Abstract

Introduction. Canines are the principal domestic reservoirs of visceral leishmaniasis in both the Old and New World. The development of highly sensitive and quantitative methods, such as real time reverse transcriptase polymerase chain reaction for measurement of canine cytokines has not been exploited in studies of visceral leishmaniasis. Objective. To standardize the relative quantification of canine IFN-γ, IL-4, IL-10, IL-12p40 and IL-12p35 using real time reverse transcriptase polymerase chain reaction. Materials and methods. RNA was isolated from PBMCs from 1 year old foxhounds and cultured with or without Con A, LPS or Staphylococcus aureus extract. This RNA was used in one-step real time reverse transcriptase polymerase chain reaction to optimize the concentrations of the cytokine primers and probes, generate standard curves for each cytokine, confirm equivalent amplification efficiency of cytokine and normalizer (18S rRNA) RNA, and to quantify the expression of the cytokine RNA. The comparative Ct method was used to determine the relative levels of gene expression in the samples, expressed as the fold-increase relative to the control samples. Results. The regression coefficient for the standard curves and the amplification efficiencies of the cytokine and normalizer RNA indicated that the quantification was reliable over a broad concentration range of input RNA. Relative to control cells, activation of PBMCs led to increased expression of IFN-γ (132-fold), IL-4 (8.8-fold), IL-10 (7.2-fold), and IL-12p40 (275-fold). Basal expression of IL-12p35 was also detected. Conclusion. This approach provides several advantages over conventional assays for cytokine measurement and can be exploited in the study of the immunopathogenesis and immunity in canine leishmaniasis.

Keywords
dogs, cytokines, reverse transcriptase polymerase chain reaction, visceral leishmaniasis, T-Lymphocytes.