THE INVESTIGATION AND MODIFICATION OF TNF REVERSE SIGNALING ON PROFESSIONAL IMMUNE CELLS

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Objectives: Professional (i.e. monocytes, macrophages) and semi-professional immune cells (i.e. endothelial cells) are both able to produce the soluble and the membrane-bound form of TNF. It is well known that receptor-ligand interactions results in physiological alterations on receptor expressing cells (forward signaling). Interestingly, receptor-ligand interactions are also able to alter the behavior of ligand expressing cells (reverse signaling). It is known that the N-terminal domain of TNF contains a putative nuclear localization signal. The treatment of TNF producing cells with TNF receptors or agonistic anti-TNF antibody (i.e. infliximab; IFX) results in the liberation of a 10kDa fragment, which is translocated in the nucleus. This fragment may has transcription factor activity and it may be involved in the fine tuning of the innate and adaptive immune response.

Methods: To analyze this phenomenon more accurately, human and mouse macrophages were pretreated with *Escherichia coli*-derived lipopolysaccharide (LPS) to mimic inflammatory conditions. Then, reverse signaling was induced by TNF neutralizing antibodies (namely IFX and certolizumab pegol; CZP, respectively). In case of T lymphocytes, phorbol 12-myristate 13-acetate (PMA) treatment was carried out to induce activation, and OKT-3 was added to mimic MHC-TCR interactions. In accordance with macrophages CZP was added for the induction of reverse signaling. Investigations were performed at relative gene expression and secreted protein level by quantitative real-time PCR (QRT-PCR) and proteome profiler, respectively.

Results: Simultaneous induction of reverse signaling by IFX or CZP stimulation resulted in marked relative gene expression decline in inflammation-related cytokines (i.e. IL-1β, IL-8, etc.) in human and mouse macrophage cell lines as compared with LPS treated cells. Furthermore, the regulatory effect of TNF reverse signaling seems to be independent from the presence of the Fc fragment, as IgG stimulation has no dramatic impact on the expression pattern of the previously mentioned effector molecules as compared with naïve controls. In case of T lymphocytes, antibody treatment resulted in relative gene expression down regulation of the previously mentioned effector molecules versus activated and OKT-3 treated cells. The analysis of cell culture supernatants by proteome profiler indicates that the induction of reverse signaling results in notable alterations of secreted cytokine levels.

Conclusions: In some pathologic conditions the strict regulation of the TNF is disturbed which results in the pathogenesis of autoimmune diseases. In our days, the application of monoclonal anti-TNF antibodies became the mainstream of biologic therapies, however little is known about their exact mechanism of action. Our results suggest that TNF blocking antibodies induce reverse signaling under inflammatory conditions in the most prominent cell types of the innate and adaptive immune system. The induction of reverse signaling has role in the beneficial effects of TNF neutralizing antibodies by interfering with cytokine production.

Abstract témája: elméleti; lehetőség szerint szóbeli előadás