Bacillus pumilus ribonuclease binase induces proinflammatory immune response in macrophages

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Background: Bacterial ribonuclease binase from *Bacillus pumilus* possesses cytotoxic activity against tumor cells. The course of malignancy progression is associated with macrophages migration towards the site of tumor development. NF-κB signaling pathway induces activation of macrophages either into the M1- or into M2- phenotype. Based on previous data from our group on NF-κB signaling pathway in leukemic cells, we propose that binase exhibits cellular activity, including promotion of NF-κB signaling in macrophages.

Material and methods: Binase was isolated as homogenous protein from cultural fluid, enzyme purity was confirmed by electrophoresis. THP-1 and RAW 264.7 cells were obtained from American Type Culture Collection (Rockville, MD). The viability of macrophages was determined using the LDH Cytotoxicity Detection Kit (Roche) and the XTT cell proliferation assay (Life Technologies). Protein expression and cytokine expression were measured by Western Blot and BD Cytometric Bead Array Mouse Inflammation Kit (BD, USA).

Results: We have shown that binase did not decreased macrophages viability. Increased expression of activated NF-κB p65 subunit in macrophages was revealed. Since no changes in MyD88 and TRIF adaptor protein expression were observed, toll-like receptors may not be involved in RNase-related NF-κB pathway activation. In addition, binase induced the release of proinflammatory cytokines IL-6, MCP-1, or TNF-α but not anti-inflammatory IL-4 and IL-10.

Conclusions: NF-κB activation is required by M1 as well as by M2 macrophages differentiation. The M1 phenotype is characterized by the expression of high levels of proinflammatory cytokines, whereas M2 macrophages demonstrate high IL-10 expression. These facts allow us to consider that binase activates macrophages of M1-biased phenotype with tumorocidal properties and stimulates antitumor immunity.

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