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3098 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)
Annexin A1 Regulates Inflammatory Response in Cutaneous Leishmaniasis Patients Caused by L. braziliensis

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Cutaneous leishmaniasis (CL) is characterized by the presence of ulcerated skin lesions. CL lesions present high levels of TNF, IL-6 and IL-1β- associated with disease progression, increased frequency of lymphocytes and mononuclear phagocytes, and few parasites. Annexin A1(ANXA1) is a calcium-dependent phospholipid-binding protein. It is induced by glucocorticoids and can regulate the production of inflammatory mediators. The anti-inflammatory effects of ANXA1 are largely mediated through its binding to the FPR2, resulting in the control of the inflammatory response. Ac2-26 is a mimetic peptide derived from the N-terminal portion of ANXA1. It reproduces many of the biological and anti-inflammatory actions of the full ANXA1 protein. To investigate the effects of ANXA1 in IL-1β, TNF, IL-10 and IL-6 production by L. braziliensis (LB)-infected macrophages and in biopsies from patients with CL. Skin biopsies from patients and healthy subjects (HS) were cultured in presence or absence of rANXA1 for 48h.Monocyte-derived macrophages from HS were infected with LB (5:1) in presence or absence of Ac2-26 and cultured for 8 and 48h. Levels of ANXA1 and IL-1β, TNF, IL-10 and IL-6 were determined in supernatants of all cultures by ELISA assay. We observed increased levels of IL-1β, IL-10, TNF and IL-6 in biopsy supernatants from CL compared with HS. In addition, we observed that rANXA1 treated supernatants of biopsies from CL patients, decreased IL-1β and IL-6 levels, but did not affect TNF or IL-10 production. LB-infected macrophages produced high levels of IL-1β, IL-10, TNF and IL-6. Finally, we found that treatment with Ac2-26 of LB-infected macrophage cultures decreased IL-1β levels without affecting the levels TNF, IL-10, IL-6 and parasite load. Our results suggest that therapy with Ac2-26 or rANXA1 downregulates IL-1β and IL-6 in patient skin and downregulates IL-1β production in infected-macrophages without affecting the ability of macrophages to kill LB.
Keywords: Cutaneous leishmaniasis;Annexin A1; Ac2-26.

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Cellular Proliferation of PBMCs from Cattle Immunized with Rhipicephalus microplus Larval Extract encapsulated in NIBDAF Nanoadjuvant: A Propesctive Comparative MTT Assay Analysis

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The NIBDAF is a biocompatible immunomodulatory target-delivery system designed to enhance immune responses without inducing cytotoxicity (intellectual property protection in progress). The use of tick-derived immunogens encapsulated in NIBDAF is a promising strategy for modulating immune responses against Rhipicephalus microplus.
To evaluate the viability and proliferative profile of PBMCs from cattle immunized with R. microplus larval extract (EL), NIBDAF, or the combination of both (NIBDAF-EL), compared to saline control groups, using the MTT assay.
PBMCs were isolated from blood of immunized Holstein calves and stimulated in vitro with PMA (mitogen), EL and R. microplus saliva antigens (both 1:1000) or saline solution (baseline control). MTT assays were performed according kit instructions (ThermoScientific), and 550nm optical density (OD) values were quantified to estimate mitochondrial activity as a proxy for cell viability and proliferation. Data were analysed using one-way ANOVA followed by Tukey's post-hoc test ($\alpha = 0.05$). PMA-stimulated PBMCs from the NIBDAF-EL group showed higher viability than the EL (mean OD: 319.8 vs. 276.5) and NIBDAF (mean OD: 364.1) groups, suggesting synergistic effects on cell activation. Under EL stimulation, the NIBDAF-EL group also demonstrated higher proliferation than the EL and saline controls (mean OD: 223.7 vs. 125.6 and 83.4, respectively; $p < 0.05$). The saline group showed basal viability, with no significant differences among immunized animals. Control animals had intermediate viability under all conditions.
In conclusion, immunization with NIBDAF-EL induced greater activation and viability in PBMCs, suggesting that the NIBDAF platform enhances the immunostimulatory capacity of larval antigens and holds promise for the development of anti-tick vaccines. Complementary studies on gene expression and cytokine profiles are ongoing to better characterize the immunomodulatory profile.
Keywords: Rhipicephalus microplus;Holstein calves;immunomodulatory.