

**IMMUNITY AND INFECTION
ORAL PRESENTATION****TUESDAY NOVEMBER, 14****9.15AM - 9.30AM****O17****PROGRESS TOWARDS INTEGRATION OF GENETIC AND IMMUNOLOGICAL STUDIES OF LEISHMANIASIS**

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Leishmaniasis is a complex disease caused by intracellular protozoan parasites of the genus *Leishmania*. Mouse infection by *Leishmania major* is a well-studied model of a disease with an immune component. Initial studies indicated that resistance or susceptibility to *L. major* might be determined by the activation of different classes of T helper lymphocytes, but later studies have revealed a complexity of responses, in relation to susceptibility to *L. major*, which include not only T lymphocytes, but also macrophages, dendritic cells, neutrophils and many other cellular and humoral components, that are not easily integrated into a simple functional model based on Th1/Th2 dichotomy.

We have mapped genes controlling clinico-pathological and immunological manifestations of *L. major*-induced disease using recombinant congenic (RC) strains. Each RC strain of CcS/Dem series carries a different random subset of 12.5% of genes of the resistant strain STS on the BALB/c (susceptible) background. We have mapped *Lmr* (*Leishmania major* response) genes in the most resistant strain CcS-5; an intermediate strain CcS-20; and a susceptible strain CcS-16 that in some parameters is even more susceptible than BALB/c. In each strain, one or two loci with strong effects were detected, whereas the other loci identified have weaker influences on pathology. *Lmr* gene effects on disease symptoms were organ specific and heterogeneous. We found 17 novel *Lmr* (*Leishmania major* response) loci: *Lmr3-19* and described their effects on organ pathology and systemic immune reactions. These *Lmr* loci control thirteen different combinations of pathological and immunological symptoms. Seven loci control both organ pathology and immunological parameters, 10 influence immunological parameters only. Two *Lmr* loci were mapped to 1.9 and 3 cM, respectively, opening way towards their positional cloning.

In conclusion, these studies revealed a network-like complexity of the combined effects of the multiple functionally diverse QTLs. Therefore the most important players (genes with the largest effect) might vary depending of genotype of the host. This may become a general paradigm for organism's response to infection.

**IMMUNITY AND INFECTION
ORAL PRESENTATION****TUESDAY NOVEMBER, 14****9.30AM - 9.45AM****O18****MPL^{-/-} MICE ARE SUSCEPTIBLE TO MALARIAL INFECTION DESPITE THEIR RESISTANT C57BL/6 BACKGROUND**V Marshall¹, C de Graaf¹, G Smyth¹, D Senyschen¹, G Panoschi¹, J Corbin¹, W Alexander¹, S Foote²¹The Walter and Eliza Hall Institute, Melbourne, Australia, ²The Menzies Research Institute, Hobart, Australia

Malaria is a nasty disease that kills between one and three million children annually. The host response to malarial infection is genetically complex due to the massive selection the disease is placing on the genome. Mouse models of malaria allow us to dissect out the host response to disease on an isogenic background. We have noticed that animals deficient in the thrombopoietin receptor, (Mpl^{-/-}), are susceptible to death to malarial infection even when the mutation is on a resistant background. We show that this phenotype is likely to be attributable to the low platelet count of these animals. We demonstrate that platelets are able to bind preferentially to red cells infected with the murine malaria *P. chabaudi*. We also show that bound platelets kill the parasite directly and that the lack of platelets in Mpl^{-/-} mice is responsible for their increased susceptibility through their inability to kill parasites. Incidentally we demonstrate that aspirin mimics the Mpl^{-/-} effect but to a lesser extent and that TUNEL staining of infected blood demonstrates less staining in Mpl^{-/-} mice than wild-type animals. During infection with many malarias, including falciparum and vivax, there is a gradual thrombocytopenia during the infection and we believe that this is due to consumption of platelets bound to infected red cells.

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ORAL PRESENTATION****TUESDAY NOVEMBER, 14****9.45AM - 10.00AM****O19****GENETIC ANALYSIS OF THE TOLL-LIKE RECEPTOR SIGNALING PATHWAYS**M Berger¹, Z Jiang¹, K Tabeta², X Du¹, K Hoebe¹, B Ortiz¹, B Beutler¹¹The Scripps Research Institute, La Jolla, CA, United States, ²Niigata University Graduate School of Medical & Dental Sciences, Niigata, Japan

The mammalian Toll-like receptors (TLRs) are key sensors for signature molecules of microbial origin. They detect infections at an early stage and ignite the immune response. We have tested the integrity of signaling from seven of the 12 TLRs known to exist in mice, measuring the secretion of biologically active tumor necrosis factor (TNF) as a readout, in macrophages harvested from >27,000 G3 ENU mutant mice. A total of 15 mutations that impair or ablate signal transduction have been identified. These mutations affect 14 genes. 13 of them have been identified. The affected proteins include three of the seven TLRs kept under surveillance, three of the four known adapter proteins that interact with TLRs, one of two protein kinases known to be required for TLR-induced TNF production, and other molecules that serve accessory roles in signaling. Based on the number of known signaling components struck, we estimate that approximately 50% phenotypic saturation has been achieved. Here we describe three mutations that have not previously been reported: *otiose*- a missense mutation in interleukin-1 receptor associated kinase 4 (IRAK4) that totally abolishes kinase activity; *languid*- a missense mutation in TLR2 that eliminates responses to all known TLR2 ligands; and *torpid*- a missense mutation in the acceptor splice site of the third exon in the *Tirap* gene, which diminishes sensing of LPS and some (but not all) TLR2 ligands. Genetic uniformity permits a detailed analysis of the transcriptional consequences of each mutation, and allows construction of a refined model of TLR signaling pathways.

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ORAL PRESENTATION****TUESDAY NOVEMBER, 14****10.00AM - 10.15AM****O20****GENOMIC INSIGHTS INTO THE RESOLUTION OF INFLAMMATION: ALTERNATE SPLICING OF KEY SIGNALLING MOLECULES IN THE TOLL-LIKE RECEPTOR CASCADE**CA Wells¹, A Chalk², S Lo¹, A Beckhouse¹, A Forrest³, P Carninci⁴, Y Hayashizaki⁴, DA Hume³, SM Grimmond³¹Eskitis ICMT, Griffith University, Brisbane, QLD, Australia, ²Karolinska Institutet, Stockholm, Sweden, ³IMB, University of QLD, Brisbane, QLD, Australia, ⁴RIKEN Genome Sciences Group, Yokohama, Japan

The Toll-Like Receptor (Tlr) pathway is a central mediator of innate immune responses to pathogens. Tlr signalling in macrophages leads to well documented transcriptional, phenotypic and biochemical changes cumulating in inflammation. The resolution of this inflammatory signal is less well understood, but vital to limit tissue damage, sepsis and chronic inflammatory disease.

Alternate splicing of key signalling molecules in the Tlr cascade has been shown to dramatically alter the signalling capacity of inflammatory cells. The production of dominant negative proteins through such splicing events has been documented for few proteins such as Irak-m and Myd88-s, yet it is not known how common this mechanism is. We exploited the FANTOM (functional annotation of the mouse genome) transcriptome dataset to systematically screen transcript variants of the Tlr cascade, Mapk, Pik3, IFN- β pathways.

Two hundred and fifty six variant transcripts were identified, including novel variants of Tlr4, Ticam1, Tollip, Rac1, Irak1, 2 and 4, Mapk14/p38, Csf1r, Atf2 and Stat1. Functional annotation of variant proteins was assessed in light of inflammatory signalling in mouse primary macrophages. Splicing arrays were used to examine the expression of each variant transcript in primary and LPS-activated macrophages. We functionally tested the expression of Tlr4 transcripts under a range of cytokine conditions via Northern and qRT-PCR. The effect of over-expressing key variant proteins on macrophage activation, inflammatory output and survival are demonstrated. Our data suggest a surprisingly common role for variant proteins in diversification/repression of inflammatory signalling.

**IMMUNITY AND INFECTION
ORAL PRESENTATION****TUESDAY NOVEMBER, 14****10.15AM - 10.30AM****O21****JINX, AN MCMV SUSCEPTIBILITY PHENOTYPE CAUSED BY DISRUPTION OF UNC13D, THE MOUSE ORTHOLOGUE OF THE TYPE 3 FAMILIAL HEMOPHAGOCYTTIC LYMPHOHISTIOCYTOSIS GENE**

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C57BL/6 mice survive challenge with 10^5 PFU of mouse cytomegalovirus (MCMV), but succumb to 10^6 PFU. Natural killer cells (NK) and other immune cells contribute to resistance, and approximately 1% of all genes may be mutated to yield susceptibility. We have screened more than 17,000 G3 ENU-mutant mice (C57BL/6 background) for susceptibility to MCMV, and have identified more than 30 phenovariants that exhibit sickness or die within seven days following inoculation. One of these, *Jinx*, is associated with obvious sickness when 10^5 PFU are administered, and death within 5 days when 2×10^5 PFU are administered despite normal or elevated cytokine responses. In *Jinx* homozygotes, activated NK cells fail to degranulate, and cytolytic T cells (CTL) show diminished ability to kill antigen-specific targets. However, the appearance of the peripheral blood and lymphoid organs are both unremarkable. *Jinx* was mapped to mouse chromosome 11 and confined to a 2 Mb critical region encompassing 70 annotated genes on a total of 246 meioses. A splicing error (creation of a novel acceptor site) was found in *Unc13d*, the mouse orthologue of the human MUNC13-4 gene, wherein mutations cause type 3 familial hemophagocytic lymphohistiocytosis (FLH3). FLH3 is a rapidly fatal disease in which massive hepatosplenomegaly is observed. Never disrupted by gene targeting, *Unc13d* encodes a protein with 1083 amino acids, containing two C2 domains and a Munc homology domain (MHD). It associates with Rab27a and is required for granule exocytosis. The remarkable phenotypic disparity between *Jinx* and FLH3 phenotypes is currently being addressed.