Abstract

Leishmaniasis is a neglected tropical disease caused by protozoan parasites of the genus *Leishmania* and transmitted by female sand flies. The outcome of *Leishmania* infection depends both on host and pathogen factors. Similarly as *L. major*, *L. tropica* very often causes cutaneous leishmaniasis in humans, but in rare occasions can also visceralize and cause systemic disease. Leishmaniasis cause by *L. tropica* has become a major public health problem in different endemic foci due to recent outbreaks in several urban areas and spread to new regions. The complications of the disease and lack of safe and effective drug and vaccine against the *L. tropica* infection require considerable attention to studies of the host-*L. tropica* interaction. Until recently, the research of leishmaniasis caused by *L. tropica* was limited due to lack of suitable inbred model and difficulties in inducing infection in animals. The aims of the present project were development of a suitable mouse model of the infection caused by *L. tropica*, and the study of mechanisms of the disease, and also mapping controlling genes/loci.

We analysed susceptibility to *L. tropica* infection using recombinant congenic (RC) CcS/Dem mouse strains. These strains differ greatly in susceptibility to *L. major* due to random distribution of 12.5% of STS genome on 87.5% BALB/c background in their genomes. Our data showed that CcS/Dem strains exhibited various susceptibility to *L. tropica* infection. We proved strong influences of parasite species, host genetic and sex on development of pathology in *L. major* and *L. tropica* infection. The CcS-16 strain not only developed the highest degree of susceptibility to *L. tropica*, but also exhibited unique transient early peaks of CCL3 and CCL5.

Further analysis of susceptibility to *L. tropica* using CcS/Dem series revealed that CcS-9 not only has large lesion but also exhibited high visceral pathology after infection with *L. tropica*. To understand the reasons of the unique symptoms of CcS-9 strain, we evaluated recruitment of different types of immune cells to the tissues, and also performed the gene expression analysis. We demonstrated the unique role of inflammatory mediators that orchestrated immune responses through modulation of infiltration of different cell types to organs and tissues reflecting the level of susceptibility to *L. tropica*.

We used CcS-16 strain to dissect the genetic susceptibility and functionally characterize the gene-loci regulating the immune responses and pathology to *L. tropica*. The present project describes the first identification of the genetic loci controlling susceptibility to *L. tropica* by mapping 8 *Ltr* (Leishmania tropica response) loci. Individual *Ltr* loci affect different subsets of the disease manifestations, exhibit organ specific effects and a separate control of parasite load and organ pathology. We observed multiple gene interactions controlling symptoms during *L. tropica* infection. *Ltr2*, *Ltr3*, *Ltr6* and *Ltr8* showed single gene effect, while *Ltr1*, *Ltr4*, *Ltr5* and

Ltr7 were detected only in gene-gene interactions with other *Ltr* loci. Interestingly, *Ltr3* exhibited the phenomenon of transgenerational parental effect on parasite numbers in spleen. *Ltr1*, which controlls parasite number in lymph nodes, was the most precisely mapped locus (4.07 Mb). Comparative analysis showed that five *Ltr* loci co-localized with previously identified loci controlling susceptibility to *L. major*, whereas three were likely *L. tropica* specific.

The related project on human samples described in the thesis, identified the $CD8^+$ T cells as the main producer of IFN-γ which might be the responsible cells for maintenance of protective immune response against human leishmaniasis.

Collectively, the thesis presents the new insight to the research of leishmaniasis caused by *L. tropica*. These studies provided experimental evidences that the host genotype and the host-*L. tropica* interactions significantly contributed to risk and development of the disease. For the first time, the present project described the role of the genes/loci that are involved in susceptibility to *L. tropica* infection. In addition, it underscored the importance of the host genetic for mechanism of the disease. These results also illustrate the contribution of inflammation and infiltration of various cells to the outcome of *L. tropica* infection. Such knowledge may also provide hints for the development of novel strategies of the disease control. Further deep characterization of *L. tropica* infection in CcS-9 and CcS-16 strains may help to understand the detailed mechanisms of the disease and would open new perspectives of the research, treatment and vaccine development against leishmaniasis caused by this parasite.

Key words: *L. tropica*, host-pathogen intraction, genetic control, *Ltr* loci, recombinant congenic strain, inflammation, skin pathology, visceral pathology, chemokine, CcS-9, CcS-16