

Interleukin 27 regulates adaptative immune responses associated with control of parasite replication in Canine Leishmaniosis

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Background

Leishmaniosis is an often neglected and potentially lethal disease when left untreated. Canine leishmaniosis (CanL) is a serious public health problem because infected dogs are potential sources of the *Leishmania* protozoan to humans through the phlebotomine vector. The progression of CanL is related to the suppression of effective immune response. IL-27 is a cytokine that exerts diverse effects on the cells of innate and adaptive immune systems. IL-27 is a heterodimeric cytokine composed of IL-27p28 and EBI3 subunits which, when combined, bind to IL-27R, leading to STAT-1 and -3 activation, playing a role in the regulation of the immune response. It was recently observed that visceral leishmaniasis patients present high levels of IL-27 in serum [1]. In mice models, secretion of IL-27 is important to drive the Th1 response but also plays a negative regulatory role in the production of IL-17 [2]. Therefore, we investigated whether IL-27 has a role in the suppression of effective immune response in CanL.

Materials and methods

This study was approved by the Committee for Ethics in Animal Experimental Research (COBEA), with the approval of the Committee for Ethics in Animal Use (CEUA) of São Paulo State University (UNESP), School of Veterinary Medicine, Araçatuba, São Paulo, Brazil. We evaluated IL-27p28 and EBI3 subunits and IL-21 in supernatant from splenic leukocytes (SL) from dogs with CanL (n= 30) compared to healthy dogs (n=5), by ELISA.

IgG anti-*L. infantum* were measured in serum by ELISA, and parasite loads were measured in SL by flow cytometry. The correlation between IL-21, IgG anti-*Leishmania*, hematological and biochemical parameters considered clinical markers of disease progression, and IL-27p28 and EBI3 subunits was investigated. Functional assay in SL of dogs with CanL followed by IL-27 blockade was performed and the production of IL-4, IL-6, IL-10, IL-12, IL-17, IL-21, TNF- α , IFN- γ were measured in the supernatant by ELISA. In addition, parasite load, and PD-1, CTLA-4, phospho-Stat-1, phospho-Stat-3, T-bet, GATA3 and nitric oxide (NO) production in splenic leukocytes were measured by flow cytometry. All statistical variables were tested for normality using the Shapiro-Wilk. The groups were compared using parametric or non-parametric tests.

Results

Our results showed that both IL-27subunits and IL-21 were increased in the culture supernatant of splenic leukocytes of dogs with CanL when compared to control dogs. EBI3 and p28 levels showed a moderate positive correlation with IL-21. EBI3 subunit was positively associated with anti-*L. infantum* IgG, parasite load and globulin. EBI3 also showed a negative correlation with albumin levels. IL-27 neutralization decreased parasite load, PD-1, CTLA-4, T-bet and NO expression in SL of dogs with CanL.

Conclusion

These findings suggest that IL-27 participates in the immune response in CanL, it regulates the adaptive response associated with negative co-stimulation signal on lymphocytes, differentiation of Th1 cells and controlling parasite replication in CanL.

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References

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