Immunity and Infection

Oral Presentation **Tuesday November 8** **9.00am – 9.15am**

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GENETIC ANALYSIS OF HOST IMMUNITY TO EXPERIMENTAL INFECTION WITH VIRULENT *MYCOBACTERIUM TUBERCULOSIS* USING MOUSE MODEL

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We have mapped a major genetic locus *sst1* (*s*uper*s*usceptibility to *t*uberculosis 1) on mouse chromosome1, and utilized positional cloning to identify a candidate gene *Ipr1*, *i*ntracellular *p*athogen *r*esistance 1, (Pan et al, *Nature* 2005, 434:767). The *Ipr1* gene controls macrophage-mediated mechanism of innate immunity to intracellular pathogens *Mycobacterium tuberculosis* (MTB) and *Listeria monocytogenes* (LM). Expression of the full length copy of this gene in the *sst1*-susceptible macrophages increased their ability to control multiplication of intracellular pathogens MTB and LM *in vitro*, prevented necrosis and turned on the apoptotic program of cell death in the infected cells. The *Ipr1* gene encodes a predicted nuclear protein that has structural homology with known eukaryotic transcriptional coactivators. The susceptible allele of *sst1* did not confer an overt immunodefficiency, but rather specifically affected progression of lung tuberculosis. Four additional genetic loci contributing to tuberculosis resistance were mapped in crosses involving the *sst1* congenic mice and their genetic interactions with the sst1 were demonstrated. While the genetic polymorphism within the *sst1* locus is due to *de novo* mutation in C3HeB/FeJ substrain of C3H, some of the novel tuberculosis resistance loci are likely to represent ancestral polymorphisms. No Ipr1 homologues were found in yeast, *C.elegans* and insects. Human homologue of the Ipr1 gene exists and has been associated with susceptibility to tuberculosis in human populations in Africa. Our studies demonstrate that mouse model of infection with MTB is useful for dissecting pathogenesis and genetic control of such complex genetic trait as susceptibility to tuberculosis. Funded by NIH NAIID and NIH HLBI.

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TLR EXPRESSION PATTERNS DURING *T. CONGOLENSE* INFECTION IN MICE

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Marked differences between inbred strains of mice in their response to trypanosomosis can be exploited to analyze the genetic basis of resistance to the disease. After QTL mapping and physical representation of the particular chromosomalfragment spanning *Tir2* and *3*, possible candidate genes were selected. Plausible candidate genes within the loci include TLR1, 5 and 6. The efficiency of clearance of the first wave of parasitemia in mice infected with *T. congolense* is positively correlated with long term survival. Rapid defense mechanisms are on the other hand provided through recognition of pathogen associated molecular patterns by receptors such as TLRs. Their expression appears to be essential for the induction of interleukins such as IL-10 and TNF-a. They act singly or in synergy in regulating macrophage activation status. Susceptible and resistant mouse strains portray differential expression of IL-10 and TNF-a over time, which might be dependent on the expression of Tolls. In order to investigate the mode of expression of the TLR, eleven groups of mice were selected and bred to maturity. Two groups of mice were sacrificed and their spleen and liver tissues collected. The other nine groups were subsequently challenged with *T. congolense* strain IL1180 and liver and spleen tissues collected at day 3, 4, 7, 10, 13 and 17 post challenge. After RNA extraction and reverse transcription, quantitative and semi quantitative PCR methods were applied to determine TLR and ß-actin mRNA expression patterns. Results were presented as a ratio of the target TLR normalized to ß–actin as a house keeping gene.

TLR1, 5 and 6 were readily detectable in cDNAs prepared the tissues from the resistant and susceptible mouse strains. Analysis of variance of the mean ratios of the TLR target normalized to the house keeping gene revealed that the genes are regulated in a statistically significant fashion. The overall TLR1 expression could significantly distinguish pre- and post-trypanosome infection status in the liver and spleen tissues. Interestingly, the data also identified two distinct groups among the liver samples, which were either expressing or not expressing TLR1 gene before and after infection which was supported by the higher expression thereafter as evident from real time PCR results. Similar trends were observed with TLR6 gene over time since the levels of TLR6 increased at the onset of the infection in all the strains but were much higher in the susceptible A/J strain than Balb/c and C57BL/6J mouse strains. TLR5 gene was clearly expressed in the three mouse strains with C57BL/6J showing weak expression through out the experimental period. The susceptible A/J and Balb/c showed increased mRNA expression of TLR5. However higher levels were observed in Balb/c as opposed to A/J. Susceptible and resistant mice infected with *T. congolense* therefore portray diverse expression patterns of the TLR genes. Up regulation of different TLR seems to coincide with up regulation of IL-10 and TNF-a in susceptible and resistant strains respectively. Response to the *T. congolense* infection therefore induces a response characterized by changes in TLR 1, 5 and 6 expression in liver and spleen tissues which in turn may influence different cytokine patterns in mice. Determination of TLR levels in resistant and susceptible livestock can therefore be exploited in the development of novel strategies for the control of trypanosomosis in man and his livestock.