Introduction

The use of vitamin D as a treatment of infections started approximately 150 years ago, although the mechanisms underneath this practice were not understood. Over a hundred years ago, Williams (1849) reported that patients with tuberculosis treated with cod-liver oil, which was shown to have a "highly nutrient material" as compared to other oils, exhibited significant improvement in their condition. Later, Niels Finsen (1903) described a method to treat patients with lupus vulgaris, a cutaneous form of tuberculosis, with ultraviolet light (UV) administration. These successful experiments granted Dr. Finsen the Nobel Prize in Medicine on 1903. In both cases, what the empirical evidence showed was that the treatments directly killed *Mycobacterium tuberculosis*. Years later it was revealed that the administration of cod-liver oil or UV light increased the levels of vitamin D which stimulated the immune response and killed *M. tuberculosis* (Martineau et al. 2007). Indeed, vitamin D has been shown to elicit different cellular functions such as differentiation (Sigmundsdottir 2011), proliferation (Cordes et al. 2012) and apoptosis (Kreutz et al. 1993). Also, it has been reported that some of the vitamin D effects directly impact in the regulation of the immune response that might be essential for the treatment of degenerative and infectious diseases. Regarding the last ones, it has been reported that vitamin D increases antimicrobial peptides which are very effective in the control of bacteria (Sato et al. 2013). However, in the case of parasitic infections, little has been reported about the role of vitamin D in the susceptibility or resistance to these infections. Parasitic infections represent a world health problem affecting mainly developing countries

and their control is complicated due to different factors such as malnutrition, vector control and resistance to drugs.

Leishmaniasis is a parasitic disease about which it is estimated that there are 12 million people infected in 88 countries (Hotez et al. 2012). The information about the role of vitamin D in the development of this disease is scarce and controversial.

Vitamin D and its receptor

Vitamin D has been frequently associated with its essential role in the metabolism of calcium (White 2008). Nevertheless, in recent times it has been determined that this micronutrient has pleiotropic actions in several tissues, including a key role in immune regulation through 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), the most active metabolite (Di Rosa et al. 2011). 1,25 (OH)2D3 is a steroid hormone which elicits its wide-spectrum functions through binding to the vitamin D receptor (VDR) (Carlberg et al. 2011). The mechanism of action of 1,25 (OH)2D3 is very similar to that of other steroid hormones. VDR is a member of the superfamily of nuclear hormone receptors, including receptors for steroid and thyroid hormones as well as retinoic acid. Once VDR binds to its ligand, it forms a heterodimer with the retinoid X receptor necessary for the regulation of vitamin D target genes. This heterodimeric complex interacts with specific DNA sequences, known as vitamin D response elements, to induce either activation or repression of transcription (DeLuca 2004; Rachez and Freedman 2000).

The actions of vitamin D, that are not related to bone metabolism, can be divided in three categories: regulation of hormone secretion, regulation of cellular proliferation and differentiation, and regulation of immune function (Bikle 2009). The immune regulation by vitamin D was first observed 25 years ago through different findings such as the expression of VDR in inflammatory cells (Provvedini 1983), the capacity of vitamin D to inhibit T cells proliferation (Rigby 1984) and the ability of macrophages from patients with sarcoidosis to produce 1,25 (OH)2D3 through an increase in the expression of CYP27B1 enzyme (Adams 1983).

Immune regulation by vitamin D

Since the role of vitamin D in the regulation of the immune system was established, a large amount of information has been reported. The first indications of a relationship between vitamin D and the immune system were obtained from the treatment of patients with tuberculosis, as has already been mentioned. Later, more evidence of the role of vitamin D in the innate and adaptive immune system were obtained from the association of deficiencies, seasonality and geographic location of degenerative and autoimmune diseases. The underlying mechanisms of how vitamin D contributes to the regulation of the immune system is nowadays under constant research. Vitamin D can act at early levels of the innate immune system, such as in the expression of toll-like receptors (TLR). It has been shown that 1,25 (OH)2D3 up-regulates TLR10 and down-regulates TLR-2, 4 and 5 in human monocytes *in vitro* (Verma et al. 2014). On the other hand, it has been documented that in cells such as macrophages, epithelial cells and keratinocytes 1,25 (OH) 2D3 increases the production of cathelicidins (antimicrobial peptides)

(Liu et al. 2006). Furthermore, it was found that vitamin D also increases the production of filaggrin by epithelial cells in the skin (Lützow-Holm et al. 1995). Taken together, these findings suggest that vitamin D contributes to the functionality of some mechanisms of the innate immune response.

On the other hand, vitamin D can also regulate the adaptive immune response by mechanisms such as the inhibition in lymphocyte proliferation, especially T-helper 1 (Th1) cells (Mangge 2013; Daniel et al. 2008), and the increase of IL-10 levels (Niino et al. 2014). These events could be very useful in the treatment of pathologies where uncontrolled inflammation is a cause of tissue destruction. Although the data about the immune regulation by vitamin D in some diseases may be controversial, the actual effect on each pathology should be explored particularly, since it could be influenced by multiple factors inherent to the disease and the individual.

Leishmania infection

Leishmaniasis is an infection caused by various species of *Leishmania* parasites, which are transmitted by Phlebotomine sand flies, where they develop as flagellated promastigotes in the digestive tract, are injected into the skin of the mammalian host during the vector bloodmeal, and escape the toxic extracellular milieu by being internalized by phagocytes such as macrophages and dendritic cells (Solbach and Laskay 2000). After internalization, promastigotes differentiate to small, non-motile amastigotes that proliferate within the host cell phagolysosome (Kane and Mosser 2000). Amastigotes perpetuate disease in the mammalian host

as a result of continuous release due to cell lysis or selective exocytic events and invasion of other cells (Rittig and Bogdan 2000). Recent data show that leishmaniasis is a global problem of health and 12 million people currently is affected by this parasitic disease with an increase of 700,000 to 1.2 million people per year (WHO 2010) (Figure 1).

It occurs as a spectrum of clinical syndromes, which are usually divided into cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL). In turn, CL presents two different manifestations: localized cutaneous leishmaniasis (LCL) characterized by a single and painless lesion and diffuse cutaneous leishmaniasis (DCL) (Figure 2), with metastatic lesions in all the skin, except for the scalp and soles of feet and hands (Silveira et al. 2004; Murray et al. 2005). In the case of VL the main organs affected are bone marrow, liver and spleen (Stanley and Engwerda 2007), with a marked hepatosplenomegaly, even palpable over the skin (Pearson and Souza 1996). On the other hand, in MCL the lesions begin with erythema and ulcerations in the nose, with a later destruction of the nasal septum and necrotic lesions and in many patients obstruction of the pharynx or larynx (Murray et al. 2005). The presence of these different clinical manifestations has been attributed to the species of the parasite and the immune response of the host, although additional mechanisms are involved that remain to be uncovered. The infection of the skin with *Leishmania* results in extensive remodeling of the tissue and CL is associated with chronic skin inflammation (Grimaldi and Tesh 1993; Reithinger et al. 2007).

In relation to the role of the species in the development of the different clinical manifestations it is noteworthy that several *Leishmania* species cause CL: *L. tropica, L. major* and *L. aethiopica* in the Old World (Africa, Asia and Europe) and *L. mexicana, L. amazonensis and L. braziliensis* complexes in the New World.

Although these parasites share similarities in their life cycle, each species shows particular characteristics in the development of the lesions. For example, *L. major* induces the fastest progression and disease resolution while, *L. aethiopica* is associated with slow disease progression and healing (WHO 2010). Many insights about the role of the host immune response in the development of the different clinical manifestations of leishmaniasis have been obtained from the murine model of human leishmaniasis. It has been shown that the mouse strain plays an important role in the outcome of the disease (Reiner and Locksley 1995). It is well documented that *L. mexicana* causes a progressive disease which rapidly spreads in the susceptible BALB/c mouse, whereas in C57BL/6 mice the disease tends to be self-limited (Villaseñor-Cardoso et al. 2008).

Evasion of the immune response

Leishmania has different mechanisms of evasion during its life cycle. Once the parasites are released after the sandfly bloodmeal, they have to enter rapidly into host cells in order to assure their survival. During invasion of promastigotes, the infective form released by sandflies, tend to resist and modulate the host immune response due in part to a dense surface glycocalyx largely composed of lipophosphoglycan (LPG). This molecule has been implicated in the attachment and release of promastigotes in the midgut of the sandfly, complement resistance,

interaction with host cell receptors and triggering of phagocytosis and signal transduction, and inhibition of oxidative burst, rendering the parasite suitable to establish successful infections (Beverley and Turco 1998).

Leishmania promastigotes are efficiently phagocytosed by macrophages and numerous studies have identified that the uptake of promastigotes by macrophages is mainly mediated by complement receptors (CR1 and CR3), although the receptors for the Fc domain of immunoglobulins (FcRs), for mannose-fucose (MR), and fibronectin are also implicated (Awasthi 2004). Interestingly, the recognition of *Leishmania* through CR3 promotes phagocytosis, but without the activation of oxidative burst (Cunningham, 2002). It has also been shown that LPG is recognized as a PAMP (pathogen-associated molecular patterns) by Toll-like receptors triggering signal transduction pathways that upregulate the production of pro-inflammatory cytokines (De Veer et al. 2003; Kavoosi et al. 2009). On the other hand, other reports have shown that *Leishmania* promastigotes can also inhibit TLR-2 activation. Specifically *L. donovani* promasitogotes suppressed the production of pro-inflammatory cytokines, despite having LPG on their surface (Srivastav et al. 2012).

Once inside host cells, *Leishmania* displays multiple evasion strategies in order to assure its survival. Some of these mechanisms are also LPG-dependent as in the case of *L. donovani* promastigotes whose LPG inhibits trafficking into lysosome- derived compartments in neutrophils allowing its survival inside these cells (Gueirard et al. 2008). The main microbicidal molecules against *Leishmania,* nitric oxide (NO) and reactive oxygen intermediates (ROI) produced by phagocytic

cells, are also affected by the parasite. It has been shown that macrophages incubated with *Leishmania* LPG or glycosylinositolphospholipids (GIPL) lose their ability to induce iNOS and subsequent generation of NO after IFN-γ and /or lipopolysaccharide (LPS) induction (Proudfoot et al. 1995). ROI generation is also inhibited by *L. donovani* and appears to be dependent on LPG and gp63 (Olivier, et al. 2005).

Furthermore, *Leishmania* can also prevent cytokine secretion that could lead to parasite killing by interfering with a broad range of signal transduction events such as the activation of protein kinase C, the IFN-γ/JAK2/STAT1 cascade, the MAP kinase-NF-kB pathway in response to phorbol esters, IFN-γ or LPS (Bogdan 2008). Opposite to this, cytokines can have a beneficial effect for the parasite during infection through the recruitment of inflammatory cells that serve as parasite reservoirs. It has been shown that the inoculation of *L. major* in air pouches of Balb/c mice can induce inflammatory mediators such as TNF-α and IL1-β 6 hours after inoculation leading to the recruitment of neutrophils monocytes/macrophages and eosinophils, important targets for parasite entry. Additionally, *L. major* can induce macrophage chemokine gene expression (i.e., MIP1-α, MIP1-β, MIP-2 and MCP-1) *in vitro* (Matte and Olivier 2002). *Leshmania* can also induce the production of high amounts of IL-10 through the ligation of macrophage FcγR by opsonized amastigotes, which prevents parasite killing by activated macrophages (Kane and Mosser, 2001).

Finally, as *Leishmania* needs to invade and multiply within host cells, it has developed several strategies to inhibit apoptosis in macrophages (Akarid et al.

2004; Moore and Matlashewski 1994), neutrophils (Aga et al 2002) and dendritic cells (Valdes-Reyes et al. 2009; Gutiérrez-Kobeh et al. 2013). The underlying mechanisms of the inhibition of apoptosis by *Leishmania* have not been fully understood. One of the signaling pathways that has been implicated in the inhibition of apoptosis is PI3K, which is activated during *Leishmania* infection (Ruhland, et al. 2007).

Vitamin D and leishmaniasis

The role of vitamin D and VDR in leishmaniasis has been scarcely investigated with only three reports published with controversial results (Ehrchen et al. 2007; Whitcomb et al. 2012; Ramos et al. 2013). Some of the differences among these works reside in the fact that different experimental models and *Leishmania* species were used. The studies by Ehrchen et al. and Whitcomb et al. employed C57/BL6 mice KO for VDR infected with *Leishmania major* and treated with vitamin D. Erchen et al. reported that the treatment with vitamin D suppressed IFN-γ production, which lead to an inhibition in NO production and concomitantly the lack of activation of macrophages, events favoring the survival of the parasite (Ehrchen et al. 2007). The results reported by Whitcomb et al. confirm that the suppressive effects of vitamin D influence the establishment of infection. They describe that in the absence of the receptor and with enzymatic depletion of vitamin D, the susceptibility to the parasite is greatly reduced. Although this work provides important information on the role of the lack of vitamin D in the course of *Leishmania* infection, the fact that VDR KO mice were used which present multiple

alterations in their organs and tissues, it is difficult to extrapolate the results to wild type mice (Whitcomb et al. 2012). On the other hand, the work by Ramos et al. used susceptible BALB/c mice infected with *L. mexicana* and supplemented with 1,25 (OH) 2D3 in order to analyze its effect in the development of skin lesions. They determined the *in situ* expression of IL-6, IL-10, IL-12, IFNγ and TGFβ in mice infected with *L. mexicana* and treated with 1,25(OH)2D3 and show that, the treatment does not affect the survival of the parasites. However, the spread of *Leishmania* is limited, the cellular architecture in the tissue is preserved and wound healing is accelerated (Ramos et al. 2013). Taken together all these works represent a very important source for the study of this steroid in the infection with the intracellular parasite *Leishmania*.

Inflammation is an immune mechanism aimed to limit and repair damage induced by physical, chemical or biological agents. In doing so, different immune cells are recruited to the site of inflammation (commonly called the inflammatory infiltrate) that will control infection and finally lead to its resolution. In the case of the cutaneous lesions caused by *L. mexicana* in mice infection, it has been shown that the inflammatory infiltrate present 12 weeks after infection consists of several parasitized cell types such as neutrophils, eosinophils, and macrophages and a large area of necrosis, which considerably increases after 24 weeks post-infection (Baldwin et al. 2007). In contrast, it has been reported that mice treated with 1,25(OH)2D3 showed lower numbers of neutrophils and infected macrophages, but a greater number of eosinophils and fibroblasts as compared to untreated animals. Interestingly, this inflammatory infiltrate is similar to what has been found in C57BL/6 mice, which are a strain of mice considered to be resistant to the infection

by some *Leishmania* species. In these mice the number of neutrophils diminishes after the 6th week of infection and virtually disappears during the 9th to 12th week post-infection (Ramos et al. 2013; Baldwin et al. 2007).

Although macrophages and dendritic cells are primarily the host cells for *Leishmania*, neutrophils play an essential role since they represent the first line of defense against the parasite. Neutrophils release a variety of molecules such as MIP-1β, a chemokine that attracts macrophages that ingest infected neutrophils. This strategy, which has been named the Trojan horse, represents another important route for the parasite to enter the host and disperse to different tissues (Sacks and Noben-Trauth 2002). Once *Leishmania* is inside macrophages, it is exposed to different microbicidal mechanisms, but as it has been exposed above, parasites exert different strategies to avoid destruction, as for example the blockage of the phagosome-lysosome fusion and hiding in non-lytic compartments (Gueirard et al. 2008). The role of neutrophils in the dispersion of *Leishmania* makes them key elements in the development of the pathology (Kaye and Scott 2011). The fact that mice treated with vitamin D showed a reduction in neutrophil numbers could be interpreted as an indication of control of the infection (Ramos et al. 2013).

The landmark in the control of *Leishmania* infection in resistant mice is the classical activation of macrophages and subsequent production of microbicidal molecules (Giudice et al. 2012). Macrophages can also be alternatively activated and produce molecules necessary for the remodeling of tissues. Relative to this, it has been reported that vitamin D neither affect the number of macrophages

recruited to the site of infection nor the ratio of infection. This suggests that vitamin D does not affect directly *Leishmania* survival thanks to the mechanisms of evasion displayed by the parasite to counteract the microbicidal effects of macrophages (Ehrchen et al. 2007; Ramos et al. 2013). This suggests that vitamin D does not have an effect directly on the parasite thanks to the evasion mechanisms exerted by *Leishmania* to counteract the microbicidal mechanisms of macrophages and manage to stay alive in intracellular compartments (Ehrchen et al. 2007; Ramos et al. 2013). Interestingly, another important difference between macrophages from mice treated with vitamin D and untreated is that in the first case macrophages remain confined in clusters surrounded by large numbers of fibroblasts, while in untreated animals they are dispersed, which implies that they have the potential for spreading infection (Ramos et al. 2013). Indeed, it has been reported that macrophages incubated with 1,25 (OH) 2D3 lose the ability to kill *Leishmania* parasites (Ehrchen et al. 2007). However, the number of parasites in animals treated with vitamin D is similar to what has been observed in C57/BL6 mice which are considered resistant to some *Leishmania* species (Ehrchen et al. 2007; Aguilar et al. 2002). On the other hand, macrophages can also be alternatively activated and produce molecules necessary for the remodeling of tissues. It has been shown that, although the treatment with vitamin D increase the proportion of macrophages alternatively activated (Zhang et al. 2014), the role of this macrophage phenotype has not been fully explored in this parasitic disease.

Another interesting difference between the inflammatory infiltrate induced by *Leishmania* infection in mice treated with vitamin D and untreated is an increased aggregation of eosinophils, which are almost absent in the latter (Ramos et al.

2013). Although the role of eosinophils in leishmaniasis has not been fully defined, it has been reported that resistant mice present larger numbers of eosinophils in the inflammatory infiltrate induced in response to infection with this parasite as compared to susceptible ones (Guerra et al. 2010). It has been described that *Leishmania* releases chemotactic factors for eosinophils and these cells could play a role in the clearance of the parasites (Solbach and Laskaym 2000). Eosinophils are involved in the elimination of pathogens through the release of their granular contents (Solbach and Laskaym 2000). Moreover, it is known that various factors released by eosinophils promote angiogenesis and wound healing (Kita 2011; Curran and Bertics 2012). Noteworthy, it has been reported that vitamin D induces the secretion of these factors to the extracellular medium (Snyman et al. 1997).

The data obtained from the analysis of the cellular infiltrate in mice treated with vitamin D has permitted a comparison between the effect of the treatment with this vitamin and the treatment with IFN-γ and antimonial drugs in humans reported by Salaiza and coworkers (1999). They showed that patients with LCL that did not receive treatment are characterized by the absence of sweat glands, large numbers of parasitized macrophages and large areas of necrosis. Interestingly, the inflammatory infiltrate was similar to the one found in BALB/c mice. On the contrary, LCL patients treated with IFN-γ and antimonials presented an infiltrate similar to the one found in mice treated with 1,25(OH)2D3, represented by a decrease in the epithelial atrophy and a great number of collagen-producing fibroblasts (Ramos et al. 2013). This type of wound healing is similar to what has been reported in chronic stages of *Leishmania* infection in resistant mice (Sakthianandeswaren et al. 2005).

Regarding the cytokines production in leishmaniasis, Rosas et al., (2005) reported a moderate production of IL-10 and IFN-γ (low with respect to resistant strains) and a similar concentration of IL-12p70 to that of resistant strains. However, Jones et al., (2000) have reported that although this cytokine is produced in the presence of this parasite is not essential for its elimination. The treatment of *Leishmania*-infected mice with 1,25(OH)2D3 also affects the profile of cytokines aforementioned. It has been shown that treated mice show a decreased expression of these cytokines, such reduction is in accordance with the already reported treatment with 1,25(OH)2D3 (Matilainen et al. 2010; Wu et al. 2011; Griffin et al. 2011). Moreover, it is known that during *L. mexicana* infection there is an over expression of TGF-β, a cytokine that down-regulates the activation of macrophages and the production of some growth factors in somatic cells, events that favor parasite survival. In this regard, it has been shown that treatment with vitamin D down-regulates TGF-β production induced by *L. mexicana* infection (Ramos et al. 2013). TNF-α has effects opposite to TGF-β promoting several inflammatory events and **is** also implicated in ischemic (Maddahi et al. 2011) and apoptotic (Kim et al. 2012) processes; therefore a decrease in TNF-α production is required for remodeling of the infected tissue, what happens with the treatment with vitamin D (Prabhu et al. 2009). Finally IL-6, inducer of acute phase proteins, is produced in large amounts in the early stages of infection by *Leishmania*. Due to its pro-inflammatory effects, a decrease in the production of this cytokine is indicative of a reduction in tissue damage. This effect has been documented in patients with CL treated with antimonial drugs (Lezama-Davila et al. 2006) and also has been found with the treatment with 1,25 (OH) 2D3 (Dickie et al. 2010).

What about the susceptibility?

Unlike other degenerative (Mathieu et al. 2012; Shao et al. 2012) and infectious diseases (Khoo et al. 2012; Arji et al. 2014) where genetic variants of molecules related to vitamin D metabolism are directly implicated with an increase in susceptibility, and although there have been reported some genetic variants associated with susceptibility to *Leishmania* infection (Castelluci et al. 2014), none of them has been related with vitamin D.

Unlike other degenerative (Mathieu et al. 2012; Shao et al. 2012) and infectious diseases (Khoo et al. 2012; Arji et al. 2014) where genetic variants of molecules related to vitamin D metabolism are directly implicated with an increase in susceptibility, none of them has been related with *Leishmania* infection (Castelluci et al. 2014). However, it has been shown that generalized nutritional deficiencies are related to an increased risk for the most severe forms of leishmaniasis (Kumar et al. 2014). Oliveira and cols. analyzed patients with different social conditions affected with the most severe forms of CL and found that about 70% of them lived under poverty with the concomitant nutritional deficiencies. This led the authors to conclude that malnutrition is a predisposing factor for the most severe forms of CL (Oliveira et al. 2013). Another study carried out by Mashayekhi-Goyal and coworkers, also analyzed the correlation between leishmaniasis and poverty. They followed the course of CL in 140 patients during one year and concluded that the socioeconomic situation of the patient correlated with a prolonged course of the disease (Mashayekhi-Goyal et al. 2014). In this regard, it has also been shown that deficiencies in protein and micronutrients, such

as vitamins, increase susceptibility to the more severe forms of CL caused by *L. mexicana* in mice (Pérez et al. 1979) and VL in hamsters caused by *L. infantum* (Carrillo et al. 2014).The role of malnutrition has also been analyzed in malnourished patients affected with VL, where the innate immunity was evaluated in response to soluble leishmanial antigens. It was shown that the migration and adherence of PMNs to the endothelial wall was diminished and there was a reduction in the generation of ROS by PMNs and monocytes. These patients also showed a reduction in the chemokines MIP-1a and IL-8, the pro-inflammatory cytokines TNF-α and IFN-γ, while the level of IL-10 was increased (Kumar, 2014). These data indicate that malnourished patients with VL do not have an appropriate innate immune response able to eliminate the parasite. Moreover, it is very important to note that the distribution of leishmaniasis in the world is largely consistent with the distribution of developing countries or with a high degree of undernourishment (Figure 3). Most likely because it is in these countries where many of the conditions necessary for *Leishmania* infection are present and malnutrition is a decisive factor for the development of this and other infectious diseases due to alterations in the immune system. In regard to vitamin D, there is still no consensus about the adequate level to efficiently activate the immune response. However, serum levels of vitamin D lesser than 20 ng/ml are considered insufficient (Bischoff-Ferrari et al. 2006). Taking all the above data it can be possible to asseverate that, although deficiencies in vitamin D do not alter susceptibility to diseases such as leishmaniasis, they can alter the immune

response, which fails to control the parasite and drives the disease to its more severe forms.

Remarks

Traditionally it has been established that vitamin D is an essential micronutrient that has a leading role in the metabolism of calcium. However, recent research has brought to light multiple effects of this vitamin on various organs and tissues, in particular, its immunoregulatory effect has been studied **in** the development and resolution of various diseases. Moreover, its precise role in leishmaniasis has not been established. This is a pathology largely associated to poverty in countries with tropical and subtropical environments affecting a large number of people where malnutrition is a predisponing factor for the most severe forms of the disease. It has been shown that vitamin D supplementation in this disease has no direct effect on the survival of the parasite, although it is a very important inducer of tissue repair. Because leishmaniasis is characterized by extensive destruction of tissue it is possible that vitamin D supplementation can act as an adjuvant in the treatment of the parasitic disease.

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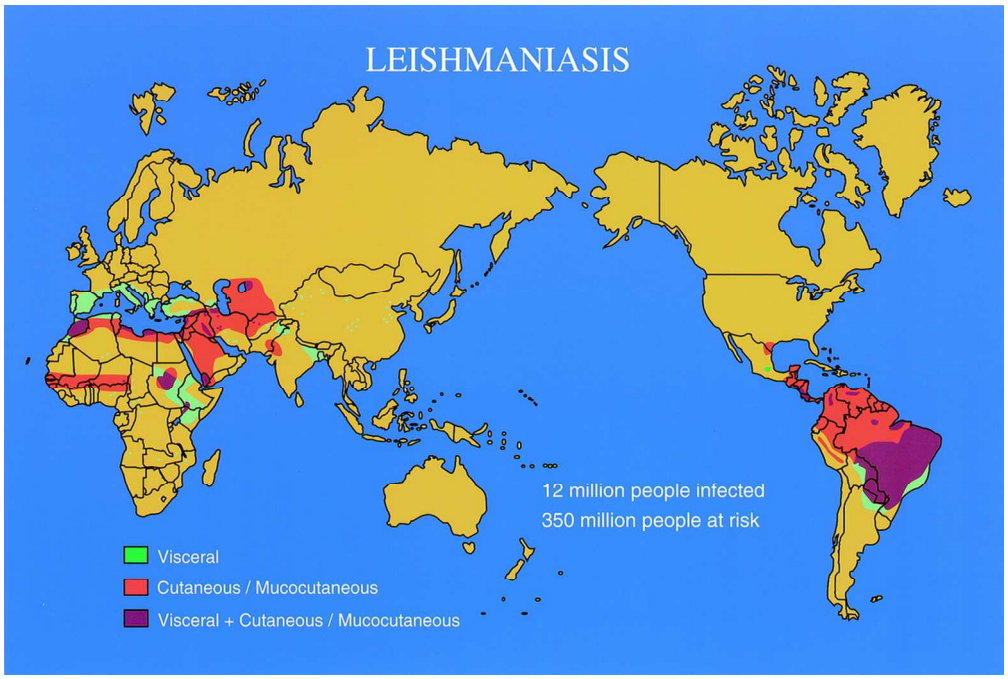
Legends:

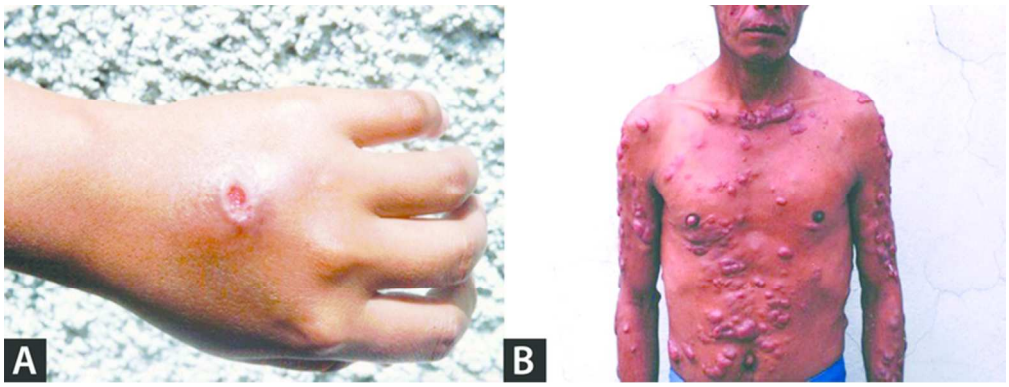
Figure 1: World map highlighting areas where cutaneous, visceral and mucocutaneous leishmaniasis is endemic, this figure is reproduced with permission from American Society for Microbiology (Handman 2001).

Figure 2: Characteristic lesions of localized cutaneous leishmaniasis (A) and disseminated cutaneous leishmaniasis (B), this figure is reproduced with permission from Villaseñor-Cardoso (Villaseñor-Cardoso 2001).

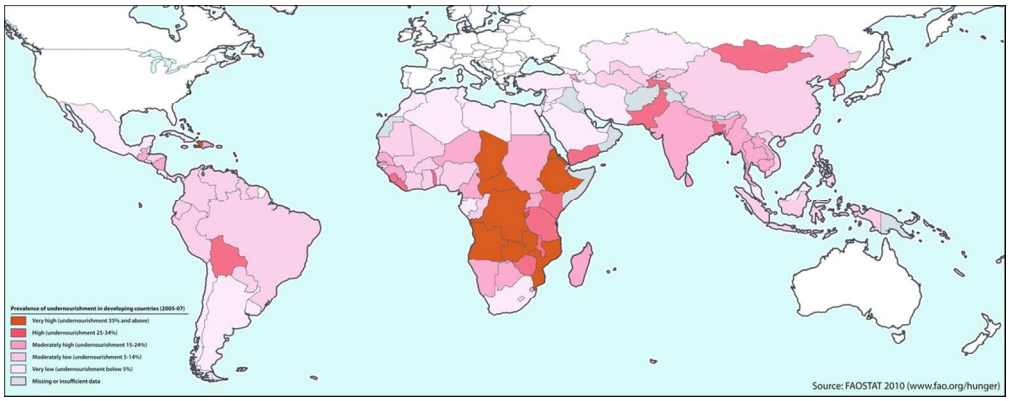
Figure 3: FAO hunger map 2010, prevalence of undernourishment in developing countries (http://www.globalsherpa.org/wp-content/uploads/2011/02/fa-hunger-map-2010.jpg)(FAO 2010).



World map highlighting areas where cutaneous, visceral and mucocutaneous leishmaniasis is endemic (Handman 2001).
133x89mm (300 x 300 DPI)



Characteristic lesions of localized cutaneous leishmaniasis (A) and disseminated cutaneous leishmaniasis (B) (Velasco et al. 1989).
71x27mm (300 x 300 DPI)



FAO hunger map 2010, prevalence of undernourishment in developing countries (FAO 2010).
110x43mm (300 x 300 DPI)