PURIFICATION OF SIMPL ANTIBODY AND IMMUNOFLUORESCENCE OF SIMPL SUB-CELLULAR LOCALIZATION IN RESPONSE TO TNF$_{\alpha}$- AND IL-1

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Abstract:

SIMPL is a transcriptional co-activator that alters the activity of transcription factor, NF-κB. In response to pathogens, cytokines such as Interleukin-1 (IL-1) and Tumor Necrosis Factor (TNF) signal through the IL-1 and TNF-α receptors, respectively, which are found on various cell types. Activation of these receptors can result in the nuclear localization of NF-κB where it enables the transcription of several different genes key in the innate immune response. Endogenous co-localization of the SIMPL protein with NF-κB in response to these same cytokine signals has yet to be demonstrated. Polyclonal antibody generated against a truncated version of the SIMPL protein was purified from the sera obtained from immunized rabbits using affinity chromatography. The antibody was found to have a high specificity for both the native and denatured form of the protein as demonstrated by the lack of nonspecific bands observed in immunoprecipitations and Western blotting. The antibody was utilized in

immunofluorescence experiments on mouse endothelial cells that were either unstimulated or were stimulated (IL-1 or TNF-α). In the absence of cytokine, SIMPL was localized in both the cytoplasm and the nucleus as opposed to NF-κB which was almost exclusively localized in the cytoplasm. In the presence of IL-1, the concentration of SIMPL in the nucleus was increased, and in the presence of TNF-α, the concentration of SIMPL in the nucleus was even greater. Results of this study identified future routes for SIMPL antibody isolation as well as to demonstrate that endogenous SIMPL protein nuclear localization may not be solely dependent upon TNF-α signaling.

Description:

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