SMP: Mammalian Models

Project: Secondary Infection Screens – The GBF Infection Challenge Platform for Mutant Mice

Andreas Lengeling & Werner Müller - GBF-National Research Center for Biotechnology, Braunschweig - lengeling@gbf.de & wmueller@gbf.de

Introduction

Acute and chronic infections are still one of the major health risk factors in poor and well-developed countries. Recent examples of re-emerging and new emerging infectious diseases like SARS and influenza infections further underline the global challenge that pathogens can impose on human health. The development of new tools for early diagnosis and interventional treatment relies heavily on a detailed understanding of the cellular, molecular and genetic basis of host-pathogen interactions. In the past, the mouse has played a pivotal role in the experimental dissection of these complex interactions. In fact, the use of genetically modified mice has generated most of our knowledge about fundamental mechanisms of immunity and pathogen defence. The optimal use of mouse models for the understanding of gene functions; and therefore for the identification of potential novel drug targets for therapeutic intervention; requires a systematic phenotyping of all resources of available mouse mutants.

Within the NGFN-1 the worldwide first phenotyping centre for mutant mice, the German Mouse Clinic (GMC) was installed at the GSF in Neuherberg. The GMC focuses on none-invasive phenotyping of mouse mutants for abnormalities in primary screens *(1)*. At the GBF an infection challenge platform (ICP) was established as a GMC outstation, which allows an extensive characterization of host responses to bacterial pathogens in secondary screens. The aim of the ICP is to screen mutant mouse lines for their infection susceptibility and to identify new genes that might be associated with genetic predisposition to infectious diseases. A further, particular focus of the ICP is on the development of novel phenotyping tools for mouse infection models. For the screening of mutant mouse lines, new protocols are needed that allow a measurement of multiple immune parameters by using small sample sizes and non-invasive detection methods. In this regard we are developing new protocols that permit a monitoring of immune responses and *in vivo* infection processes without stressing of the host immune system during the course of the infection.

Results/Project Status

Within the last three years we established a platform and the infrastructure that allows a systematic phenotyping of mice under high-quality hygiene conditions with standardized operation procedures (SOPs). Our phenotyping efforts are focussed on three bacterial pathogens. We established a mouse infection model for gram-positive induced septic shock by using the extracellular pathogen *Streptococcus pyogenes*. In addition, we established infection screens with the facultative intracellular, gram-positive pathogen *Listeria monocytogenes*. More recently, we developed an additional mouse infection model by using a non-invasive strain of the gram-negative pathogen *Yersinia enterocolitica* (serotype E40:O9). This model allows a specific investigation of mucosal immune responses in challenged mice and of their ability to clear a local infection of the gastro-intestinal system. For all three pathogens phenotyping routines were developed to test the susceptibility and resistance of mice after infection. These include for example parameters such as survival after infection, pathogen growth kinetics in target organs, pathogen tissue dissemination, extensive histo-pathological analysis, and quantification of cytokines and immune effector cells in the peripheral blood after infection. We used these approaches successfully to establish base-line data for different inbred strains of mice and to phenotype different knockout mice for defects in pathogen and immune defence. Examples of at the ICP analysed mutant mouse strains, are *Dnase2*-, *Nrf*-, *Ptdsr*-, *Il10*-, *Itgb7*, *Madcam*-, *Vcam*-, *Srf*-, and *Vdr*-knockout mice. Some of our most recent findings on the biology of the immune defence to the different pathogens are discussed below in more detail.

Fig 1: *The GBF Mouse Infection Challenge Platform allows to tackle complex questions about the function of the immune system in the infected host. A crucial infrastructure for the project is the new S2 mouse infection facility in which experiments can be performed under highly standardized hygiene conditions.*

Sex differences in susceptibility to *Listeria* infections are mediated by interleukine-10

It is well documented that sex-dependent factors affect susceptibility to infection, with most mouse models demonstrating higher resistance in females. In collaboration with the Immunology Screen at the GMC (headed by Dirk Busch, GSF, Neuherberg and Technical University of Munich) we made the unexpected observation that infection of mice with *Listeria monocytogenes* shows an opposite pattern: female mice are significantly more susceptible to *L. monocytogenes* infection than male mice.

L. monocytogenes is an intracellular, gram-positive bacterium that causes disease in immunocompromized individuals and pregnant women, often with deleterious consequences for the fetus. We analysed sex-related susceptibility patterns of listeriosis in four commonly used inbred strains of mice (C57BL/6J, BALB/cJ, C3H/HeN and CBA/J). We consistently found that females of all mouse strains were significantly more susceptible to *Listeria* infections than males. The pronounced sensitivity of females

to *L. monocytogenes* was correlated with higher lethality rates, increased bacterial growth in organ tissues and several changes in immunological parameters in the peripheral blood. Most interestingly, the increased severity of infection in females correlated with elevated interleukine-10 (IL-10) levels in plasma but not with interferon-γ. *L. monocytogenes* infection experiments in *Il10*-knockout mice revealed a loss of this sex dependence in the absence of the cytokine, demonstrating the crucial role of IL-10 for the outcome of the disease in females *(2).* We think that our findings might be of substantial clinical importance, since similar sex differences in infection with *L. monocytogenes* and other intracellular pathogens have been reported in humans.

Phenotyping of vitamin D receptor knockout mice revealed a new function of the steroid hormone in macrophage responses

The vitamin D hormone is best known for its well-established importance in regulating calcium homeostasis and bone mineralization. More recently, evidence has accumulated that the hormone has as well important functions in the immune system. It could be demonstrated that vitamin D3 can regulate adaptive immune responses by acting immunosuppressive on dendritic and T helper cells.
In contrast to its well-characterized effects on adaptive immune responses, much less is known about the effects of vitamin D3 on effectors of innate immunity, especially on macrophages. It has been demonstrated that macrophages can produce vitamin D3 upon activation with interferon-γ (IFN-γ), although little is understood about the biological significance of this response. We found that vitamin D3 is a potent suppressor of IFN-γ-mediated macrophage activation. Vitamin D3 can downregulate IFN-γ responsive genes in activated macrophages. This has major consequences on macrophage effector functions such as bactericidal killing activity and regulation of inflammatory responses. Among these are the suppression of *Listeria* killing activity, the inhibition of phagocyte oxidase mediated oxidative burst, and the suppression of important IFN-γ-induced cytokine and chemokine genes. These effects of vitamin D3 are specific for IFN-γ-activated macrophages, they are strictly dependent on a functional vitamin D receptor, and are distinct from previously described macrophage deactivation mechanisms. We therefore hypothesize that the production of vitamin D3 by IFN-γ-activated macrophages might be an important negative feedback mechanism to control innate and inflammatory responses of macrophages *(5).*

Genetic control of susceptibility against *Streptococcus pyogenes* infections in mice

The group A streptococcus (GAS) – also known as *Streptococcus pyogenes* – is among the most flexible and prevalent of human pathogens. It is responsible for a wide spectrum of human diseases, ranging from mild, self-limiting infections such as pharyngitis, scarlet fever and impetigo to extremely severe and life-threatening invasive diseases, such as necrotizing fascitis and streptococcal toxic shock-like syndrome. By phenotyping different inbred mouse strains for their susceptibility to *S. pyogenes* infections we could establish a mouse model of GAS-induced sepsis. BALB/c mice display a superior resistance to GAS infection (>1000-fold) in comparison to C3H/HeN mice after intravenous or subcutaneous inoculation with *S. pyogenes (4)*. The enhanced susceptibility of C3H/HeN mice correlates with an inability to control bacterial growth after infection and in addition with the development of an overshooting inflammatory reaction in response to the infection that leads finally to organ damage and death. Within the last years we could determine that susceptibility / resistance against *Streptococcus pyogenes* infection is under multigenic control in both selected inbred strains of mice. By using a backcross between C3H/HeN and BALB/c mice ([BALB/c x C3H/HeN] F_1 x BALB/c) we could map three quantitative trait loci on mouse chromosomes 2, 7 and 17 (together with Eva Medina and Oliver Goldmann; *5*). To increase the current map resolution and statistical power of our linkage analysis, we generated more backcross progenies for further infection experiments. In addition, we have analyzed different lines of congenic mice, which harbour the susceptibility locus of the C3H/HeN parental mice on proximal mouse chromosome 17 in the resistant BALB/c genetic background. This allowed us to narrow down the critical gene interval to 8.1 cM through detection of new recombination events. Currently, we are investigating the first candidate genes within this chromosomal region.

Development of new phenotyping tools – immuno-proteomics assays for detection of serum factors

Bacterial infections are in part resolved by the production of antibodies directed against bacterial proteins. Some of these antibodies may be protective for a secondary infection. Using glass chip protein arrays we are measuring antibody responses against *Y. enterocolitica* derived proteins. We have started to use gel-free proteomics approaches to determine serum components that might be indicative for responses towards bacterial infections. By the use of nano-HPLC and mass spectrometry (MS) analysis we determine changes in serum components of the mice upon infection. Target proteins identified by this approach will be later on used to generate new quantification assays in the BioPlex (Luminex) detection system.

Outlook

Susceptibility to infections is determined by the complex interplay of environmental, host and pathogen factors. The goal of our studies is to better understand the genetic and environmental factors that can influence the course of an infection, and to develop new preventive and therapeutic strategies to fight infectious diseases in humans. In this context, the mouse represents an essential *in vivo* model system to understand the complex biological processes during an infection and to elucidate the molecular mechanisms underlying host-pathogen interactions. To accomplish this new innovative tools for the phenotyping and monitoring of immune responses under infection challenge conditions are needed. In the future, we will extend our efforts to develop novel serum analytic tools that permit the analysis of surrogate markers indicative for disease development and progression. In addition, we will continue to use the mouse as a genetic model system to understand the biological functions of genes and regulatory networks that are crucial for pathogen defence.

*Lit.: **1.** Gailus-Durner V et al. Introducing the German Mouse Clinic: Open access platform for standardized phenotyping. Nat. Methods 2005 June 2 (6): 403-4. **2.** Pasche B et al. Sex dependent susceptibility pattern to Listeria monocytogenes infection is mediated by differential IL-10 production. Infect. Immun. 2005 in press. **3.** Goldmann O et al. The role of the major histocompatibility complex on resistance to Group A Streptococci in mice. J. Immunol. 2005 in press. **4.** Medina E. and Lengeling, A. Genetic regulation of host responses to Group A Streptococcus in mice. Brief. Funct. Genomic Proteomics 2005 in press. **5.** Helming et al. 1α,25 dihydroxyvitamin D_3 is a potent suppressor of interferon-γ mediated macrophage activation. Blood in press.*