



Tuesday November 7

10.00am – 12.30pm

Poster Session 3

Immunity and Infection Pages 190 - 194

Posters P-169 – P-178

Immunity and Infection

P-169 STANDARDIZED IMMUNOLOGICAL PHENOTYPING OF LABORATORY MICE

S Kalaydjiev², T J Franz², V Gailus-Durner¹, H Fuchs¹, M Hrabé de Angelis¹, D H Busch²

¹ German Mouse Clinic, Institute of Experimental Genetics, GSF Research Center, Munich, Germany,

² Institute of Medical Microbiology, Immunology and Hygiene, Technical University, Munich, Germany

P-170 WILD-DERIVED INBRED MICE AS TOOLS TO STUDY INNATE IMMUNOLOGY: GENETIC ANALYSIS OF CPG HYPO-RESPONSIVE MOLF/EI MICE

K Stephan, A Poltorak

Tufts University, Boston, MA, United States

P-171 THE GENETICS OF SUSCEPTIBILITY TO SYSTEMIC PNEUMOCOCCAL INFECTION

L Boubbane¹, A Haynes¹, S Moyes¹, E Hopes¹, N Gingles², K, W Broman³, W McPheat⁵, J Morten⁴, J Alexander², P W Andrew², S D M Brown¹, P Denny¹

¹MRC Mammalian Genetics Unit, Harwell, Oxon, United Kingdom, ²Department of Microbiology and Immunology, University of Leicester, Leicester, UK, United Kingdom, ³John Hopkins University, MD, United States, ⁴ AstraZeneca, Cheshire, United Kingdom, ⁵ AstraZeneca, Sweden, Sweden, Sweden

P-172 SCREEN OF PERIPHERAL LYMPHOID ORGAN DEVELOPMENTAL DEFECT MUTANT FROM ENU-INDUCED MUTANT POOL

H Yoshida, T Yasuda

RIKEN, RCAI, Yokoham, Japan

P-173 INFECTION OF MICE BY A LOW PATHOGENIC STRAIN OF YERSINIA ENTEROCOLITICA, A SECONDARY SCREEN TO SEARCH FOR SUSCEPTIBILITY GENES

U Frischmann, M Greweling, S Mateika, A Gruber, S Schippers, W Müller

¹ GBF, Braunschweig, Germany, ² TIHO, Hannover, Germany

P-174 NOVEL GENES IN *MUS CASTANEUS* RESPONSIBLE FOR RESISTANCE TO INFECTION BY POLYTROPIC MOUSE LEUKAEMIA VIRUSES

T Wu¹, M S Lyu², E Shaffer³, Y Yan³, C A Kozak³

¹ Laboratory of Molecular Genetics, National Institute of Child Health and Development, Bethesda, MD, United States, ² Center for Cancer Research, National Cancer Institute, Bethesda, MD, United States, ³ Laboratory of Molecular Microbiology, Bethesda, MD, Australia

P-175 AGING ALTERS THE GENETIC BASIS OF RESISTANCE TO *BABESIA MICROTI*, A PATHOGEN OF RED BLOOD CELLS

J Yuan¹, J Spiegler¹, A Pliego¹, A Thambundit¹, S R Telford², S Shah³, A Spielman⁴, J A Gelfand³, W F Dietrich⁵, H H Wortis³, E Vannier¹

¹ Tufts-New England Medical Center, Boston, MA, United States, ² Tufts Univ. School Vet. Medicine, Grafton, MA, United States, ³ Tufts Univ. School Medicine, Boston, MA, United States, ⁴ Harvard School Public Health, Boston, MA, United States, ⁵ Harvard Medical School, Boston, MA, United States

P-176 DISSECTION OF GENETIC SUSCEPTIBILITY TO PRION INFECTION

R Giri¹, I Lee², D Hwang², H Yoo², E Yi², D Baxter², B Ogata², C Ebeling¹, W Miller¹, R Kumar¹, R Young¹, R Pitstick¹, LE Hood², G A Carlson¹

¹ McLaughlin Research Institute, Great Falls, Montana, United States, ² Institute for Systems Biology, Seattle, Washington, United States

P-177 STUDENT ORAL ABSTRACT S-17

PYRUVATE KINASE DEFICIENCY AND AN ADDITIONAL LOCUS ON CHROMOSOME 10 CONFER RESISTANCE TO MALARIA IN ACB55 RECOMBINANT CONGENIC MICE

G Min-Oo, A Fortin, M Tam, MM Stevenson, P Gros

McGill University, Montreal, Canada

P-178 STUDENT ORAL ABSTRACT S-18

AGING ALTERS THE GENETIC BASIS OF RESISTANCE TO *BABESIA MICROTI*, A PATHOGEN OF RED BLOOD CELLS

J Yuan¹, J Spiegler¹, A Pliego¹, A Thambundit¹, S Telford², S Shah³, A Spielman⁴, J Gelfand³, W Dietrich⁵, H Wortis³, E Vannier¹

¹ Tufts-New England Medical Center, Boston, MA, United States, ² Tufts Univ. School Vet. Medicine, Grafton, MA, United States, ³ Tufts Univ. School of Medicine, Boston, MA, United States, ⁴ Harvard School Public Health, Boston, MA, United States, ⁵ Harvard Medical School, Boston, MA, United States

P-169**STANDARDIZED IMMUNOLOGICAL PHENOTYPING OF LABORATORY MICE**

S Kalaydjiev², T J Franz², V Gailus-Durner¹, H Fuchs¹, M Hrabé de Angelis¹, D H Busch²

¹ German Mouse Clinic, Institute of Experimental Genetics, GSF Research Center, Munich, Germany,

² Institute of Medical Microbiology, Immunology and Hygiene, Technical University, Munich, Germany

The maintenance of immunological homeostasis and immune function is attributed to complex interactions of various signaling pathways, known to be regulated by multiple genes. Since most of these genes are still unknown, experimental mouse models are largely used to find new immune regulators involved in human disease. Although the generation of mouse models has been well-established and even transferred to large-scale mutant production via ENU mutagenesis, gene-trap, or conditional knock-in/knock-out technologies, the detailed and standardized phenotypic analysis for the identification of defined alterations in the immune system has remained a major bottleneck.

Within the German Mouse Clinic (GMC) we have established a comprehensive standardized high-throughput immunological screening approach for detecting immunodeficiencies in the mouse. Peripheral blood samples are analyzed by multi-color 11-parameter flow cytometry (Cyan, DakoCytomation) and bead arrays (Bioplex, Biorad) specifically developed in our lab. To improve the management of high sample numbers and minimize the impact of human errors, sample handling has been automated - at the GMC we have implemented a Quadra 3 pipetting robot (Tomtec) for sample preparation. High throughput sample acquisition was accomplished by combining sensitive analysis hardware with automated sampling devices (for example, automated microplate sampler, AMS (Cytex) with Cyan flow cytometer). All screening protocols have been validated, and standard operation procedures were defined.

Our approach has allowed, for a period of two years, to identify and establish out of an ENU screen over 50 mutant lines with abnormalities of the immune system, and attribute novel unsuspected immunological phenotypes to 24 well-established mutant lines.

P-170**WILD-DERIVED INBRED MICE AS TOOLS TO STUDY INNATE IMMUNOLOGY: GENETIC ANALYSIS OF CPG HYPO-RESPONSIVE MOLF/EI MICE**

K Stephan, A Poltorak

Tufts University, Boston, MA, United States

The mammalian innate immune system is able to detect bacterial and viral nucleic acids through the recognition of unmethylated DNA motifs referred to as CpG motifs. These motifs can activate B cells, natural killer cells, dendritic cells, and monocytes, thus promoting both innate and antigen-specific adaptive immune responses. Despite the profound impact of host recognition and uptake of CpG on the magnitude of the immune response, the molecular basis of these activities is not completely understood. Our long-term goal is to understand the signal transduction pathways that underlie innate immune responses. Current evidence suggests the response to CpG is mediated by Toll-like Receptor 9 (TLR9), although evidence is mounting that a TLR9-independent response to CpG sequences may also occur.

In our attempt to find new components of TLR-mediated signaling, we initiated innate immune screens on a panel of wild-derived inbred mouse strains. These strains were chosen because wild-derived strains show stronger immune responses, and possess greater genetic diversity than classical inbred mice. Our central hypothesis is that the greater genetic diversity of wild-derived mouse strains will allow for the discovery of new components central to innate immune pathways stimulated by CpG. Indeed, we found several mutant immunological phenotypes among this panel. Among them, we discovered a hypo-response to CpG in the MOLF/Ei mouse strain, and established that it is not linked to *Tlr9*, but to another locus. This phenotype is a dominant, monogenic trait and we are currently mapping the gene of interest using meiotic recombination.

P-171**THE GENETICS OF SUSCEPTIBILITY TO SYSTEMIC PNEUMOCOCCAL INFECTION**

L Boubbane¹, A Haynes¹, S Moyes¹, E Hopes¹, N Gingles², K W Broman³, W McPheat⁵, J Morten⁴, J Alexander², P W Andrew², S D M Brown¹, P Denny¹

¹MRC Mammalian Genetics Unit, Harwell, Oxon, United Kingdom, ² Department of Microbiology and Immunology, University of Leicester, , Leicester, UK, United Kingdom, ³ John Hopkins University, MD, United States, ⁴ AstraZeneca, Cheshire, United Kingdom, ⁵ AstraZeneca, Sweden, Sweden, Sweden

The pneumococcus, *Streptococcus pneumoniae*, is an important human pathogen causing pneumonia, bacteraemia and meningitis and is associated with very high morbidity and mortality. It is likely that host genetic factors play a significant role in susceptibility to pneumococcal disease, as is clearly the case for other infectious diseases, such as malaria and tuberculosis. However, genetic linkage analysis is impractical in pneumococcal disease due to the paucity of sibling cases or multiple-case families. As an alternative approach to the identification of candidate disease genes, we have developed a murine model of genetic susceptibility to pneumococcal infection

Nine inbred strains of mice were infected intranasally with *Streptococcus pneumoniae* D39 and BALB/c and CBA/Ca were found to be resistant and susceptible respectively. A genome scan for loci responsible for this difference in susceptibility to invasive infection was conducted in the progeny of an F2 intercross. A major QTL called *S.pneumoniae* infection resistance 1; *Spir1* was mapped to proximal chromosome 7, in a region of approximately 11 megabases.

In order to prioritise candidate genes, microarrays are being used to investigate differential gene expression in an infection time course of parental BALB/c and CBA/Ca mice. The critical region containing the *Spir1* locus will also be reduced by congenic mapping. We will report on progress in positional candidate cloning of this major QTL.

P-172**SCREEN OF PERIPHERAL LYMPHOID ORGAN DEVELOPMENTAL DEFECT MUTANT FROM ENU-INDUCED MUTANT POOL**

H Yoshida, T Yasuda

RIKEN, RCAI, Yokoham, Japan

Peripheral lymphoid organs (PLO), such as lymph node or Peyer's patch are the tissue in which the foreign particles are trapped and presented as antigen for lymphoid cells. With the wide spread of genetic manipulation technique, many molecules were found to have important roles in those organ development. Especially, interaction between lymphoid tissue inducer cells which express lymphotoxins and lymphoid tissue organizer cells are those respond to lymphotoxins and express various adhesion molecule is a pivotal event in PLO development. Therefore, PLO developmental defect is detectable by organizer cells' VCAM-1 expression absence during fetal stage.

In order to find out the new PLO developmental defect mutant by phenotype screen, we injected ENU into C57BL/6 male and obtained the next generation male mice each as the independent founders, and obtained the third next generation to produce the recessive mutant pool of each pedigree. Since we produced every mutant generation by in vitro fertilization and embryo transfer (IVF-ET), we could easily obtain a large number of mice of each pedigree at the same developmental stage.

We screened the mutant mice pool at 17.5 embryonic days to detect the developmental defect in thymus, fetal liver or PLO by means of flowcytometry, wholemount-immunostaining, and pathological study. In the first 14 months study, we analyzed 1526 mice from 24 pedigrees. We found 11 independent mutant lines those have defect in PLO development by detecting the VCAM-1 expression in peripheral lymphoid organ anlagen. All the mutant we detected seemed to be different from any known PLO defective mutant mouse.

Here we summarize the strategy and result of one-year screen for mutant with peripheral lymphoid organ development.

P-173**INFECTION OF MICE BY A LOW PATHOGENIC STRAIN OF YERSINIA ENTEROCOLITICA, A SECONDARY SCREEN TO SEARCH FOR SUSCEPTIBILITY GENES**

U Frischmann, M Greweling, S Mateika, A Gruber, S Schippers, W Müller

¹ GBF, Braunschweig, Germany, ² TIHO, Hannover, Germany

We have established an infection model to screen mouse mutants for resistance or susceptibility towards *Yersinia enterocolitica* infections. We are using a low virulence strain of *Yersinia* that only leads to a local infection in the gut and does not disseminate to other internal organs. This model not only allows the analysis of inbred mouse strains, mutants and recombinant inbred but also allows the analysis of long-term consequences of a *Yersinia enterocolitica* infection.

We have tested extensively four inbred strains of mice, C57BL/6, BALB/c, 129 Sv and C3H and analysed colony-forming units on day 3, 9 and 21 and performed a quantitative morphological analysis of the local lesions in the gut of the infected mice. While C57BL/6 completely eliminate the *Yersinia* within 21 days, BALB/c mice still carry *Yersinia* in the gut and are unable to completely clear the infection within this time frame. In addition to these four Inbred strains mouse mutants deficient for cytokine genes or cell adhesion molecules were analysed in this infection model.

This work is supported by EUMORPHIA and NGFN

P-174**NOVEL GENES IN *MUS CASTANEUS* RESPONSIBLE FOR RESISTANCE TO INFECTION BY POLYTROPIC MOUSE LEUKAEMIA VIRUSES**

T Wu¹, M S Lyu², E Shaffer³, Y Yan³, C A Kozak³

¹Laboratory of Molecular Genetics, National Institute of Child Health and Development, Bethesda, MD, United States, ²Center for Cancer Research, National Cancer Institute, Bethesda, MD, United States, ³Laboratory of Molecular Microbiology, Bethesda, MD, Australia

Mus castaneus is unique among wild mouse species in that it harbors infectious mouse gammaretroviruses of the ecotropic (mouse-tropic) and nonectropic host range groups. *M. castaneus* is also unique in its resistance to infection by the leukemogenic nonectropic polytropic mouse leukemia viruses (P-MLVs). Two factors are responsible for this resistance: 1) a defective XPR1 cell surface receptor for P-MLVs, and 2) a resistance factor detectable only in interspecies hybrids between *M. castaneus* and mice carrying a variant of XPR1 that permits infection by the xenotropic host range MLV group (X-MLVs) as well as P-MLVs. Inheritance of this second virus resistance phenotype is associated with expression of viral envelope (Env) in resistant cells. This suggests that resistance is due to interference by one or more of the multiple chromosomally integrated X-MLV *env* genes carried by *M. castaneus*. The specific proviral sequence responsible for resistance in CAST/EiJ, *Rmcf2*, was identified and characterized as a full length X-MLV integrated on distal Chr 18 in these mice. Use of viral pseudotypes confirmed that *Rmcf2* prevents entry of P-MLVs.

Two additional laboratory stocks of *M. castaneus* were also found to be resistant to P-MLV infection and were screened for *Rmcf2*. CAST/Rp carries *Rmcf2*, but CAST/Ncr does not. Resistance in CAST/Ncr is due to inheritance of two novel unlinked genes, *Rmcf3* and *Rmcf4*. Co-opting proviral *env* genes may thus be a common defense strategy in natural populations in which survival is dependent on adaptation to widespread endemic infection.

P-175**AGING ALTERS THE GENETIC BASIS OF RESISTANCE TO *BABESIA MICROTI*, A PATHOGEN OF RED BLOOD CELLS**

J Yuan¹, J Spiegler¹, A Pliego¹, A Thambundit¹, S R Telford², S Shah³, A Spielman⁴, J A Gelfand³, W F Dietrich⁵, H H Wortis³, E Vannier¹

¹Tufts-New England Medical Center, Boston, MA, United States, ²Tufts Univ. School Vet. Medicine, Grafton, MA, United States, ³Tufts Univ. School Medicine, Boston, MA, United States, ⁴Harvard School Public Health, Boston, MA, United States, ⁵Harvard Medical School, Boston, MA, United States

Human babesiosis is an emerging infectious disease in New England. Age is the major risk factor for babesiosis, i.e., severe disease is most often seen in individuals over the age of 50. To determine the genetic basis of resistance, we developed a mouse model of infection with a human isolate of *B. microti*. Mice were infected i.p. with 10⁵ parasitized red blood cells. Parasitemia was measured at three-to-four day intervals, and defined as the frequency of nucleic acid positive red blood cells. Two-month old DBA/2 mice developed an intense parasitemia whereas B10.D2 mice were resistant. Male mice from reciprocal (DBA/2 x B10.D2) F₁ crosses failed to develop parasitemia, indicating that resistance is a dominant trait conferred by autosomal genes. Segregation analyses of 141 F₂ mice infected at young age mapped a major locus of resistance (*Babesiosis resistance locus-1*, *Brl-1*; LOD 13.2) on proximal chr.9 that accounted for 38% of phenotypic variance. A weaker linkage was detected on distal chr.4 (*Brl-2*; LOD 4.0). When infected at 18 months of age, DBA/2 displayed a greater susceptibility whereas B10.D2 remained resistant. Segregation analyses of F₂ mice infected at 18 months indicated a lesser role for *Brl-1* and *Brl-2* (LOD <3.0), and revealed a novel locus *Brl-3* (LOD 4.5) on distal chr.9. These studies establish that i) B10.D2 alleles at *Brl-1* and *Brl-2* are determinants of resistance to *B. microti* at young age and ii) aging alters the genetic basis of resistance by recruiting *Brl-3*.

(Supported by National Institute on Aging, NIH, R01AG19781)

P-176**DISSECTION OF GENETIC SUSCEPTIBILITY TO PRION INFECTION**

R Giri¹, I Lee², D Hwang², H Yoo², E Yi², D Baxter², B Ogata², C Ebeling¹, W Miller¹, R Kumar¹, R Young¹, R Pitstick¹, LE Hood², GA Carlson¹

¹McLaughlin Research Institute, Great Falls, Montana, United States, ²Institute for Systems Biology, Seattle, Washington, United States

Allelic coding variants of the prion protein (PrP) gene dramatically affect prion incubation time and disease susceptibility in mice and humans. However, genes other than *Prnp* also affect prion incubation time. An intercross between C57BL/6J (B6) and MA/MyJ (MA) mice that encode identical PrP molecules was analyzed to localize disease modifiers. B6 mice die 143 ± 4 days after inoculation with the RML isolate of murine scrapie, MA mice at 190 ± 5 days, while their F1 hybrids survive to 215 ± 3 days. Survival of F2 intercross offspring ranged from 93 to 267 days after prion inoculation. Analysis of age at death as a quantitative trait revealed highly significant linkage (LOD = 6.77) to an interval on Chromosome 2 that contains the *Prnp* gene. Levels of PrP or its regional distribution in the brain can affect prion incubation time, but other genes in this genetic interval might also influence prion disease susceptibility. Differential expression analysis of genes at the mRNA and protein levels provides tools for prioritization of candidate genes. However, the mechanisms responsible for genetic modulation of prion disease by *Prnp* or by other genes have been difficult to address given the lack of genetically tractable *in vitro* models. We have established neurosphere/CNS stem cell lines from various inbred strains and transgenic lines of mice. These cultures are susceptible to prion infection, providing a new system for mechanistic analysis of the bases for genetic differences in prion susceptibility. *Supported by grants from the NIAID and the USAMRMC.*

See page 30 for P-177 and P-178