Abstract of Thesis

Title of thesis: Functional Studies On The Host Pathogen Interaction In Experimental Leishmania Donovani Infection
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Leishmaniasis is a disease complex caused by 17 different species of protozoan parasites belonging to the genus *Leishmania*. The parasites are transmitted between mammalian hosts by phlebotomine sandflies. Leishmaniasis has a worldwide distribution with important foci of infection in Central and South America, southern Europe, North and East Africa, the Middle East, and the Indian subcontinent. Most forms of leishmaniasis are zoonotic, human beings affected only secondarily, but two species of *Leishmania* can maintain arthropod-arthropod cycle. These are *L. donovani*, the species responsible for VL in the Indian subcontinent and East Africa, and *L. tropica*, which is responsible for CL in the old World. VL, also known as kala azar, is a protozoan systemic infection, which is almost always fatal if left untreated. Since there is no antileishmanial vaccine in clinical use, control of VL relies almost exclusively on chemotherapy.

For almost seven decades pentavalent antimonials constituted the standard antileishmanial treatment worldwide, however the last 15 years their clinical value was jeopardized due to the widespread emergence of resistance to these agents in Bihar, India, where half of VL cases occur globally. The last decade novel formulations of conventional antileishmanials as well as new drugs, including the oral agent miltefosine, became available or are under investigation. In practice, however, their wide use in poor countries is hampered mainly due to high costs and also due to concerns of toxicity and emergence of resistance. This thesis will focus on the factors that cause variation among the parasites in response to antileishmanial chemotherapy, evaluate the problems and mechanism associated with clinical resistance.

Quest for a simple marker that specifies difference in responsiveness of *Leishmania donovani* isolates to wide variety of antileishmanial drugs is of fundamental importance to provide appropriate treatment to the patients. The first part of the first chapter deals with a number of biochemical parameters were studied to find out the difference, if any, among the recent *Leishmania donovani* isolates, and in this way assign the parasites as sensitive or resistant to antileishmanial drugs. The extent of resistivity to a particular drug among the field isolates has been assessed by the degree of clearance of amastigotes from infected macrophages (MΦ) represented by the EC\(_{50}\) values of the four drugs Sodium stibogluconate (SSG), Miltefosine (Mil), Amphotericin B (AmB) and Paromomycin (Paro). Fifteen of the isolates showed decreased sensitivity towards SSG with an average EC\(_{50}\) of 25.7 ± 4.5 µg/ml pentavalent antimony (SbV) (defined as antimony resistant (Sb\(_{R}\)LD)), whereas nine showed considerable sensitivity with an average EC\(_{50}\) of 4.6 ± 1.7 µg/ml (defined as antimony sensitive (Sb\(_{S}\)LD)). Compared with the Sb\(_{S}\)LD, Sb\(_{R}\)LD isolates showed enhanced expression of thiol metabolising enzymes in varying degrees coupled with increased intracellular non-protein thiol content and over-expression of the terminal glycoconjugates (N-acetyl-D-galactosaminyl residue). MΦs infected with Sb\(_{R}\)LD but not with Sb\(_{S}\)LD showed up-regulation of ATP Binding Cassette transporters like Multidrug resistant protein 1 (MDR1). The above results reinforced the notion that Sb\(_{R}\)LD parasites have undergone a number of biochemical changes as part of their adaptation to ensure their survival in the host.

In order to combat the pressing problem of drug resistance, the metabolic pathways of antimony unresponsive and sensitive parasites are required to be studied. Sb\(_{R}\)LD are known to show more aggressive infection as compared to Sb\(_{S}\)LD in infected patients because of higher number of metacyclics in Sb\(_{R}\)LD.
parasites as compared to Sb⁸LD. In the second part of my first chapter, such observation was established with clinical isolates. To probe the possible reason for such an aggressiveness we observed that Sb⁸LD shows faster cell cycle kinetics. Sb⁸LD also show a higher rate of O₂ consumption as compared to Sb⁵LD. This might be due to their requirement of energy for rapid division. Surprisingly enough, Sb⁸LD show significantly low ATP content as compared to Sb⁵LD. A probable hypothesis for this is that to maintain rapid cell cycle kinetics, parasites require to synthesise nucleic acid at a faster rate, at the cost of ATP. However, for nucleic acid synthesis, more pentose sugar is necessary than Sb⁵LD which is important for replication. Therefore, the expression of the rate limiting step enzyme of glycolytic and pentose phosphate pathway, which uses the same substrate, glucose-6-phosphate, was compared in Sb⁸LD and Sb⁵LD. However, knock-down of autophagy related gene _atg5_ in antimony resistant strains resulted in a phenotype similar to Sb⁸LD excepting the number of metacyclics, which remained similar to Sb⁵LD. Overall, this work provided an understanding regarding the biochemical difference in the Glycolytic and Pentose phosphate pathway in antimony resistant and sensitive clinical isolates thereby providing a platform for designing appropriate drug targets and drugs to solve the problem of antimony resistance.

The differences in the biochemical parameters between these isolates were not sufficient to understand their disease pathogenesis in host. Therefore, the molecular mechanism of Sb⁸LD driven upregulation of MDR1 in infected Mφ has been investigated in my second chapter. Sb⁸LD but not Sb⁵LD express a unique glycan with N-acetylgalactosamine as terminal sugar removal of which compromise the ability to induce above effect. The enzyme galactosyl transferase (Gal-T) is responsible for adding the terminal sugar on parasite surface. Infection of Mφs with Gal-T knock-down Sb⁸LD enhanced the sensitivity towards antimonials as compared to infection with wild type Sb⁸LD. Although, from various pharmacological inhibitors, it was evident that Sb⁸LD mediated MDR1 upregulation is mediated by PI3K/Akt and JNK pathway, from studies with IL-10⁻/⁻ mice it was evident that IL-10 surge is a prerequisite for MDR1 upregulation. The transcription factor important for IL-10 driven MDR1 upregulation is c-Fos/c-Jun as evident from studies with promoter mapping with deletion constructs. This result indicates that Sb⁸LD, as opposed to Sb⁵LD, differentially interacts with host cells and gives rise to significant differences in the outcome of pathogenesis.

Another major feature in the mode of interaction with the host cells is the ability of Sb⁸LD to induce host cell autophagy. My last chapter deals with the mechanism of Sb⁸LD induced transient autophagy in host cell followed by apoptosis which leads to parasite egress. Autophagy, a major cellular pathway for the degradation of cytoplasmic macromolecules and organelles, is crucial for cell survival in response to starvation and for preventing intracellular accumulation of abnormal protein aggregates. However, intracellular Sb⁸LD have evolved distinct mechanisms to survive and multiply within membrane-bound compartments. Our studies elucidated that Sb⁸LD but not Sb⁵LD induce host cell autophagy at early time-points of infection as evident from the presence of LC3B-II puncta in Sb⁸LD infected Mφ. Sb⁸LD mediated autophagy induction in host cell is brought about by synergistic regulation of E2F and NRF2 transcription factors on beclin 1 promoter. It was observed that at very late timepoint post Sb⁸LD infection, host cells gradually become apoptotic. Increased intracellular calcium in Sb⁸LD infected cells acts as the switch from autophagic to apoptotic pathway. This study provides insight into the mechanism by which Sb⁸LD exploit host cell autophagy for more aggressive infection as compared to sensitive parasites, leading to apoptosis of the host cells which might aid in parasite egress. Developing means of selectively inhibiting autophagy in infected cells should therefore be viewed as a new window of opportunity in dealing with hard-to-eliminate intracellular drug resistant pathogens.