Summary

In the context of viral infection, specialized cells of the immune system are able to release interferon alpha (IFN-α) upon contact with virus or viral components. Due to the activation of antiviral mechanisms IFN-α plays a key role in innate defence against viral infections. Moreover, this cytokine is characterized by a broad spectrum of immune modulatory effects that contribute to basic alignment and development of the adaptive immune system. Essential aspects of the IFN-α induction by viruses are still not clarified. Therefore the aim of the study was to gain insights into molecular mechanisms of the IFN-α induction process. Non-infectious induction pathways by viral components were the main focus of the investigation. Analysis of viral IFN-α induction mechanisms was performed either with mononuclear cells from the peripheral blood (PBMC), with myeloid dendritic cells (mDC) or with plasmacytoid dendritic cells (pDC). The pDC as main producers of IFN-α and mDC were isolated from PBMC by magnetic cell sorting.

Additionally not only the amount of released IFN-α was determinated, but also transcriptional activation of IFN-α genes was measured to estimate the quality and quantity of the IFN-α response. To enable the determination of relative amounts of some highly homologous IFN-α subtype transcripts, different quantitative real-time PCR-assays were established during this work. Due to the highly sensitive method, it was possible to measure a low level of IFN-α transcription in unstimulated cells, to detect the expression of different IFN-α subtypes shortly after stimulation and to track the course of the IFN-α response. Although the composition of the IFN-α subtype profile remained constant, irrespective of the kind of viral inducer, significant differences in the course and the strength of IFN-α transcription between single virus preparations were detectable.

The amount of released IFN-α not only depended on the kind of inducer but could additionally be influenced by modulatory factors. One of these influencing factors was IFN-α itself. It was able to accelerate the IFN-α response after cellular stimulation in an autocrine manner. Depending on the viral stimulus the autologous blood plasma, representing the natural environment of PBMC, also significantly influenced the amount of released IFN-α.

Without having to be infected themselves, IFN-α producing cells are able to secrete IFN-α after interaction with envelope proteins of some viruses. It should be investigated in this work, if this was also true for the Respiratory syncytial virus (RSV), that is a pathogen for humans. IFN-α production could be induced in co-cultures of PBMC and fixed, RSV-infected epithelial cells, presenting proteins of the virus on their cell surfaces. Potential remaining infectious viral particles...
were not detected. These results indicated that detection of RSV-proteins alone is an adequate stimulus for the activation of IFN-α transcription.

Molecular requirements of an IFN-α induction by the interaction of viral envelope proteins with surface structures on IFN-α producing cells are not fully understood. Functional involvement of glycostructures from viral proteins in triggering an IFN-α response could be demonstrated by using monosaccharids in competition experiments, chemical modification of sugar side chains and by reduction of cellular glycostructures. Besides this non-infectious IFN-α induction by envelope glycoproteins, the relevance of double stranded ribonucleic acid (dsRNA), an intermediate of viral replication, as an IFN-α inducer could be demonstrated. To achieve this, dsRNA-fragments derived from genomic sequences of the Newcastle disease virus (NDV) were generated in vitro and extracellular administrated to the cells. Functional comparison with a synthetic dsRNA-analogue showed a superior IFN-α induction capacity when using the in vitro produced dsRNA-fragments due to the fact that only these molecules were able to activate pDC. Complexation of dsRNA with a transfection reagent led to an increase of IFN-α induction efficiency. Involvement of toll-like immune receptors (TLR) for the dsRNA mediated IFN-α induction in pDC could not be verified.

Innate immune system possesses multiple recognition strategies to detect viral infections at an early state. Beside cellular infection, especially the recognition of non-infectious viral components like glycoproteins of viral envelopes or intra- and extracellular dsRNA plays an important role for IFN-α induction. The strength and course of an IFN-α response not only depend on the kind of inducer but are also subjected to modulatory influences of the extracellular environment. These complex and redundant IFN-α induction mechanisms allow the immune system to establish a “defence line” against a broad spectrum of viruses rapidly and effectively.