Abstract: The intraperitoneal infection with Toxoplasma gondii (T. gondii) caused accumulation of γδ T, NK, NK1.1+T-like (NKT) cells at inflamed sites. To clarify the roles of these cells in protection against T. gondii at the inflamed sites, BALB/c mice were depleted of γδ T, NK, NK and NKT cells by treatment with antibody against TCR-γδ, asialoGM1 or Interleukin-2 receptor β-chain (IL-2 Rβ), respectively, prior to infection. Mice treated with anti-TCR-γδ monoclonal antibody (mAb) became more susceptible to infection, whereas mice treated with anti-IL-2Rβ mAb acquired resistance. Treatment with anti-asialoGM1 Ab showed no effect. We previously reported that heat shock protein 65 (HSP65) in macrophages induced by γδ T cells plays an essential role in protective immunity against T. gondii infection, by preventing apoptotic death of infected macrophages. In the present study, we showed that treatment with anti-IL-2Rβ mAb, but not with anti-asialoGM1 Ab, enhanced the HSP65 induction in macrophages, and inhibited Interleukin-4 (IL-4) expression in nonadherent peritoneal exudate cells. Furthermore, neutralization of endogenous IL-4 by anti-IL-4 mAb enhanced the HSP65 induction in macrophages. These findings suggest that NKT cells, but not NK cells, negatively regulate the protective immunity against T. gondii infection possibly by producing IL-4 and suppressing HSP65 induction.

Keywords: Toxoplasma gondii, HSP65, NKT cells
Cell preparation

Animals and parasites

Antibodies

In vivo cell depletion

Western blotting
Flow cytometric analysis

Flow cytometric analysis of γδ T, NK, and NKT cells in peritoneal cavity after T. gondii infection

Cell sorting

Cell sorting analysis of γδ T, NK, and NKT cells in peritoneal cavity after T. gondii infection

RT-PCR

RT-PCR analysis of γδ T, NK, and NKT cells in peritoneal cavity after T. gondii infection

Effects of treatment with antibodies specific for TCR-γδ, asialoGM1 and IL-2Rβ on resistance against infection with T. gondii.
Effects of treatment with antibody on HSP65 expression in the peritoneal macrophages

A. 

B. 

C. 

Effects of treatment with antibody on HSP65 expression in the peritoneal macrophages
Effects of $\gamma\delta$ T, NK, or NKT cell depletion on expression of IL-4 and IFN-$\gamma$ mRNAs in nonadherent PEC

Effects of $\gamma\delta$ T, NK, or NKT cell depletion on expression of IL-4 and IFN-$\gamma$ mRNAs in nonadherent PEC
Effect of neutralization of endogenous IL-4 on HSP65 expression in peritoneal macrophages

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in vitro and in vivo experiments in the form of a brief description of the methods and materials used. The results obtained were analyzed statistically using the Student's t-test. The statistical significance level was set at p < 0.05.

In vitro experiments

The in vitro experiments were performed using several strains of human macrophages, including J774A.1, THP-1, and U937. The cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. The cell cultures were infected with T. gondii tachyzoites at a multiplicity of infection (MOI) of 10:1. The infected cells were incubated for 48 hours, and the parasite growth was monitored by measuring the intracellular parasite load.

In vivo experiments

The in vivo experiments were performed in a BALB/c mouse model infected with T. gondii tachyzoites. The infected mice were divided into two groups: one treated with a drug and the other serving as control. The drug treatment was administered daily for 10 days, and the parasite loads were monitored by measuring the levels of anti-T. gondii antibodies in the sera.

Discussion

The results of the in vitro experiments showed that the drug treatment significantly decreased the intracellular parasite load in all cell lines tested. The in vivo experiments confirmed these results, with a significant reduction in parasite loads in the drug-treated group compared to the control group. These findings suggest that the drug has potential antiparasitic activity against T. gondii.

Conclusion

The results of this study provide evidence that the drug has significant antiparasitic activity against T. gondii. Further studies are needed to evaluate the drug's safety and efficacy in human trials.

References

Innate immune cells in *T. gondii* infection

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Toxoplasma gondii is a protozoan parasite that infects a wide range of hosts, including humans. Its lifecycle involves a complex interplay between host and parasite, and the innate immune system plays a crucial role in both the establishment and control of infection.

**Leishmania major** is a member of the Leishmania genus and is responsible for cutaneous leishmaniasis. Like *T. gondii*, it utilizes the host immune system to its advantage, often evading immune recognition and control.

**Salmonella** serovars are a diverse group of bacteria that cause a range of infections, from mild gastroenteritis to invasive diseases. Salmonella Salmonella enterica serovar Typhi, for example, is the causative agent of typhoid fever.

**Trypanosoma cruzi** is the etiological agent of Chagas disease, a serious infectious disease that primarily affects the heart and gastrointestinal system. The parasite uses complex mechanisms to evade and subvert the host's immune response.

The γδ T cells are a subset of T cells that are known for their rapid response and broad reactivity. They play a crucial role in the early innate immune response to pathogens, including *T. gondii*.

The γδ T cells are activated by pathogen-derived molecules that are expressed on infected cells, leading to the production of cytokines that drive the inflammatory response. This response is critical for the elimination of *T. gondii* from the host.

In conclusion, the γδ T cells are an integral part of the innate immune response to *T. gondii* infection. Their rapid activation and broad reactivity make them a key component in the early defense against this parasite.