MASTER PROJECT OFFER:
Specific Cytokine-Degrading Enzymes:
A Novel Way of Immunoregulation Influenced by Nutrients

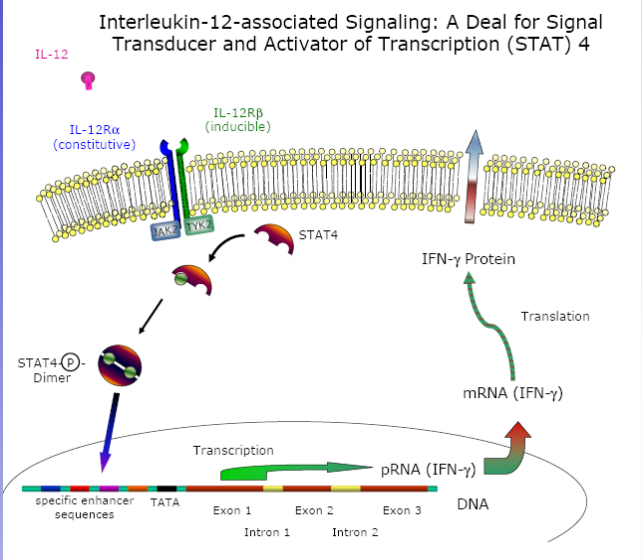
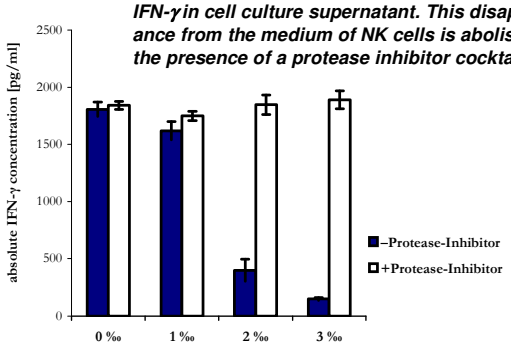


Fig. 1:
Regulation of interleukin-12 (IL-12)-induced IFN-γ production. We know already that none of these factors is responsible for disappearance of IFN-γ from the supernatant of stimulated immuno-competent cells (NK) if treated with ethanol.

Background:

- **Studies on immuno-regulation mostly concentrate on the expression of single factors of immune mediators (cytokines and other small molecules). Of course, cytokines, which are very potent regulators of immune response, remain in the system where they are produced (i. e. in the body).**
- **Seemingly, the immunocompetent cells are also capable to secrete enzymes (proteases) that are specific for the degradation of specific cytokines only.**
- **One of the most important cytokines regulating the immune response – and hereby the resistance to pathogens – is interferon gamma (IFN-γ).**

Fig. 2:
Already at 0.3 %, ethanol leads to disappearance of IFN-γ in cell culture supernatant. This disappearance from the medium of NK cells is abolished in the presence of a protease inhibitor cocktail.



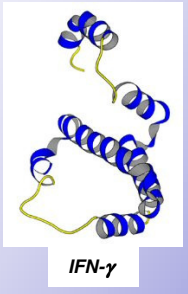
What we know:

1. **After treatment of stimulated immunocompetent cells (NK-cells) with some nutrients (e.g. alcohol), IFN-γ (but no other cytokines) is vanishing completely from the medium of these cells.**
2. **This disappearance is NOT linked to any changes of intracellular signaling leading to IFN-γ production (such as receptor density, transcription factor activation, binding of transcription factors to promoting sequences, amount of mRNA for IFN-γ being produced, Figure 1).**
3. **The factor responsible for disappearance of IFN-γ is secreted into the medium and is not associated with the cells.**
4. **Application of a protease inhibitor abolishes the ethanol-induced disappearance of IFN-γ (Fig. 2).**

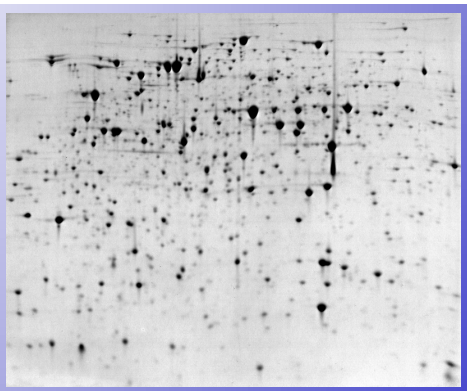
=> Most likely, IFN-γ is removed by proteolytic cleavage, but: by which protease?

Methods:

1. Cell culture
2. Analysis/isolation of specific immune cells from human blood (Flow-associated cell sorting, FACS)
3. Protein isolation
4. Enzyme incubation assays
5. 1-D/2-D gel electrophoresis with novel detection techniques, evtl. combined with mass spectrometry
6. ELISA



IFN-γ



Aim: To identify the protease secreted by NK cells being responsible for cleavage of IFN-γ.

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