MOLECULAR PARASITOLOGY LABORATORY

Research Area: Leishmaniasis

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Collaborators:

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Our group is involved in studies on certain clinical and basic aspects of Leishmaniasis including Visceral leishmaniasis (VL) or Kala-azar (KA), Post kala-azar dermal leishmaniasis (PKDL) and Cutaneous Leishmaniasis (CL). The emphasis in the laboratory is on the following areas:-

Drug resistance: Resistance to the first line drug sodium antimony gluconate has reached alarming proportions in the high endemicity regions (HR) of KA in India, and studies with Indian field isolates are warranted to address this issue. Our studies with field isolates of *L.donovani* from KA and PKDL patients provided evidence of high incidence of antimony refractoriness in Indian kala-azar being contributed by anthroponotic transmission via PKDL. Global gene expression profiling using genomic microarray identified certain genes upregulated in antimony resistance. Analysis of selected genes at both RNA and protein level in a large number of field isolates revealed their potential as biomarkers for antimony resistance. Susceptibility of Indian field isolates to Miltefosine was found to be highly variable, ED50 of isolates from HR zones being significantly higher than isolates from LR zones. Studies to understand mechanism of Miltefosine resistance in Indian field isolates have been initiated. The *in vitro* sensitivity of other available antileishmanial drugs, paromomycin and sitamaquine, are also being explored in SAG sensitive and resistant KA field isolates.

Vaccine development: Our studies under Indo-US Vaccine Action Program led to identification of an important gene of *Leishmania donovani*- centrin, for the first time in a protozoan parasite. Centrin null mutants were awarded US patent owing to the vaccine potential of these genetically modified parasites. Studies are underway to define the immune responses elicited by this attenuated parasite in animals and in human PBMC. Genomic approaches to understand the process of differentiation of *Leishmania* parasite has led to the identification of several virulence related *Leishmania* genes that are being pursued as targets for development of vaccines. Characterizations of candidate genes

identified in this study (Parasite Surface Antigen, Arginosuccinate synthetase, a novel trypanosomatid specific protein P27, Ubiquitin activating enzymeUBA -5) are underway.

Diagnostics: Our studies have culminated in the development of sensitive and specific molecular and immunological tests for diagnosis of both KA and PKDL. We have developed a species- specific PCR test for non-invasive diagnosis of KA which was validated in the field and granted a US patent. Immunological tests based on recombinant and indigenous promastigote and amastigote antigens were developed for PKDL and KA diagnosis. Our group has evaluated the diagnostic and prognostic potential of recombinant Lepp12 antigen in *L. donovani* infections and found it to be a specific diagnostic and prognostic marker for KA. It was also identified that the *L. donovani* species was responsible for Indian mucosal leishmaniasis. Recently, in collaboration with FDA, USA, *Leishmania* minicircle DNA footprint assay was developed for species identification and for detecting *Leishmania* in asymptomatic blood donors.

Population genetics in Indian *L. donovani:* Our group is involved in genotyping of parasites of PKDL and KA origin, under an Indo-German project. Genetic polymorphisms were analyzed using molecular methods: PCR-RFLP and PCR-SSCP analysis of Internal Transcribed Spacers-1 (ITS-1) region, SSCP analysis of tandemly repeated antigen coding regions of gp63 and the more recent multi locus microsatellite typing assays. Our study demonstrated that both SAG resistant and sensitive *L. donovani* strains causing VL or PKDL were genetically homogenous at a number of genetic loci tested unlike those from African countries or Mediterranean regions where intra-species variations among *L. donovani* isolates are known to exist. Therefore, *L donovani* strains causing the two types of pathologies (KA or PKDL) in India, as well as the SAG resistant or sensitive strains, depict genetic monomorphism. Further, the Indian *L. donovani* strains were genetically closest to the strains from Kenya.

PKDL Immunobiology: Investigation of localized immune responses in dermal lesions of PKDL patients identified IFN-γ, TNF-α and IL-6 as the major host immune determinants. The diminished expression of their respective receptors IFN-γR1 and TNFR1, may affect the coordination of signaling to mount effective immune responses against the pathogen. Ongoing studies include the global analysis of host immuno-determinants involved in the pathogenesis of KA and PKDL, employing cDNA arrays and elucidation of the defect in signaling pathway using pathway based real-time PCR arrays.

Awards/Honours

1. Member of the WHO Expert Advisory Panel on Parasitic Diseases (Leishmaniasis) in 2009.

2. Elected Fellow of the National Academy of Sciences, India, in the year 2008.
3. Basanti Devi Amir Chand Award conferred by ICMR for the year 2006.
4. ICMR International fellowship for Senior Biomedical Scientists for the year 2006.
5. Awarded Courtesy Fellowship by CBER, FDA, USA in Sep 2005.
6. Awarded fellowship by National Foundation of Infectious Diseases, USA in 2005.
7. Prof. BK Aikat Award conferred by Indian Council of Medical Research for 2004.
8. Granted ICMR Award for Excellent Research output in July 2004.
9. Silver Jubilee award by Indian Association of Medical Microbiology in 2003.
10. Awarded Courtesy Fellowship by CBER, FDA, USA in Dec 2003
11. Kshanika Oration Award, a National award for Eminent Woman Scientist, conferred by Indian Council of Medical Research in 2002.
12. National Science Talent Scholarship awarded by N.C.E.R.T. , New Delhi

Patents

1. Awarded US Patent No. 6,855,522 for "Species-specific PCR assay for detection of *Leishmania donovani* in clinical samples of kala-azar and post kala-azar dermal leishmaniasis".
2. US patent no. 20060240046 for "Live attenuated *Leishmania* vaccines"

PROJECTS:

Current Ongoing Projects

International

1. Principal investigator in the Project funded by **European Commission** entitled "New tools for monitoring drug resistance and treatment response in visceral Leishmaniasis in the Indian subcontinent" (2009-2012).

2. Principal investigator (India) in the Project funded by **European Commission** entitled "Pre-clinical studies of a PSA based human vaccine candidate targeting visceral , cutaneous and muco- cutaneous leishmaniasis and development of the associated procedures for further clinical trials" (2009-2012).

National

3. Principal Investigator in project funded by **ICMR** entitled "Transcriptome profiling for identification and characterization of miltefosine resistance associated genes of *Leishmania donovani*" (2009-2012).

4. Principal Investigator in project entitled, "Parasite surface antigen-2(PSA-2) of *Leishmania donovani*: Studies on its role in parasite virulence, drug resistance and modulation of host macrophage function" funded by **Department of Science and Technology** (2008-2011).
5. Principal Investigator in project funded by **ICMR** entitled "Analysis of host immuno-determinants involved in the pathogenesis of Indian Cutaneous leishmaniasis exploiting cDNA microarray" (2007-2010)

Projects Completed

6. Principal Investigator in project funded by Ministry of Defence entitled "Evaluation of host immunodeterminants involved in the pathogenesis of kala azar and Post-Kala-azar Dermal Leishmaniasis using cDNA array" (2006-2009).

7. Indo-German project entitled, "Molecular Characterization of leishmania parasites isolated from dermal lesions of PKDL patients in India" funded by Department of Science and Technology (2005-2008).

8. Principal Investigator in project funded by Indo-U S Vaccine Action Program on "Discovery of virulence-related genes in *Leishmania donovani* using a genomic microarray" (2004 - 2007).

9. Principal Investigator in project funded by Department of Science and Technology on "Molecular cloning of differentially expressed genes in *Leishmania donovani* isolated from patients of post-kala-azar dermal leishmaniasis" (2003-2006).

10. Principal Investigator in project on "Evaluation of cellular immune responses in kala-azar and PKDL" funded by DRDO, Ministry of Defence (2002-2005).

11. Principal Investigator in ICMR Task-Force approved project on "Application of DNA microarray technology for identification of differentially expressed genes in parasite isolates from Kala-azar and PKDL patients" (2002-2005).

12. Principal Investigator in project funded by Indo-US Vaccine Action Program on "Identification and characterization of genes with stage specific expression in *Leishmania donovani* isolated from kala-azar patients" (1999 - 2003).

13. Co-investigator in project funded by Department of Biotechnology on “Cloning and expression of a mutagenized variant of protective antigen” (1995 -1998).

14. Co-investigator in project entitled “Production of a recombinant vaccine against anthrax” funded by Ministry of Defence (1995 -1998).

15. Co-investigator in DAE (Department of Atomic Energy) project entitled “Development of an immunotoxin against filariasis” (1994 - 1996).

16. Principal Investigator in DST (Department of Science & Technology) project entitled “Role of stress proteins in Leishmania” (1991 - 1993).