TITLE: ANALYSIS OF THE INVOLVEMENT OF TYPE I INTERFERON IN ERYTHEMA NODOSUM LEPROSUM.

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ABSTRACT: The course of the multibacillary leprosy may be interrupted by acute inflammatory episodes known as erythema nodosum leprosum (ENL). Elevated levels of inflammatory cytokines and other immunological factors have been associated with ENL episodes. Recent data from our group demonstrated increased TLR-9 expression in both the lesions and in the circulating mononuclear cells of ENL patients. The levels of endogenous DNA and mycobacterial histone-like protein, TLR-9 ligands, were also high in the circulation of ENL patients. An important consequence of the activation of the TLR-9 pathway is the production of type-I interferon (IFN-I). This cytokine has been seen as a potential target in several chronic inflammatory diseases and infections. Based on the above-mentioned data, this study aimed to analyze the participation of IFN-I in the pathogenesis of ENL. To achieve this goal, blood samples and skin lesions from nonreactional multibacillary patients (LL / BL group) and patients diagnosed with ENL before starting thalidomide treatment (ENL D0 group) were collected. We also analyzed clinical samples from ENL patients after 7 days of thalidomide treatment (ENL D7 group) to assess the impact of thalidomide on the IFN-I pathway. Immunofluorescence and real-time PCR assays were performed to investigate, respectively, the presence of IFN-α and the expression of IFN-I signature genes (IFIT-1, MX-1 and EIF2AK2). In addition, MX1 protein levels in the skin lesion were analyzed by western blotting. Our gene expression data indicate higher levels of EIF2AK2 mRNA in both skin lesions and blood of ENH D0 patients. Regarding the expression of MX1 and IFIT1 genes, no difference between the groups was observed. Thalidomide treatment resulted in decreased expression of EIF2AK2 and MX1 genes. Protein levels of MX1 were elevated in LL/BL patients and ENL D0, with a decrease after thalidomide treatment. Immunofluorescence images suggested the presence of a greater number of IFN-α producing cells in the lesions of ENL D0 patients compared to LL/BL, and with a drastic decrease in production after 7 days of thalidomide treatment. Finally, we confirmed that IFN-α has the ability to induce NETose in neutrophils from healthy individuals. Taken together, our data suggest the involvement of type I IFN in the pathogenesis of ENL, with perspectives for the identification of biomarkers for early diagnosis and new therapeutic targets for a better management of this reactional episode.

Keywords: *mycobacterium leprae*, ENL, type-I interferon.
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