

Leishmania Donovanii: How it makes Fool to our Immune System

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Abstract- Visceral Leishmaniasis (Leish'ma NIGH a sis) is a vector-borne anthrozoontic disease caused by obligate intracellular macrophage protozoan *Leishmanian donovani* belongs to genus within the family *Trypanosomatidae*. This is very serious disease and endemic in warmer part of the world covering almost 88 countries (16 developed and 72 developing or 66 countries in the Old World while 22 countries located in the New World). A total of 350 million people at risk and 12 million cases of infection every year. Out of 500,000 cases of Visceral Leishmaniasis, more than 90 percent are reported from India, Bangladesh, Southern Sudan, Nepal and Northeast Brazil.

The protozoan parasite *Leishmania* exists at least in two alternative forms in two different hosts, 1. Amastigote form 2. Promastigote form

Promastigotes of *Leishmania* (infective stage in human) are released in human blood by female sand fly (*Phelobotomus argentipes*). After entering, these parasites are engulfed by the macrophages of the patients for killing them but due to several protective mechanisms leishmanian promastigotes are not only protected himself inside the macrophages but also rapidly divide within it and releases about ~20 amastigotes form each infected macrophages by bursting them.

Index Terms- *Leishmania donovani*, Visceral Leishmaniasis, Sand Fly, Amastigotes, Promastigotes.

I. INTRODUCTION

Visceral Leishmaniasis (Leish'ma NIGH a sis) is a vector-borne anthrozoontic disease caused by obligate intracellular macrophage protozoan, *Leishmania donovani*, a genus within the family *Trypanosomatidae*. Visceral Leishmaniasis (VL) also known as Kala-Azar (Hindi: kala means black, azar means sickness), was first described by Leishman and Donovan in 1903. They separately demonstrated VL parasites in stained smear prepared from the spleen of patients suffering from a malaria-like illness. The disease is endemic, covering almost the five continents, in 88 countries with a total of 350 million people at risk and 12 million cases of infection. Out of 500,000 cases of visceral Leishmaniasis, more than 90 percent are reported from India, Bangladesh, southern Sudan, Nepal and northeast Brazil.

II. CLINICAL SPECTRUM

Visceral leishmaniasis (VL):

Visceral Leishmaniasis (VL) also known as Kala-azar (Hindi: kala means black, azar means sickness, also known as Assam fever, Dumdum fever, Sikari disease, Burdwan fever, Shahib's disease and tropical splenomegaly. It is second fatal disease after malaria reported by World Health Organization. In this disease, the parasite enters into spleen, liver, bone marrow and the consequences are usually with an almost 100% mortality rate if left untreated. The involvement of Visceral Leishmaniasis with HIV infection indicates that Visceral Leishmaniasis is an opportunistic infection.

Post kala-azar dermal leishmaniasis (PKDL):

It is a dermatropic form of leishmaniasis developed by part of the ex-VL patients (WHO, 1990), but there are also few which don't have any previous history of VL (El-Hassan et al., 1992). Post kala-azar dermal leishmaniasis (PKDL) is a dermal complication, caused as a consequence to VL. In Indian perspectives, it manifests in 5-15 per cent of VL cases after months or several years of remission from infection, while in global perspective (Sudan), it develops within weeks or months in 50-60 per cent of cured VL cases. Post Kala azar Dermal Leishmaniasis was first described by Brahmchari in 1922 in cured VL patients with outbreak and sign in the skin. In India, PKDL develops as a dermatosis in a small percentage of treated VL patients with a usual interval of 2-3 yr but it may occur much earlier (*i.e.*, after 6 months) or much later (up to 32 yr). In 15-20 per cent of PKDL cases no preceding history of VL is available, suggestive of subclinical infection.

Cutaneous leishmaniasis (CL):

It is known, as 'little sister' in some countries that the disease is so common that is part of the family. In the Old World is known as oriental sore. It produces skin lesions, sometimes as many as 200 on the face, arms and legs, causing serious disability and permanent scars (WHO, 1998). In the Old World is caused by *Leishmania major*, *Leishmania tropica* and *Leishmania aethiopica*. In the New World CL is caused by *L. mexicana* and *L. braziliensis* complexes.

Diffuse cutaneous leishmaniasis (DCL):

It is less common disease, chronic in evolution and particularly difficult to treat. Lesions produce by this disease is similar to leprosy, and due to lack of immune response can't be

heal spontaneously, Reported by Desjeux, 1996; WHO, 1998). DCL is caused by to *L. aethiopica* and *L. amazonensis* (Desjeux, 1996).

Mucocutaneous leishmaniasis (MCL):

Also called 'espundia', it produces disfiguring lesions to the face, it especially affects the mucous membranes of the nose, mouth and throat (Desjeux, 1996; WHO, 1998). It is mostly related to Leishmania species of the New World such as *L. braziliensis*, *L. panamensis* and *L. guyanensis*, but mucosal lesions have been reported in the Old World due to *L. donovani*, *L. major* and *L. infantum* in immunosuppressed patients (Desjeux, 1996). 90% of all cases of MCL occur in Bolivia, Brazil and Peru (WHO, 1996).

Morphology of the parasite:

The protozoan parasite *Leishmania* exists at least in two alternative forms in two different hosts:

- **Amastigote form:** formerly called Leishmanial form. *Amastigotes*, occurs in Man, are ovoid and non-flagellated form of *Leishmania*, measuring 3-5 μm in length.
- **Promastigote form:** formerly called Leptomonad form, found in sand fly. These have short oval or pear shaped bodies, measuring 5 to 10 μm in length by 2 to 3 μm in breadth. Later on, after development the parasites becomes elongated, slender and spindle shaped bodies measuring 15 to 20 μm in length and 1 to 2 μm in breadth, which occurs in the gut of sand fly (*Phelbotomus argentipes*).

Transmission:

The blood sucking insects (Sand flies) are 2-3 mm long and are found through-out the tropical and temperate parts of the world. Larval development of sand fly is required organic matter, specific range of temperature, and moistures. This condition of environment is very common in house-hold garbage, bay of old trees, burrows of old trees and in cracks in house walls. The sand flies feeds on the host at night (Arias et al, 1996). Different species of sand fly transmits disease in different countries.

P. argentipes: India and sub continents).

P. martini and *P. orientalis*: Africa & Mediterranean basin

P. chinensis and *P. alexandri*: China

Lutzomyia logipalpis: New world (Americas)

Life cycle of Leishmania donovani:

Leishmania donovani, the vector of the VL, is digenetic i.e. complete its life cycle in two hosts (Invertebrate host: sand fly & Vertebrate host: Man).

- **In Sand fly:** Female sand fly (*Phelbotomus argentipes*) receives infected cell containing *amastigotes* on sucking the blood from the infected patients. The *amastigotes* are released in the mid-gut of the insect. At this place *amastigotes* stage transforms into the procyclic stage and start multiplying actively without penetrating the hemocoel. After few days, numerous procyclic triumphs are visible over the gut of

the insect and the elongated procyclic promastigote attach to the mid-gut epithelium by inserting their long flagella in microvilli present on the lining of mid-gut. At this stage they are capable to migrate into cardiac valve, where they are transformed into short, spherical, non-dividing promastigotes. These parasites are released from the midgut and penetrate the pharynx (proboscis) as metacyclic promastigotes, also termed as *paramastigote*. From proboscis the metacyclic promastigotes are ousted to the new mammalian host.

- **Infection in man:** Metacyclic promastigotes enter into the skin of the vertebrate host (man) when the infected sand fly bites and suck out its blood as meal. It may inoculate 10-200 *promastigotes* into the dermis in a bite. They rapidly transform into *amastigotes* inside the macrophages and other related cells and reside at same place. They develop and multiply with in the macrophages and at the time when 20 or more *amastigotes* are developed, macrophages are bursts and free *amastigotes* released into the blood to infect other uninfected cells. Now, these infected macrophages and other cells move from the skin to other tissues like spleen, Liver and Bone marrow.

Intracellular survival of Leishmania: *Leishmania donovani* promastigotes stay alive and multiply in phagolysosomal compartment of macrophages, which is a very gracious setting for the parasite to live.

Normal action of Macrophages on pathogens: Macrophages usually kill the parasite by a succession of the reaction / mechanisms as follows:

➤ Oxidative burst

When the macrophage engulfs any foreign particle like bacteria, or another pathogens, an enzyme located on plasma membrane of macrophages known as NAD(P)H Oxidase becomes activated and generate the reactive superoxide and hydroxyl radicals. These two radicals not only enhance the permeability of pathogen's membrane but also capable of damaging the pathogen's macromolecules like DNA.

➤ Acidification

After fusion of phagosome with the endosomal compartment it leads to acidification of phagosome. This process is facilitated by the action of a proton ATPase located on the plasma membrane, which leads to a significant drop in pH (pH 5.0). As a consequence proteins start to denature and unfold and in due course they are hydrolyses by the action of hydrolases.

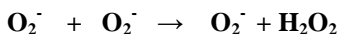
➤ Digestion

After acidification of phagosome / endosomal compartment, it get fused with primary lysosomes and forms a phagolysosomal compartment. Here, the release of enzyme acid hydrolases (digestive enzymes) causes the degradation / digestion of macromolecules such as DNA, RNA, proteins and carbohydrates.

Adaptation in Leishmania donovani promastigotes against the normal action of Macrophages

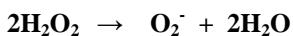
Oxygen-defense mechanisms: All pathogen have a number of mechanisms that protect themselves against oxidative pressure. These are superoxide dismutase (SOD), catalase, peroxidase and glutathione. Following systems prevent pathogen from membrane and DNA damage.

Superoxide dismutase (SOD) is act as the first line of defense mechanism against the toxic intermediates of oxygen. It extensively accelerates the supeoxide dismutation reaction spontaneously.



Superoxide dismutase (SOD) is among one of the highest known enzyme with the production rate is 10×10^6 / s.

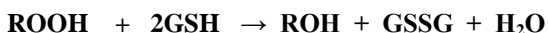
Catalase is the second defensive enzyme against the noxious intermediates of oxygen. It has the capability to inactivate the hydrogen peroxide via following reaction:



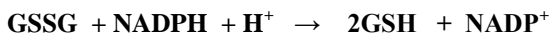
Peroxidases are the third defensive enzymes against the toxicity of oxygen. They have catalyze the following type of reaction:



Glutathione (Gamma-glutamyl cysteyl glycine or GSH) is another important protective agent against oxidant stress of oxygen. It readily reacts non-enzymatically with peroxides according to the following reaction:



A similar reaction can also be catalyzed by the enzyme glutathione peroxidase. Oxidized glutathione (GSSG) is then enzymatically reduced by the enzyme glutathione reductase:



The reducing equivalents required to maintain glutathione in the reduced form come from the pentose-phosphate shunt where the enzymes glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase produce NADPH. Red blood cells that are loaded with oxygen-carrying hem and thus are exposed to high concentration of oxygen have an important pentose-phosphate shunt and high activities of catalase and superoxide dismutase.

1. Acid Phosphatase Mechanism:

Acid Phosphatase is produced by the surface of amastigotes which restrain the action of superoxide and hydroxyl radical and prevent them from disintegration. Due to this activity of the parasite, macrophages are unable to digest amastigotes. In addition to these properties, parasite also possess a proton pump in their cell surface, this proton pump maintains the intercellular pH towards neutral that prevents it from lysosomal digestion, since lysosomal enzymes work only at acidic pH. Parasites also

have a Lipophosphoglycan (LPG) on its surface, which prevents them from lysosomal enzymes.

2. Complement deactivation:

The complement protein C3b, present in the blood, is the important factor for compelling immune complex. It binds with foreign material and promotes its uptake by phagocytic cells. Leishmanian promastigotes possess a *gp63* protein on their cell surface, which possess the capability to convert C₃b into iC₃b (inactive form of C₃b). iC₃b is unable to develop immune response.

Recently, Indian scientists have recognized a key protein **cytosolic trypanredoxin peroxidase (cTXNPx)** that plays an important role in regulating the survival, infectivity and drug response of the *Leishmania donovani*. It belongs to the group of enzymes, which detoxify peroxides, chemicals that are toxic to *L. donovani*. Parasites with higher levels of cTXNPx were more able to withstand high levels of hydrogen peroxide, and were also resistant to an antileishmanial drug.

III. CONCLUSION

Our immune system is very smart in recognizing the foreign antigens but the leishmania, the pathogen, is very clever in the sense that it takes over the control of immune defense mechanism (cell mediated and humoral immune defense) after entering into the host. The pathogen – leishmania - remains alive encapsulated within macrophages and not affected by our immune system / antibodies. When parasite enters in the body, the production of antibody starts from plasma cells (a form of B lymphocyte) but these antibodies are unable to interact with parasitic antigens because before the interaction, parasite already get entered into the macrophages and the antigens is covered within macrophages. Macrophages itself are unable to kill them due to different protective mechanism of parasite i.e. they produce Acid Phosphatase on their surface, which restrain the action of superoxide and hydroxyl radicals on parasite and thus, prevent them from disintegration. Thus, these are the reason also why any of the detection tests can detect the diseases with 100% surety.

REFERENCES

- [1] Parasitic disease, 1982. Katz, Despommier, Gwadz, Springer.verlag, Newyork.
- [2] Lee M B, Gilbert. Current approaches to Leishmaniasis, Infect. Med 1999; 16 (1): 34-75.
- [3] University of East London: Leishmaniasis and the Immune system.
- [4] <http://www.uel.ac.uk/pers/2738m/tim4.htm>
- [5] Immunity to Leishmaniasis
- [6] <http://www.cnrs.fr/cnrspresse/en31a1.htm>
- [7] How Leishmaniasis Fools the Immune System
- [8] <http://www.cnrs.fr/Cnrspresse/en31a1.html>
- [9] Neuroimmune Interactions in Cutaneous Leishmaniasis
- [10] <http://diss.kib.ki.se/1998/19980925ahme.cfm>
- [11] Kemp M, Kurtzhals JAL, Bendtzen K Poulsen LK, Hansen MB, Koech DK, Kharazmi A, Theander TG. Leishmania donovani-reactive Th1- and Th2-like T-cell clones from individuals who have recovered from visceral leishmaniasis.

- [13] Infect Immun 1993; 61:1069-1073. Reiner SL, Locksley RM. The regulation of immunity to *Leishmania major*. Annu Rev Immunol. 1995;13:151-77. Review.
- [14] Jitesh P. Iyer†, Anees Kaprakkaden†, Manohar L. Choudhary, Chandrima Shaha Crucial role of cytosolic trypanothione peroxidase in *Leishmania donovani* survival, drug response and virulence.
- [15] Immunity to Leishmaniasis <http://www.icp.ucl.ac.be/~opperd/parasites/>
- [16] Reiner SL, Locksley RM. The regulation of immunity to *Leishmania major*. Annu Rev Immunol. 1995;13:151-77. Review.
- [17] Locksley RM, Heinzel FP, Holaday BJ, Mutha SS, Reiner SL, Sadick MD. Induction of Th1 and Th2 CD4+ subsets during murine *Leishmania major* infection. Res Immunol. 1991 Jan; 142(1):28-32.
- [18] Lehmann J, Enssle KH, Lehmann I, Emmendorfer A, Lohmann-Matthes ML. The capacity to produce IFN-gamma rather than the presence of interleukin-4 determines the resistance and the degree of susceptibility to *Leishmania donovani* infection in mice. J Interferon Cytokine Res. 2000 Jan;20(1):63-77.
- [19] Matthews DJ, Emson CL, McKenzie GJ, Jolin HE, Blackwell JM, McKenzie AN. IL-13 is a susceptibility factor for *Leishmania major* infection. J Immunol. 2000 Feb 1;164(3):1458-62.
- [20] Lohoff M, Gessner A, Bogdan C, Rollinghoff M. Experimental murine leishmaniasis and the Th1/Th2 cell concept. J Exp Clin Med. 1998 Dec;23(6):347-50.
- [21] Heinzel FP, Maier RA Jr. Interleukin-4-independent acceleration of cutaneous leishmaniasis in susceptible BALB/c mice following treatment with anti-CTLA4 antibody. Infect Immun. 1999 Dec;67(12):6454-60.
- [22] Murphy ML, Cotterell SE, Gorak PM, Engwerda CR, Kaye PM. Blockade of CTLA-4 enhances host resistance to the intracellular pathogen, *Leishmania donovani*. J Immunol. 1998 Oct 15;161(8):4153-60.
- [23] Frau Nahla O. A . El Tai, Molecular approaches to direct diagnosis and characterization of *Leishmania donovani* in clinical isolates dissertation 26.09.2002
- [24] <http://edoc.hu-berlin.de/dissertationen/tai-nahla-omer-ahmed-el-2002-09-26/HTML>
- [25] Abdallah, R. E.; Ali, M.; Wasfi, A. I. & El-Hassan, A. M. 1973. Cutaneous leishmaniasis in the Sudan. Trans. R. Soc. Trop. Med. Hyg. ., 67 : 549-559.
- [26] Abdallah, R. E. & Sherif, H. 1978. Epidemics of cutaneous leishmaniasis in northern Sudan. Ann. Trop. Med. Parasitol. ., 72 : 349-352.
- [27] <http://leishmaniasis.askdefine.com/>

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