Kamlesh Gidwani

Dr. Gidwani has been developing his scientific career at the Infectious Disease Research Laboratory of Institute of Medical Sciences, BHU-India, working on marker of Leishmania donovani parasite infection in asymptomatic infected individuals and also marker of exposer of its vector sand fly in endemic region of Visceral Leishmaniasis. In his initial three year of doctorate work he was involved in a European Commission KALANET project in which he used sand fly saliva ELISA to measure the efficacy of insecticidal bed nets in intervention clusters incomparision to controls. Later, he took on the challenge to adapt the Quantiferon commercial assay into an application for leishmaniasis. He will be working with an immune response in the saliva of sand fly at the London School of Tropical Medicine and Hygiene.

This research was supported with a grant from the International Society for Infectious Diseases (ISID).

ISID Fellowship Report

Immune response of humans to sand fly *P. argentipes* saliva: Western blot approach to identify novel salivary peptides with serum of asymptomatic infection in VL.

Kamlesh Gidwani
PI Dr. Shyam Sundar, Institute of Medical Sciences, Banaras Hindu University, Varanasi India
PI Dr. Matthew Edwards Rogers, London School Hygiene Tropical Medicine, London, UK

Training Program at the *London School Hygiene Tropical Medicine, London, UK*

Background

In Indian subcontinents visceral leishmaniasis (VL or kala-azar) caused by obligate intracellular parasite *Leishmania donovani* is transmitted exclusively by the bite of female *Phlebotomus argentipes* sand flies (Swaminath CS, et al 1942). The disease is fatal if not treated, and it is responsible for an estimated 60,000 deaths per year worldwide (WHO 1998). The annual incidence of kala-azar cases is estimated to be 0.5 million and the prevalence to be 2.5 million (WHO, 1998). More than 90% of the world's reported VL cases are in Indian subcontinents, Sudan and Brazil. Every year, more than 100,000 cases of VL occur in India alone, with the state of Bihar accounting for more than 90% of these cases, followed by West Bengal and Eastern Uttar Pradesh (WHO 2009). Importantly 80-90% of *L. donovani* infections living in endemic areas are asymptomatic usually associated with strong cell-mediated immunity (Blackwell JM et al 2009). Annual incidence rates and prevalence of asymptomatic infection of *L. donovani* are known to be much higher than the frequency of clinical cases (Bern C et al and Bart O et al 2011).

Sand fly always injects saliva to the host during a bite irrespective of uninfected or infected with leishmania parasites. It contains a variety of anti-haemostatic, vasodilatory and immunomodulatory compounds. Sand fly saliva antibodies correlate with sand fly exposure in: Turkey (*P. sergenti*), Tunisia (*P. papatasi*), Brazil (*Lu. longipalpis*) (Rohousova I *et al* 2005, Barral A *et al* 2000 and Volf P 2001). Sand fly saliva antibodies in children correlate with 'protection' to VL in Brazil (seroconversion from a-saliva –ve to +ve correlates with a-*L. infantum* DTH) (Gomes RB et al 2002). Vaccination with either whole saliva, defined salivary proteins (PpSP15) or pre-exposure to the bites of uninfected sand fly have all shown to protect against *L. major* infection (Kamhawi, S et al 2000).

Recently, we developed pre-adsorbed *P. argentipes* saliva ELISA method as a marker of exposure which distinguishes people from a sand fly free country (UK) or people from urban areas of India where VL does not exist, from those sera collected from the VL-endemic State of Bihar. Densities of *P. argentipes* and *P. papatasi* correlated well with the antibody response to saliva of sand flies (Clements et al 2009). And in preliminary study we found highest anti salivary protein antibodies among asymptomatic individuals (Rogers ME *et al* Unpublished). So it was proposed by us to compare the western blot profile against saliva of sand fly by using different groups of serum including asymptomatic and to relate it to a protective profile. For better specificity we also want to replace the crude saliva with recombinant salivary protein for better marker of exposer.

To increase the participation rate of blood sampling for any community trial it is better to use finger prick blood on filter paper in comparison to venous blood, for that it is necessary to evaluate the recovery of antibodies against the saliva of the sand fly from blood spots archived on filter paper. To improve the specificity of this assay it is necessary to replace the crude saliva by its recombinant antigen by using western blot profile with different group's sera of endemic and non- endemic region.

continued on next page

The specific aim of small grant ISID fellowship:

1) To evaluate the recovery of antibodies against the saliva of the sand fly *Phlebotomus argentipes* from blood spots archived on filter paper.

2) To analyse the reactivity of Indian sera from a variety of groups exposed to sand flies and VL patients against sand fly saliva by Western blot to accurately determine the molecular weight of interesting protein bands that were (a) markers of exposure to *P. argentipes* bites and (b) unique to asymptomatic VL patients.

Training Method

To investigate whether recoveries of anti salivary protein antibodies from archived blood spots on FP, we used washed blood cells from heparinized venous blood of healthy volunteer mixed with equal volume of hyper immune sera (known high titer of anti salivary protein Abs) and spotted on what man filter paper number-3 (FP) allowed drying at room temperature then the FP blood spots eluted in diluent at 4C for overnight. FP elute were serially diluted (1:20 to 1:1280) parallel with same hyper immune sera alone and anti *P. argentipes* saliva antibodies detected through ELISA (Clements M. et al 2010).

For specific aim 2, saliva from colonized *P. argentipes* (LSHTM, London UK) was collected from female flies five days old post-emergence and maintained on 70% sucrose solution given *ad libitum* . Pools of 20 sand fly salivary gland pairs were collected in 100 µL PBS on ice and individually pierced to release their saliva. After centrifugation (1,800*g* for 5 minutes), the saliva was collected from the supernatant fraction and frozen at −70°C until used. Saliva of 3-4 flies was run in each lane of SDS PAGE, precast gradient gel (4-12%) used and the protein bands were transfer on nitrocellulose membrane for blotting with different groups of serum and was finally develop it to substrate observe protein band profile and identify the peptide band which only present Indian people and which only in asymptomatic individuals among Indian groups for marker of exposure and protection respectively.

Group of serum samples used for western blot were (a) VL-contact (serologically positive) asymptomatic, sub clinically infected (b) VL-contact (serologically negative); (c) Active VL; (d) 6 month Post-treated VL; (e) Non Endemic (Healthy) Control as negative control (f) UK resident.

Results

Recovery of anti salivary Abs from serially diluted elute of blood spots on FP was excellent and the trend of OD was almost similar to the serially diluted same serum samples with our saliva ELISA. In our another aimSaliva of *P. argentipes* were run on SDS PAGE (precast gradient gel) and after transferring on nitrocellulose membrane blots with different groups of serum mentioned above. A number of antigenic bands were recognized by the serum sample of different groups with different frequency and intensity. In addition, major bands of 15 kDa, 20 kDa, 35 kDa and 62 kDa were frequently recognized. While most of these frequently recognized bands were also reactive with sera from patients with kala-azar and patients who had been cured of kala-azar . Differences in reactivity were observed for the 15 kDa and 62 kda bands, against sera of Indian people while it was absent in UK sera, further 35 and 20 kda band was only present in asymptomatic individuals and absent in all other groups. Among the controls very faint cross-reactions were observed.

Conclusion

In recent years major advances have been made in the development of diagnostic methodologies that focus on evaluation of the patient's antibody response to determine whether infection or exposure has taken place. In continuation of this diagnostic development, we are also developing the salivary protein based diagnostic tools for identification of asymptomatic individuals. Because of the conditions prevailing in areas of endemicity, any sophisticated method cannot be employed on a wider scale. There is a need for a simple rapid and accurate test with good sensitivity and specificity, which can be used without any specific expertise.

continued on next page

ISID Report of Kamlesh Gidwani

Immune response of humans to sand fly *P. argentipes* saliva: Western blot approach to identify a novel salivary peptides with serum of asymptomatic infection in VL

This research was supported with a grant from the International Society for Infectious Diseases (ISID).

The experiments of recovery of anti salivary Abs from FP blood spots confirmed that we can use this methodology for study of exposer of sand fly on archived finger prick blood samples on FP for vector control measure programs on larger series of samples which was not possible on venous blood samples.

Recombinant antigen(s) specially based on 15 and 62 kda bands shown in our western blot experiments with Indian serum samples while it was absent with UK sera can be replaced the whole *P.argentipes* saliva for saliva ELISA for better marker of exposer. This will improve the specificity and sensitivity of the assay and this approach will not require labor-intensive sand fly rearing and salivary gland dissection, this modification will make the assay more amenable for large-scale application.

In endemic region of VL most of the leishmania infected population remains healthy and showed serology and PCR positive. We also used such asymptomatic sera to see the western blot profile against saliva and we identify 20 and 35 kda bands was only present in asymptomatic groups which can be further used as a marker of protection for vaccine candidates.

The results of this fellowship will allow us to (i) publish a new methodology of utilising dried blood spots for studying exposure to sand fly bites and (ii) identify specific sand fly salivary proteins that reflect exposure to *P. argentipes* to improve the specificity and sensitivity of the current saliva ELISA by developing recombinant salivary antigens.

References

1. WHO, 2009. Neglected tropical diseases, hidden successes, emerging opportunities. World Health Organization, Geneva.

2. WHO, 1998. Life in the Twenty-first Century: A Vision for All. The World Health Report. World Health Organization, Geneva.

3. Barral A, Honda E, Caldas A, Costa J, Vinhas V, Rowton ED, Valenzuela JG, Charlab R, Barral-Netto M, Ribeiro JM, 2000. Human immune response to sand fly salivary gland antigens: A useful epidemiological marker. *Am J Trop Med Hyg* 62:740–745.

4. Gomes RB, Brodskyn C, de Oliveira CI, Costa J, Miranda JC, Caldas A, Valenzuela JG, Barral-Netto M, Barral A, 2002. Seroconversion against *Lutzomyia longipalpis* saliva concurrent with the development of anti-*Leishmania chagasi* delayed-type hypersensitivity. *J Infect Dis* 186:1530–1534.

5. Rohousova I, Ozensoy S, Ozbel Y, Volf, P, 2005. Detection of species-specific antibody response of humans and mice bitten by sand flies. *Parasitol* 130:493–499.

6. Gomes RB, Mendonça IL, Silva VC, Ruas J, Silva MB, Cruz MS, Barral A, Costa CH, 2007. Antibodies against *Lutzomyia longipalpis* saliva in the fox *Cerdocyon thous* and the sylvatic cycle of *Leishmania chagasi*. *Trans Royal Soc Trop Med Hyg* 101:127–33.

7. Rohousová I, Volf P, 2006. Sand fly saliva: effects on host immune response and *Leishmania* transmission. *Folia Parasitol (Praha)* 53:161–171.

8. Volf P, Rohousova I, 2001. Species-specific antigens in salivary glands of phlebotomine sandflies. *Parasitol* 122:37–41.

9. Kamhawi, S., Y. Belkaid, G. Modi, E. Rowton, and D. Sacks**.** 2000. Protection against cutaneous leishmaniasis resulting from bites of uninfected sand flies. *Science* 290:1351–1354.

10. M. Clements*****, Gidwani K***,** Kumar R*****, Hostomska J, Dinesh DS, Kumar V, Das P, Müller I, Hamilton G, Volfova V, Boelaert M, Das M, Rijal S, Picado A, Volf P, Sundar S, Davies CR, Rogers ME 2009. Human antibody responses to sand fly saliva can measure recent exposure to *Phlebotomus argentipes* the vector of Indian visceral leishmaniasis. *American Journal of Tropical Medicine and Hygiene*. 2010 May; 82(5):801–807.

11. J.M.Blackwell, M. Fakiola, ME Ibrahim, SE jamieson, SB Jeronimo, EN Miller, A Mishra, HS Mohamed, CS Peacock, M Raju, S. Sundar and ME Wilson. Genetics and visceral leishmaniasis: of mice and man. *Parasite Immunology*. 2009. 31:254–266.

ISID Report of Kamlesh Gidwani

Immune response of humans to sand fly *P. argentipes* saliva: Western blot approach to identify a novel salivary peptides with serum of asymptomatic infection in VL

This research was supported with a grant from the International Society for Infectious Diseases (ISID).