Abstract

Leishmaniasis is a neglected tropical disease, which belongs to the top health problems because it is endemic in 98 countries in Asia, Africa, the Americas and the Mediterranean region, and is gradually expanding to new areas, including Central Europe and USA. Clinical manifestations of leishmaniasis include a diverse range of forms, ranging from non-lethal cutaneous leishmaniasis to potentially lethal visceral leishmaniasis. Asymptomatic cases are known to exist in endemic areas. Different species of *Leishmania* induce distinct symptoms, but even the patients infected by the same species develop different symptoms and may respond differently to the treatment. Thus, one of the challenges is to explain the observed variability of leishmaniasis that cannot be attributed to the currently known factors. To find novel regulatory factors of the disease we tested molecules that were shown to play role in other infections and mapped loci controlling parasite load after *L. major* infection. We also determined genetic control of survival after infection with tick-borne encephalitis virus (TBEV) in order to establish whether there are common elements in response to *L. major* and TBEV.

Interferon-induced GTPases (guanylate-binding proteins, GBPs) play an important role in inflammasome activation and mediate innate resistance to many intracellular pathogens, but little is known about their role in leishmaniasis. Fcγ receptor IV (FCGR4), the receptor for the Fc fragment of immunoglobulin G (IgG), participates in IgG2a- and IgG2b-dependent effector functions in immune response. Experiments with mice bearing knockouts of other Fcγ receptors have shown that the genetic background of the host controls their role in response to *Leishmania* parasites, leading either to protective

immunity or to progression of disease. However, the information about the role of FCGR4 in leishmaniasis is missing.

We therefore studied expression of *Gbp2b*/*Gbp1*, *Gbp5* and *Fcgr4* mRNA in skin, inguinal lymph nodes, spleen and liver after *L. major* infection and in uninfected controls. We used two different groups of related mouse strains, which were classified on basis of size of infection-induced skin lesions as highly susceptible (BALB/c, CcS-16), susceptible (B10.O20), intermediate (CcS-20), and resistant (STS, O20, B10, OcB-9, OcB-43).

We observed strong genetic influence on *Gbp2b*/*Gbp1* and *Gbp5* mRNA levels. Some strains differed in *Gbp2b*/*Gbp1* and *Gbp5* expression prior to infection. Several of them differed in *Gbp2b*/*Gbp1* and/or *Gbp5* expression although they carried the same *Gbp2b*/*Gbp1* and/or *Gbp5* alleles, indicating their trans-regulation.

Infection resulted in approximately 10x upregulation of *Gbp2b*/*Gbp1* and *Gbp5* mRNAs in organs of both susceptible and resistant strains. It was most pronounced in skin. Co-localization of GBP2b protein with most *L. major* parasites in skin of resistant and intermediate strains, but not in highly susceptible BALB/c mice suggests that this molecule might play role in defense against leishmaniasis.

Similarly as in study of *Gbps* expression, we observed strong genetic influence on *Fcgr4* mRNA levels, as well as trans-regulation in both uninfected and infected mice. Infection caused a varying degree of up-regulation (up to 50x) of *Fcgr4* in organs of mouse strains irrespective of their susceptibility or resistance. The up-regulation of FCGR4 after infection was confirmed by immunohistochemistry. We have compared localization of the parasites and FCGR4 in skin and liver of selected strains and found their partial co-

localization. These findings suggest relationship of this molecule to the response to *L. major* infection.

The development of visceral leishmaniasis, which is lethal if untreated, is not yet understood. Therefore we analyzed the genetics of parasite load, spread to internal organs, and ensuing visceral pathology. Quantification of *Leishmania* parasites in lymph nodes, spleen and liver of infected F_2 hybrids between BALB/c and recombinant congenic strains CcS-9 and CcS-16 allowed us to describe a network-like set of interacting genetic loci that control parasite load in different organs. We mapped two novel loci controlling parasite load: *L. major* response 24 and 27 (*Lmr24* and *Lmr27*). We also detected parasite-controlling role of the previously described loci *Lmr4*, *Lmr11*, *Lmr13*, *Lmr14*, *Lmr15* and *Lmr25*, and describe 8 genetic interactions between them. *Lmr14*, *Lmr15*, *Lmr25* and *Lmr27* controlled parasite load in liver and lymph nodes. In addition, *Leishmania* burden in lymph nodes but not liver was influenced by *Lmr4* and *Lmr24*. In spleen, parasite load was controlled by *Lmr11* and *Lmr13*. We detected a strong effect of sex on some of these genes.

In the last part of presented thesis we explored genetic control of susceptibility to TBEV. Our previous data shown that the strain CcS-11 carrying 12.5% of the STS genome on the background of the genome of the strain BALB/c, is even more susceptible than parental strains. Therefore, we have generated a F_2 intercross between BALB/c and CcS-11 and performed a linkage and bioinformatics analysis. These studies revealed a novel suggestive locus on mouse chromosome 7 containing 9 potential candidate genes for TBEV response. Interestingly, on the mouse chromosome 7 was in the strain CcS-11 mapped locus *Lmr21* that controls susceptibility to *L. major*. However, this locus is mapped on a long chromosomal segment, thus other gene(s) might be responsible for its effect.

Collectively, the thesis presents the new insight on the response to *L. major* parasites. For the first time, we described the role of *Gbps* and *Fcgr4* as inflammatory markers of *L. major* infection. Our results also point out that expression of *Gbps* and *Fcgr4* was increased even in organs of resistant mice, which might suggest a hidden inflammation. It remains to be established whether the clinically asymptomatic infection might represent danger in predisposing organism to other diseases. We also provided a first systematic a genome wide search of the genetic control of parasite load in mammalian organs after *L. major* infection. Host genes controlling *L. major* revealed a wide variety of heterogeneous effects that included distinct organ-specific control, single-gene effects, gene-gene interactions and sex dependent control.

Further deep characterization of role of *Gbps*, *Fcgr4* genes and fine mapping of newly identified loci involved in controlling parasite burden in different organs after *Leishmania* infection may help to understand the detailed mechanisms of the disease and would open new perspectives of the research, treatment and vaccine development against leishmaniasis.