Summary

The inflammatory process

The entire host reaction to pathophysiological stimuli, including bacterial infections, parasite infestation or exposure to irritant environmental substances and conditions, is named inflammation. Many distinct inflammatory pathways exist, each of them involving a different set of cells and mediators. Individual events in course of the inflammatory process are controlled by cytokines and other small regulatory molecules which in this context are named inflammatory mediators. A given mediator may exert its effect directly or indirectly by regulating the activity of other mediators. This complicated interplay of the network mediators gives rise to an integrated response. Although the mechanisms constituting inflammation evolved to eliminate injurious substances or limit their spreading through the organism, they may be the cause of excessive damage in inflamed tissue when injury is severe or when they become misregulated. In the course of sepsis, it is not the inducing pathogens, but the excessive immune inflammatory responses that are responsible for severe health problems. Allergic reactions are examples of excessive immune inflammatory response against innocuous substances such as dust, pollen, food or drugs. The same mechanisms of immunological inflammation are partly responsible for pathogenic consequences of autoimmune diseases.

Mast cells and their granules

Mast cells are tissue-dwelling cells that are predominantly located at the interfaces of the organism and the exterior, such as skin, gut mucosal membranes and lung. They are evolutionarily old cells that play multiple roles in many modes of immune response, including innate and antibody-dependent reactions. Ultrastructural analysis of mast cell granules reveals several subtypes such as scroll-containing, crystal-containing, particle-containing and homogeneously electron-dense content-containing granules although the functional importance of this heterogeneity is not clear. The granules were initially considered a storage organelle for the products of a cell; these products could be rapidly released upon appropriate stimulation. Many highly bioactive mediators are found in mast cell granules; apart from the long-known ones such as chymase, tryptase, histamine or heparin an increasing number of cytokines are being discovered: bFGF, SCF, VEGF, IL-4 and TNF, to name just a few.

It is often observed that mast cells secrete bioactive compounds, including cytokines, without full degranulation. This mode of granule cargo release was shown to be mediated by vesicular transport and apparently involves additional regulatory checkpoints. The mechanisms responsible for selective release of granule cargo without the full degranulation remain largely unclear.

TNF intracellular trafficking in mast cells

In order to gain insight into the process of intracellular TNF-alpha trafficking in mast cells we have employed a strategy involving expression of constructs coding for TNF-alpha-EGFP fusion protein transiently expressed in RBL-2H3 rat mast cells (chapter 2). The EGFP fusion protein containing the entire sequence of the transmembrane TNF-alpha accumulated predominantly in cytoplasmic secretory granules. The unequivocal identification of the compartment in which TNF-alpha fusion protein accumulated is supported by colocalization with three independent markers for secretory granules. The kinetics of appearance of the full TNF-EGFP fusion protein in subsequent compartments of transfected mast cells supports the hypothesis that it is sorted into cytoplasmic granules via a ER/Golgi secretory pathway. Additionally, ectopically expressed TNF-EGFP fusion protein is not only sorted to mast cell granules, but also efficiently released. Inhibitor studies confirmed that TNF is sorted with the use of ER/Golgi pathway and that it is retrieved from the secretory pathway at the stage of late Golgi. This retrieval seems to be mediated by an MPR (mannose-6-phosphate receptor) system. Inhibition of N-linked glycosylation by tunicamycin or mutation (N86S) results in abrogation of granular sorting indicating that this process is carbohydrate-dependent and supporting the hypothesis involving MPRs.

In human mast cells, however, carbohydrate-dependent trafficking does not seem to be in operation since the N86 residue is not conserved and deglycosylation studies seem to indicate that TNF is not glycosylated in human mast cells (chapter 3). Analysis of the trafficking patterns of deletion mutants of TNF-EGFP revealed that residues 21-46 are critical for ER entry of TNF and its subsequent sorting. In contrast, majority of the cytoplasmic tail of TNF is expendable for its granular sorting. Surface biotinylation experiments showed that TNF is transiently exposed on the outer membrane and re-endocytosed on its way to the granules and that this process is dependent on the lumenal/extracellular part of the TNF molecule, suggesting an interaction with a receptor or an accessory protein. These important differences in modes of TNF trafficking between rodent and human systems stress potential weaknesses of rodent models when TNF biology in mast cells is considered.

Regulation of IL-4 expression in mast cells by environmental stress

Mast cells play an important regulatory role in the course of inflammatory process. Until recently they were considered the cells that, when activated, will augment the development of inflammatory state. Recent reports, however, indicate that mast cells may also contribute to immune tolerance and the resolution of an inflammatory state. This prompted our research concerning the regulation of IL-4, a potentially anti-inflammatory cytokine expression in mast cells.

In many pathological situations mast cells are exposed to hypoxic condition. Mast cells contribution to inflammation developing in such tissue has been established and attributed, at least in part, to prestored TNF that is released upon reperfusion. However, there is a separate, degranulation independent, phase of cytokine production that involves *de novo* synthesis and release of these mediators. In chapter 4 we

show that in hypoxic conditions IL-4 (and IL-13) expression and release is induced, as opposed to IL-6, TNF and IFN-gamma. This induction, under various experimental conditions, does not correlate with stabilization of HIF-1 alpha, a major transducer of hypoxic signalling. Instead, we show that regulation of IL-4 promoter activity in hypoxia is mediated by Akt/GSK-3 and calcineurin pathways that converge at the level of NFAT, a known regulator of IL-4 and IL-13 promoter activity in mast cells.

Treatment with mercuric ions has long been used as a model system for systemic autoimmune inflammation including arthritis, vasculitis and glomerulonephritis. Such treatment causes polyclonal B cells activation and enhanced IgE and IL-4 production. The involvement of mast cells and mast cell-derived IL-4 in full elicitation of these responses has been suggested. The molecular mechanism underlying mercury-induced IL-4 expression is not known and therefore in chapter 5 we aimed at establishing signal transduction pathways responsible for this induction. We have shown that in murine mast cells Hg activates IL-4 production and this effect is dose-dependent, additive to IgE/antigen stimulation and critically depends on P1 NFAT binding site in IL-4 promoter. Inhibitor and dominant-negative mutant studies demonstrated involvement of calcineurin in the regulation of mercury-induced IL-4 expression; this hypothesis was further reinforced by the *in vitro* assay of calcineurin activity in the presence of mercuric ions.

General conclusions

Mast cells, as discussed above, are cells that in some situations are regulated by unique signal transduction pathways. They also are tissue resident cells that places them close to the interface of the organism and the exterior. This, in conjunction with the fact that mast cells store large amounts of highly bioactive compounds in their granules makes them perfect candidates for initiating immune or non-immune response in case of microbial infection, parasite infestation or an exposure to biophysical stimulus such as UV, hypoxia or environmental pollutions (exemplified by mercuric compounds). It has been demonstrated in several setups that mast cells have the potential of setting the course of the immune response they initiate. Considering the increasing body of evidence that these cell may act in both pro- and anti-inflammatory manner, mast cells should be regarded an important point in immunoregulation and target in therapy of inflammatory diseases and immunodeficiencies.