Chapter 1

General introduction

The inflammatory process

Immune responses are often accompanied by other cellular, physiological and biochemical processes. Many of these are evolutionarily primitive processes that can be induced with or without immune activation. One example is acute injury that induces response in local vasculature resulting in redness, warmth and swelling. Many types of host immune cells can migrate into the affected area forming what is microscopically visible as an infiltrate. Tissue reactions may include activation of complement or kinin cascades; systemic manifestations may include fever, malaise or aching. If injury is severe, fibroblasts and endothelial cells may proliferate locally and form a scar.

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. The outcome of an inflammatory process depends on many factors, including the characteristics of the host and the nature of the stimulus. This outcome may be regarded as beneficial, detrimental or both. 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.

TNF ligands superfamily

TNF is a prototype member of a group of proteins known as TNF ligand superfamily. This group is comprised of at least 18 type II (lumenal N-terminus) transmembrane proteins, including such well-known members as TNF (formerly known as cachectin), LT-α (lymphotoxin-α), Fas ligand (FasL) and CD40 ligand, as well as increasing number of newly discovered members, including APRIL (a proliferation-inducing ligand), TRAIL (TNF-related apoptosis inducing ligand), TWEAK (TNF-like and weak inducer of apoptosis), RANKL (RANK ligand) and BLyS (B lymphocyte stimulator). The biological activities of TNF were first described in the 1960s and 70s with the identification of a macrophage- and lymphocyte-derived factor that caused hemorrhagic necrosis in solid tumors (1). Since then a plethora of biological functions of TNF have been identified, many of them extending beyond the immune system. The functions of

the members of TNF superfamily often overlap, but they also display considerable diversity. In fact many members of the family are not so much involved in immune response, but rather in development and organogenesis (2-5). Not surprisingly, also the receptors for numerous TNF superfamily ligands comprise a superfamily of related genes with distinct and common structure and signal transduction pathways (summarized in Table 1).

TNF SUPERFAMILY			**TNF RECEPTOR SUPERFAMILY**		
Systematic Name	Alternative Names	Cells	Systematic name	Alternative names	Cells
TNFSF1	LT, LTα, TNFB	NK, T, B	TNFRSF1A	TNF-R, TNFAR, TNFR60, TNF-R-I, CD120a, TNF-R55, TNFR1	All cells
			TNFRSF1B	TNFBR, TNFR80, TNF-R75, TNF-R-II, p75, CD120b, TNFR2	E, Immune cells
TNFSF2	TNF, TNFA, DIF	NK, T, B	TNFRSF1A		
			TNFRSF1B		
TNFSF3	LTβ, TNFC, p33	NK, T, B, DC, M	TNFRSF3	TNFCR, TNFR-RP, TNFR2-RP, TNF-R-III, LTBR	NK, T CD4/8+
TNFSF4	OX-40L, gp34, CD252,TXGP1	T, B	TNFRSF4	ACT35, OX40, CD134, TXGP1L	T
TNFSF5	CD40L, TRAP, gp39, hCD40L, CD154, HIGM1, IMD3	T, B	TNFRSF5	p50, Bp50, CD40	B
TNFSF6	FasL, CD178, APT1LG1, FASLG	T*	TNFRSF6	CD95, APO-1, FAS1, APT1, FAS	Nucl. Cells
			TNFRSF6B	DcR3, DCR3, TR6, M68	Lung, colon
TNFSF7	CD70, CD27LG, CD27L	NK, T, B	TNFRSF7	S152, Tp55, CD27	
TNFSF8	CD153, CD30LG	T, Mo	TNFRSF8	CD30, D1S166E, Kl-1	
TNFSF9	4-1BB-L	B, DC, M	TNFRSF9	CD137, 4-1BB, ILA	T*, Mo, NK
TNFSF10	TRAIL, Apo-2L, TL2, CD253	Lc, DC	TNFRSF10A	DR4, Apo2, TRAILR-1, CD261	Most cells
			TNFRSF10B	DR5, KILLER, TRICK2A, TRAIL-R2, TRICKB, CD262	Most cells
			TNFRSF10C	DcR1, TRAILR3, LIT, TRID, CD263	Most cells
			TNFRSF10D	DcR2, TRUNDD, TRAILR4, CD264	Most cells
TNFSF11	TRANCE, RANKL, OPGL, ODF, CD254	T*, Ob	TNFRSF11A	RANK, CD265	preOc
			TNFRSF11B	OCIF, TR1, OPG	preOc, E
TNFSF12	TWEAK, DR3LG, APO3L	Mo	TNFRSF12	DR3, TRAMP, WSL-1, LARD, WSL-LR, DDR3, TR3, APO-3	T*
			TNFRSF12A	FN14, TweakR, CD266	E, F

TNFSF13	APRIL, CD256	SLO	TNFRSF13B	TACI, CD267	T, B
TNFS-F13B	BAFF, THANK, BLYS, TALL-1, TALL1, CD257	T, DC, M, Mo	TNFRSF13C	BAFFR, CD268	B
TNFSF14	LIGHT, LTg, HVEM-L, CD258	T, DC, Mo, Gr	TNFRSF14	HVEM, ATAR, TR2, LIGHTR, HVEA	T, B, Mo
TNFSF15	TL1, VEGI	E			
			TNFRSF16	NGFR, CD271	
			TNFRSF17	BCM, CD269, BCMA	
TNFSF18	AITRL, TL6, hGITRL	E, T*	TNFRSF18	AITR, GITR	E, T*
			TNFRSF19	TAJ-alpha, TROY, TAJ, TRADE	Ep, ES, Hair, Brain
			TNFRSF19L	RELT	LyT
			TNFRSF21	DR6	T
			TNFRSF27	XEDAR, EDA-A2R, EDAA2R, EDA2R	Ec

Table 1 Members of TNF ligand and receptor superfamilies and their occurrence. T-T cells (*-activated), B-B cells, Lc-lymphocytes, DC-dendritic cells, NK-NK cells, M-macrophages, Mo-monocytes, Ob-osteoblasts, preOc-osteoclast precursors, SLO-secondary lymphoid organs, Gr-granulocytes, E-endothelial cells, F-fibroblasts. Based on HUGO Gene Nomenclature Committee recommendations at http://www.gene.ucl.ac.uk/nomenclature/

The diversity of functions of TNF ligand superfamily members has only begun to be appreciated, and already there is general recognition that they control and regulate inflammatory and immune responses. For instance lymphotoxin-α, RANKL and TNF are critical for the development of peripheral lymphoid organs (6). The differentiation of several lymphocyte and myelocyte cell lineages is also dependent on regulated and timed release of TNF superfamily ligands, such as BLyS (7), CD40 ligand (8), 4-1BB ligand (9), OX40 ligand (10) or CD27 ligand (11). Equally important is the role that several ligands of the superfamily, including TNF, FasL and TRAIL play in cytotoxic activity of effector cells or the removal, via activation-induced apoptosis, of lymphocyte populations and homeostasis restoration (12-14). Some of the ligands, notably lymphotoxins, TNF and LIGHT, provide communication between immune cells and the surrounding stromal and parenchymal cells, creating the signalling network that is necessary for innate and adaptive immune response (15).

Involvement of TNF ligand superfamily members in many disorders, especially those connected to inflammation has been shown. Rheumatoid arthritis and inflammatory bowel disease are caused by excessive local production of TNF and three specific TNF inhibitors (etanercept, infliximab and adalimumab) are used in therapy of RA. Misregulated TNF expression has also been implicated in pathogenesis of a number of other chronic and acute inflammatory diseases, such as septic shock, meningococcemia, adult-respiratory distress syndrome, otitis media, hepatitis B and C infection, Reyes' syndrome, and cerebral malaria, among others. Several hereditary diseases of the immune system are also associated with mutations in genes or receptors of TNF superfamily, including hyper IgM syndrome (CD40L) (16),

autoimmune lymphoproliferative syndrome (FasL/FAS) (17), and the TNF receptor associated periodic fever syndrome (TNF) (18). Mutations in ED1 or EDAR lead to severe impairment of skin, hair and teeth development associated with ectodermal dysplasia syndrome (19).

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. Mast cells derive their name from the original name given by Paul Ehrlich, *Mastzellen* (well-fed cells) reflecting the fact that a mature mast cell contains large number of cytoplasmic granules. These granules are specialized organelles, found primarily in granulocytes, which possess the unique capability of rapid release. 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. Many granules contain a mixture of these patterns. The granules were initially considered a storage organelle for the products of a cell; these products could be rapidly released upon appropriate stimulation. Initial studies of human mast cell granule composition by enzyme-affinity labelling and ultrastructural immunocytochemical techniques allowed for identification of proteases chymase and tryptase (20), a proinflammatory biogenic amine histamine (21) and the proteoglycan heparin (22). Subsequently, new techniques allowed for establishing granular localization of many more mediators, among them several cytokines such as bFGF (23), SCF (24), VEGF (25), IL-4 (26) and TNF (27). The presence of cytokines in granules adds a new dimension to a role of mast cells in cytokine biology. Perhaps the best example of novel mechanisms in cytokine biology is the role of mast cell granule-derived TNF. This pool of the cytokine plays critical role in host defence against bacterial infections (28, 29) and its lack results in drastically reduced neutrophil influx and significantly increased mortality of experimental animals. This unique capability of fast releasing of a considerable amount of preformed cytokines might enable mast cells to influence the course of the immune processes being initiated, directing it towards inflammatory or allergic response, depending on the profile of cytokines released.

It is often observed that mast cells secrete bioactive compounds, including cytokines, without full degranulation (30, 31). This process is interchangeably named "differential" or "selective" release; ultrastructural analysis of the process resulted with a term "piecemeal degranulation" as opposed to "anaphylactic degranulation" meaning full release of the granules. This mode of granule cargo release was shown to be mediated by vesicular transport (32) and apparently involves additional regulatory checkpoints. On the functional level this has been demonstrated by differential sensitivity of degranulation and TNF release to a H1 histamine inhibitor azelastine (33). The mechanisms responsible for selective release of granule cargo without the full degranulation remain largely unclear.

Another interesting aspect of granule biology concerns its potential role in protein biosynthesis. It has been reported that in mast cells undergoing piecemeal degranulation or recovering from anaphylactic degranulation, when the cargo rebuilding process requires high protein synthesis, many ribosomes are observed in perigranular space, on the surface and in the lumen of granules (34). In addition mRNA has been detected in the lumen of granules (35). The accumulating evidence of the synthesizing machinery in secretory granules may suggest more complex role for these organelles than previously recognized, possibly including protein synthesis and sequestration of excessive mRNA.

Mast cells in immune response

General remarks

Mast cells are an important element of both innate and acquired immunity. They derive from a distinct precursor in bone marrow (36, 37) and differentiate under the influence of SCF (38). They express numerous receptors that, when stimulated, may induce production of plethora of mediators. These receptors include IgE and IgG, complement, IL-1, TNF and several Toll-like receptors, to name just a few most important (39). Upon stimulation mast cells undergo activation and, in some cases, degranulation, releasing and synthesizing highly bioactive, proinflammatory, vasodilative, chemotactic, and cytotoxic substances. These cells are crucial for the function of several biologically and clinically important mechanisms of immune response such as allergy, inflammation and, as shown recently, also immune tolerance (40). Some of the more important mediators prestored and synthesized by mast cells and their major patophysiological effects are summarized in Table 2. Mast cells play multiple roles without inducing anaphylactic shock and this requires mechanisms of differential activation; indeed mast cells are rarely seen to degranulate during autoimmune (30) or inflammatory responses (31). The mechanism of selective stimulated release was first observed in case of serotonin and histamine and named "differential" or "selective" release (41). The profile of mediators released depends on the type of mast cell and the stimulus which clearly shows there is a complicated network of signalling pathways regulating secretion; several examples of differential release and their physiological importance are presented in Table 3.

Innate immunity

Looking from a broader perspective of host defence, mast cells play several roles in innate and acquired immunity. Although there is some evidence of mast cells exhibiting directly germicidal activity by phagocytosis (42) or bactericidal peptide release (43), several lines of evidence suggest that the most important way by which mast cells contribute to innate immune response is initiation and regulation of the magnitude of leukocyte infiltration into the site of inflammation. It has been demonstrated using mast-cell deficient mice that at least TNF and leukotrienes are important factors in neutrophil recruitment towards sites of bacterial infection and mast cells deficiency correlates with much worse prognosis in experimentally infected mice (28, 44). Experiments in other model systems have shown that secretion of TNF (45) and leukotrienes (46) in acute phase of inflammatory process may also

promote influx of leukocytes other than neutrophils, such as T cells or macrophages which are typical for chronic inflammatory state. Such leukocytes can then initiate and maintain features characteristic of chronic inflammatory state.

MEDIATOR	MAJOR PATHOPHYSIOLOGIC EFFECT
Prestored	
Biogenic amines	
Histamine	Vasodilation, angiogenesis, mitogenesis, suppressor T-cell activation
5-HT	Leukocyte regulation, vasoconstriction, pain
Chemokines (IL-8, MCP-1, MCP-3, MCP-4, RANTES)	Chemoattraction and tissue infiltration of leukocytes
Enzymes	
Chymase	Tissue damage, pain, angiotensin II synthesis
Tryptase	Activation of PAR, inflammation, pain, tissue damage, degradation of antigens and peptides
Kinogenases	Synthesis of kinins, pain
Nitric oxide synthase	NO production
Polypeptides	
CRH	Inflammation, vasodilation, mast cell VEGF release
Endothelin	Sepsis
Kinins	Inflammation, pain, vasodilation, mast cell trigger
Somatostatin (SRIF)	Anti-inflammatory (?), mast cell trigger
VEGF	Neovascularization, vasodilation
Proteoglycans	
Chondroitin sulfate	Connective tissue component, anti-inflammatory, mast cell inhibitor
De novo synthesized	
Cytokines	
IL-1, -3, -4, -5, -6, -9, -10, -13, -16, IFN-γ, MIF, TNF	Multiple roles
Growth Factors	
SCF, GM-CSF, GnRH-I β-FGF, NGF, VEGF	Growth of a variety of cells, mast cell proliferation
Phospholipid metabolites	
LTB4	Leukocyte chemotaxis
LTC4	Vasoconstriction, pain
PAF	Platelet activation, vasodilation, inflammation
PGD2	Bronchoconstriction, pain
NO	Vasodilation, neuromodulation

Table 2 Selected mast cell mediators and their major pathophysiologic activities. β-FGF, fibroblast growth factor; GnRH, gonadotropin-releasing hormone-I; LTB4, leukotriene B4; MIF, macrophage inflammatory factor; NO, nitric oxide; PAF, platelet-activating factor; PGD2, prostaglandin D2; SRIF, somatomedin release inhibitory factor, somatostatin; TGF-β, transforming growth factor-β. Adapted from (88)

Another function mast cell play in innate response is limiting the toxicity of certain substances generated by the host which have adverse effects when present in high concentrations. An example of such activity is degradation of endothelin-1, a peptide that is involved in sepsis (47, 48), by the proteases released from mast cell granules (47). Mast cells are also capable of releasing mediators influencing (positively or negatively) the transition from innate to acquired immunity (38). It has been

reported that mast-cell derived TNF plays a role in draining lymph node hypertrophy and T cell recruitment to these nodes in a model of *E. coli* infection in mouse (45). In a model of FITC challenge in the skin of mast cell-deficient mice dendritic cell migration to lymph nodes was significantly decreased 24 hours after the challenge, but no defect in migration was detectable 48 hours after the challenge (49). This indicates that while there is a mast cell-dependent component in the development of adaptive immune response, the mechanisms are likely to be more redundant as compared to innate response. It is of note, however, that in certain circumstances mast cells can have suppressive effect on antigen sensitization and this effect seems to be attributable, at least in part, to histamine (50-52).

Stimulus	Mast cell type	Mediators released	Mediators not released	Physiological importance
Endogenous				
CD8 ligands	RPMC	TNF, NO	H	T cell interactions
Endothelin-1-3	RMMC	TNF, IL-12↑	IL-4, IL-10, IL-13↓	Th1 immunity
IL-1	hCBMC	IL-6, IL-8, TNF	H, tryptase	Inflammation
IL-12	RPMC	IFN-γ	H	Th1 immunity
Monomeric IgE	BMMC	IL-6	H, LTC4	Mast cell survival
PGE2	hCBMC	MCP-1	No degranulation	Angiogenesis
Suboptimal FcεRI stimulation	BMMC	MCP-1, low H	IL-10, H	Chemokines >> Cytokines
Exogenous				
Cholera toxin	RPMC	IL-6	TNF	Inflammation
CpG DNA	BMMC	TNF, IL-6	H, IL-4, IL-12, GM-CSF, IFN-γ	Host response to bacteria
H. pylori VacA toxin	BMMC	IL-6, IL-8, TNF	H	Gastric injury
LPS	RPMC	IL-6	H	Bacterial infection

Table 3 Selected examples of selective release of mediators from mast cells. Adapted from (88)

IgE-associated adaptive responses

Another aspect of mast cells contribution to immune response is their involvement in adaptive immunity. Originally, these activities were connected to antigen-specific IgE that, when bound to FcεRI and crosslinked by an antigen, activate multiple pathways in the mast cell. Recent findings demonstrate, that IgE at high concentrations have more than just passively sensitizing activity. Some antibodies are able to elicit full response in the absence of antigen while other only upregulate FcεRI and enhance mast cells survival (53). This survival enhancement is mediated by autocrine IL-3 stimulation and activation of Bcl-xL/Bcl-2 (54). The extent of mast cell activation in absence of antigen depends on a particular antibody, although the molecular determinants of this anti-apoptotic activities are not defined (55). Additionally, the increased survival after FcεRI stimulation differs between mast cell subpopulations (56).

It's been widely accepted that mast cells contribute significantly to acute inflammatory reactions to antigens/allergens against which the host bears antibodies of the IgE class. Mast cells are responsible for virtually all of the increased

vascular permeability and tissue swelling during IgE-dependent passive cutaneous anaphylactic response (57). If the stimulation is of more persistent or more severe nature, acute response may undergo transition into late-phase reaction (LPR) which, except for the time scale ranging from few to several hours from initial antigen challenge, is characterized by recruitment of leukocytes to the site of inflammation. Apart from the role played by mast cells in recruitment of other immune cells to the site of inflammation (58), they can also influence the phenotype and function of T cells and other leukocytes; these regulatory activities may be attributed to both soluble mediators, such as TNF, and cell-to-cell interactions involving costimulatory molecules, e.g. OX40 ligand (59, 60). Many of the clinical symptoms of LPR are thought to result from the actions of the cells recruited to these sites rather than from direct activity of the mediators released by mast cells during the acute phase (30, 61). It has to be noted, however, that not in all cases LPR are preceded by the acute allergic reactions *in situ* (62). Such sequence of events corresponds well with many reports concerning differential release of mast cell mediators, an example being Bcl10-MALT-1 adapter complex-dependent specific release of IL-6 and TNF without degranulation following FcεRI stimulation (63). This is consistent with the fact that of many mast cell products, TNF has been most clearly implicated in leukocyte recruitment and the development of other LPR features (58, 64). Mast cells are also capable of providing mediators and direct intercellular contact-mediated functions at sites of long-term, IgE-associated chronic allergic inflammation characteristic for disorders like asthma, allergic rhinitis and atopic dermatitis (60, 65, 66). It has been shown recently, that optimal expression of ovoalbumin-induced asthma in mice is mast cell-dependent (67). In this model many features of the disorder were markedly reduced in mice whose mast cells lacked FcRγ and thus could not be activated by IgE or IgG1. Interestingly, certain features of this disorder were found mast cell-dependent, but FcRγ-independent. In this view, a wide range of innate and IgE-associated immune responses appears to represent a situation in which mast cells activity, depending on particular circumstances, may be either beneficial or detrimental to the host.

IgE-independent responses

Apart from IgE-dependent responses, mast cells have been implicated in pathogenesis of several autoimmune diseases (30), including multiple sclerosis (68) and rheumatoid arthritis (69) in humans and experimental autoimmune encephalomyelitis (EAE) (70) and IgG1 antibody-dependent autoimmune arthritis in mice (71). Moreover, under some experimental conditions mast cells are necessary for complete elicitation of inflammation associated with hapten-induced contact hypersensitivity (CHS) (72) or asthma (73) and inflammatory bowel disease (74). In addition, an effector role for mast cells has been proposed in murine models of fibrosis associated with chronic inflammatory states e.g. scleroderma. In a model of chronic graft versus host disease (CGVHD) based on injection of spleen cells from B10.D2 mice into irradiated BALB/c recipients, mast cells present in affected skin were found depleted of cytoplasmic granules which leaded to hypothesis defining mast cells activation as an important factor for fibrosis that is a major feature of

both CGVHD and scleroderma (75). Injecting bleomycin into skin of wild type and mast cell-deficient mice for 1 week elicits dermal sclerosis only in the former; after 4 weeks however this state is equally developed in both populations, indicating that while mast cells are not necessary for the development of skin fibrosis they may accelerate its development (76).

Mast cell-T cell interactions

As mentioned above mast cells can exert their activities by being effector cells or response initiators. There is, however, yet another important way for these cells to shape immune response: regulation of T cells function. These interactions may be of both direct and indirect nature. Due to relatively low numbers of mast cells in secondary lymphoid organs their presence was not considered important for T cell differentiation. However, under inflammatory conditions mast cells can additionally migrate to spleen and lymph nodes and modulate the immune response (77, 78). The proximity of mast cells and naïve T cells in these organs allows for cytokines released by the former influence priming of the latter for polarized differentiation. In some settings mast cell-derived IL-4 may directly skew Th2 responses. Mast cells are also good sources of TGF-β and IL-6, cytokines known to favour Th17 development. The expression of surface proteins that display stimulatory or inhibitory activity towards T cells may also be relevant in these settings (38). Under some conditions, mast cells express the costimulatory molecules CD80 (B7-1) and CD86 (B7-2) and the adhesion molecules CD54 (ICAM-1) and CD18 (β2 integrin chain of leukocyte function-associated antigen-1), all of which are involved in T-cell activation. More recently expression of members of the TNF/TNF receptor superfamily, including OX40L, CD153, Fas, CD137, and glucocorticoid-induced TNF receptor, by mast cells and the importance of these molecules for T cell activation has been demonstrated (60). Although the range of mediators expressed by mast cells suggests a broad ability of mast cells to direct T cell differentiation, only limited evidence for such activity was demonstrated in *in vitro* co-culture system (59, 60, 79). The development of *in vivo* models to evaluate the importance of these interactions will be a major progress in mast cell and T cell biology.

Another feature of mast cell enabling them to influence T cell fate is their ability to act as antigen-presenting cells. They express both MHC-I and MHC-II and have been shown to present antigen to T cells in an *in vitro* system (42, 80). Mast cell can also influence trafficking of T cells into the site of infection or inflammation. Leukotriene B4, that is essential chemotactic factor for CD4+ Th1 and Th2 cells and for CD8+ effector T cells, is produced by mast cells upon activation (46). Released mediators, including TNF, upregulate expression of endothelial adhesion markers such as ICAM-1 and vascular adhesion molecule-1 (81) and vasoactive amines, such as histamine, increase endothelial permeability as well as P selectin expression on endothelial cells, allowing for an influx of activated T cells.

Mast cells in limitation of inflammation

Although there is a solid body of evidence that mast cells exert predominantly proinflammatory activities, there are a few reports stating otherwise. Examination of biological activity of mast cell mediators, indicates that some of them, including TGF-β (82), IL-4 (83), IL-10 (84) and histamine (85) have potentially anti-inflammatory activity, although until now very few studies have demonstrated this *in vivo*. The first report of such activity of mast cells in knockin mouse concerned UV-induced suppression of contact hypersensivity to TNCB that was, at least partly, mediated by histamine (86). It has also been demonstrated, that mice that were bitten by a mosquito display lowered antigen-specific T cell responses in the model of delayed hypersensitivity to OVA and that this phenomenon requires mast cells at the site of the bite (87). The mechanism of this regulatory activity remains unknown. The results of yet another study show, that mast cells are necessary for peripheral tolerance in skin allografts (40). In tolerant mice considerable increase of mast cell-specific transcripts and number of mast cells was observed. This increase correlates with the influx to the graft of IL-9-producing CD4+Foxp3+ T cells. Mast cell-deficient mice cannot be tolerized and experience rapid graft rejection, which can be prevented by local skin reconstitution with mast cells. IL-9 released by Tregs is the major mediator of mast cells recruitment and activation in the dermis of these tolerant grafts. Mast cells may then act by limiting the influx of inflammatory T cells or cooperating with dermal Tregs. Unexpected as it sounds, mast cells do contain TGF-β that is a major Tregs inducing factor. In conclusion, mast cell activators may yield pro- or anti-inflammatory responses; under IL-9 stimulation mast cells seem to rather suppress than induce the inflammatory processes.

Conclusions on mast cell role in regulation of inflammatory process

The regulatory activities of mast cells related to induction and resolution of the inflammatory process seem to favour defining them as proinflammatory cells. While in many settings such activity is detrimental to the host it has to be remembered, that in the view of host defence inflammatory process is often crucial to restoring homeostasis. Additionally, there is gradually accumulating evidence, that in some settings mast cells may exhibit anti-inflammatory activity which makes them potentially attractive therapy target in disorders involving chronic inflammation. As far as the perspective of inventing a chemical compound specifically targeting selected mast cell functions is rather improbable, this kind of precise manipulation seems to be feasible with the use of biologicals. The new track of research concerning mast cells as anti-inflammatory regulatory cells is certainly worth pursuing.

Outline of this thesis

Mast cells as an important regulator of immune response have long been underestimated. There is, however, growing body of evidence indicating, that these cells are crucial for induction and perhaps attenuation of inflammatory response. This thesis focuses on analysis of expression of TNF and IL-4, cytokines regarded pro- and anti-inflammatory, respectively, by mast cells.

Given the unique physiological properties of the pool of TNF that is stored in mast cell granules, we analyzed intracellular trafficking pathways leading to the storage of this cytokine in mast cell secretory compartment. We examined amino acid motifs and posttranslational modifications necessary for proper targeting of TNF. The differences between mouse and human TNFs were analyzed revealing potential weaknesses of murine model when TNF biology is considered (chapters 2 and 3)

Inflammatory processes elicited by non-immune stimuli may take a course that is detrimental to the host. We analyzed potential involvement of mast cells in the regulation of immune response in such situations. Models of hypoxia and heavy metal ions exposure were utilized in order to find out how IL-4, that is a potential inflammation limiting factor, is regulated in mast cells under these conditions (chapters 4 and 5).

References

1. Carswell, E. A., L. J. Old, R. L. Kassel, S. Green, N. Fiore, and B. Williamson. 1975. An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci U S A 72:3666.
2. Griffith, T. S., and D. H. Lynch. 1998. TRAIL: a molecule with multiple receptors and control mechanisms. *Curr Opin Immunol 10:559*.
3. Idriss, H. T., and J. H. Naismith. 2000. TNF alpha and the TNF receptor superfamily: structure-function relationship(s). *Microsc Res Tech 50:184*.
4. Mackay, F., and S. L. Kalled. 2002. TNF ligands and receptors in autoimmunity: an update. *Curr Opin Immunol 14:783*.
5. Schluter, D., and M. Deckert. 2000. The divergent role of tumor necrosis factor receptors in infectious diseases. *Microbes Infect 2:1285*.
6. Ettinger, R., R. Mebius, J. L. Browning, S. A. Michie, S. van Tuijl, G. Kraal, W. van Ewijk, and H. O. McDevitt. 1998. Effects of tumor necrosis factor and lymphotoxin on peripheral lymphoid tissue development. *Int Immunol 10:727*.
7. Miller, J. P., J. E. Stadanlick, and M. P. Cancro. 2006. Space, selection, and surveillance: setting boundaries with BLyS. *J Immunol 176:6405*.
8. Kilmon, M. A., N. J. Wagner, A. L. Garland, L. Lin, K. Aviszus, L. J. Wysocki, and B. J. Vilen. 2007. Macrophages prevent the differentiation of autoreactive B cells by secreting CD40 ligand and IL-6. *Blood*.
9. Kim, Y. J., G. Li, and H. E. Broxmeyer. 2002. 4-1BB ligand stimulation enhances myeloid dendritic cell maturation from human umbilical cord blood CD34+ progenitor cells. *J Hematother Stem Cell Res 11:895*.
10. Ito, T., Y. H. Wang, O. Duramad, S. Hanabuchi, O. A. Perng, M. Gilliet, F. X. Qin, and Y. J. Liu. 2006. OX40 ligand shuts down IL-10-producing regulatory T cells. *Proc Natl Acad Sci U S A 103:13138*.
11. Hendriks, J., Y. Xiao, and J. Borst. 2003. CD27 promotes survival of activated T cells and complements CD28 in generation and establishment of the effector T cell pool. *J Exp Med 198:1369*.
12. Nagane, M., H. J. Huang, and W. K. Cavenee. 2001. The potential of TRAIL for cancer chemotherapy. *Apoptosis 6:191*.
13. Fiers, W., R. Beyaert, E. Boone, S. Cornelis, W. Declercq, E. Decoster, G. Denecker, B. Depuydt, D. De Valck, G. De Wilde, V. Goossens, J. Grooten, G. Haegeman, K. Heyninck, L. Penning, S. Plaisance, K. Vancompernolle, W. Van Criekinge, P. Vandenabeele, W. Vanden Berghe, M. Van de Craen, V. Vandevoorde, and D. Vercammen. 1995. TNF-induced intracellular signaling leading to gene induction or to cytotoxicity by necrosis or by apoptosis. *J Inflamm 47:67*.
14. Screpanti, V., R. P. Wallin, A. Grandien, and H. G. Ljunggren. 2005. Impact of FASL-induced apoptosis in the elimination of tumor cells by NK cells. *Mol Immunol 42:495*.
15. Ware, C. F. 2005. Network communications: lymphotoxins, LIGHT, and TNF. *Annu Rev Immunol 23:787*.
16. Kroczek, R. A., D. Graf, D. Brugnoni, S. Giliani, U. Korthuer, A. Ugazio, G. Senger, H. W. Mages, A. Villa, and L. D. Notarangelo. 1994. Defective expression of CD40 ligand on T cells causes "X-linked immunodeficiency with hyper-IgM (HIGM1)". *Immunol Rev 138:39*.
17. Del-Rey, M., J. Ruiz-Contreras, A. Bosque, S. Calleja, J. Gomez-Rial, E. Roldan, P. Morales, A. Serrano, A. Anel, E. Paz-Artal, and L. M. Allende. 2006. A homozygous Fas ligand gene mutation in a patient causes a new type of autoimmune lymphoproliferative syndrome. *Blood 108:1306*.
18. Rezaei, N. 2006. TNF-receptor-associated periodic syndrome (TRAPS): an autosomal dominant multisystem disorder. *Clin Rheumatol 25:773*.
19. Monreal, A. W., B. M. Ferguson, D. J. Headon, S. L. Street, P. A. Overbeek, and J. Zonana. 1999. Mutations in the human homologue of mouse dl cause autosomal recessive and dominant hypohidrotic ectodermal dysplasia. *Nat Genet 22:366*.
20. Craig, S. S., N. M. Schechter, and L. B. Schwartz. 1988. Ultrastructural analysis of human T and TC mast cells identified by immunoelectron microscopy. *Lab Invest 58:682*.
21. Dvorak, A. M. 1997. Ultrastructural localization of histamine in human basophils and mast cells; changes associated with anaphylactic degranulation and recovery demonstrated with a diamine oxidase-gold probe. *Allergy 52:14*.
22. Craig, S. S., A. M. Irani, D. D. Metcalfe, and L. B. Schwartz. 1993. Ultrastructural localization of heparin to human mast cells of the MCTC and MCT types by labeling with antithrombin III-gold. *Lab Invest 69:552*.
23. Dvorak, A. M., E. S. Morgan, and P. F. Weller. 2001. Ultrastructural immunolocalization of basic fibroblast growth factor to lipid bodies and secretory granules in human mast cells. *Histochem J 33:397*.
24. de Paulis, A., G. Minopoli, E. Arbustini, G. de Crescenzo, F. Dal Piaz, P. Pucci, T. Russo, and G. Marone. 1999. Stem cell factor is localized in, released from, and cleaved by human mast cells. *J Immunol 163:2799*.

25. Grutzkau, A., S. Kruger-Krasagakes, H. Baumeister, C. Schwarz, H. Kogel, P. Welker, U. Lippert, B. M. Henz, and A. Moller. 1998. Synthesis, storage, and release of vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) by human mast cells: implications for the biological significance of VEGF206. *Mol Biol Cell 9:875*.
26. Wilson, S. J., J. K. Shute, S. T. Holgate, P. H. Howarth, and P. Bradding. 2000. Localization of interleukin (IL) -4 but not IL-5 to human mast cell secretory granules by immunoelectron microscopy. *Clin Exp Allergy 30:493*.
27. Beil, W. J., P. F. Weller, M. A. Peppercorn, S. J. Galli, and A. M. Dvorak. 1995. Ultrastructural immunogold localization of subcellular sites of TNF-alpha in colonic Crohn's disease. *J Leukoc Biol 58:284*.
28. Echtenacher, B., D. N. Mannel, and L. Hultner. 1996. Critical protective role of mast cells in a model of acute septic peritonitis. *Nature 381:75*.
29. Malaviya, R., T. Ikeda, E. Ross, and S. N. Abraham. 1996. Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-alpha. *Nature 381:77*.
30. Benoist, C., and D. Mathis. 2002. Mast cells in autoimmune disease. *Nature 420:875*.
31. Woolley, D. E. 2003. The mast cell in inflammatory arthritis. *N Engl J Med 348:1709*.
32. Dvorak, A. M. 2005. Piecemeal degranulation of basophils and mast cells is effected by vesicular transport of stored secretory granule contents. *Chem Immunol Allergy 85:135*.
33. Hide, I., N. Toriu, T. Nuibe, A. Inoue, M. Hide, S. Yamamoto, and Y. Nakata. 1997. Suppression of TNF-alpha secretion by azelastine in a rat mast (RBL-2H3) cell line: evidence for differential regulation of TNF-alpha release, transcription, and degranulation. *J Immunol 159:2932*.
34. Dvorak, A. M. 2005. Mast cell secretory granules and lipid bodies contain the necessary machinery important for the in situ synthesis of proteins. *Chem Immunol Allergy 85:252*.
35. Dvorak, A. M., and E. S. Morgan. 2000. Ultrastructural cytochemical, immunocytochemical and in situ hybridization methods with polyuridine probes detect mRNA in human mast cell granules. *Histochem J 32:423*.
36. Chen, C. C., M. A. Grimbaldeston, M. Tsai, I. L. Weissman, and S. J. Galli. 2005. Identification of mast cell progenitors in adult mice. *Proc Natl Acad Sci U S A 102:11408*.
37. Rodewald, H. R., M. Dessing, A. M. Dvorak, and S. J. Galli. 1996. Identification of a committed precursor for the mast cell lineage. *Science 271:818*.
38. Galli, S. J., S. Nakae, and M. Tsai. 2005. Mast cells in the development of adaptive immune responses. *Nat Immunol 6:135*.
39. Valent, P., G. H. Schernthaner, W. R. Sperr, G. Fritsch, H. Agis, M. Willheim, H. J. Buhring, A. Orfao, and L. Escribano. 2001. Variable expression of activation-linked surface antigens on human mast cells in health and disease. *Immunol Rev 179:74*.
40. Lu, L. F., E. F. Lind, D. C. Gondek, K. A. Bennett, M. W. Gleeson, K. Pino-Lagos, Z. A. Scott, A. J. Coyle, J. L. Reed, J. Van Snick, T. B. Strom, X. X. Zheng, and R. J. Noelle. 2006. Mast cells are essential intermediaries in regulatory T-cell tolerance. *Nature 442:997*.
41. Theoharides, T. C., P. K. Bondy, N. D. Tsakalos, and P. W. Askenase. 1982. Differential release of serotonin and histamine from mast cells. *Nature 297:229*.
42. Malaviya, R., N. J. Twesten, E. A. Ross, S. N. Abraham, and J. D. Pfeifer. 1996. Mast cells process bacterial Ags through a phagocytic route for class I MHC presentation to T cells. *J Immunol 156:1490*.
43. Di Nardo, A., A. Vitiello, and R. L. Gallo. 2003. Cutting edge: mast cell antimicrobial activity is mediated by expression of cathelicidin antimicrobial peptide. *J Immunol 170:2274*.
44. Malaviya, R., and S. N. Abraham. 2000. Role of mast cell leukotrienes in neutrophil recruitment and bacterial clearance in infectious peritonitis. *J Leukoc Biol 67:841*.
45. McLachlan, J. B., J. P. Hart, S. V. Pizzo, C. P. Shelburne, H. F. Staats, M. D. Gunn, and S. N. Abraham. 2003. Mast cell-derived tumor necrosis factor induces hypertrophy of draining lymph nodes during infection. *Nat Immunol 4:1199*.
46. Ott, V. L., J. C. Cambier, J. Kappler, P. Marrack, and B. J. Swanson. 2003. Mast cell-dependent migration of effector CD8+ T cells through production of leukotriene B4. *Nat Immunol 4:974*.
47. Maurer, M., J. Wedemeyer, M. Metz, A. M. Piliponsky, K. Weller, D. Chatterjea, D. E. Clouthier, M. M. Yanagisawa, M. Tsai, and S. J. Galli. 2004. Mast cells promote homeostasis by limiting endothelin-1-induced toxicity. *Nature 432:512*.
48. Wanecek, M., E. Weitzberg, A. Rudehill, and A. Oldner. 2000. The endothelin system in septic and endotoxin shock. *Eur J Pharmacol 407:1*.
49. Suto, H., S. Nakae, M. Kakurai, J. D. Sedgwick, M. Tsai, and S. J. Galli. 2006. Mast cell-associated TNF promotes dendritic cell migration. *J Immunol 176:4102*.
50. Caron, G., Y. Delneste, E. Roelandts, C. Duez, J. Y. Bonnefoy, J. Pestel, and P. Jeannin. 2001. Histamine polarizes human dendritic cells into Th2 cell-promoting effector dendritic cells. *J Immunol 167:3682*.
51. Cumberbatch, M., R. J. Dearman, C. E. Griffiths, and I. Kimber. 2000. Langerhans cell migration. *Clin Exp Dermatol 25:413*.

52. Jutel, M., K. Blaser, and C. A. Akdis. 2005. Histamine in allergic inflammation and immune modulation. *Int Arch Allergy Immunol 137:82*.
53. Kawakami, T., and S. J. Galli. 2002. Regulation of mast-cell and basophil function and survival by IgE. *Nat Rev Immunol 2:773*.
54. Kohno, M., S. Yamasaki, V. L. Tybulewicz, and T. Saito. 2005. Rapid and large amount of autocrine IL-3 production is responsible for mast cell survival by IgE in the absence of antigen. *Blood 105:2059*.
55. Kawakami, T., and J. Kitaura. 2005. Mast cell survival and activation by IgE in the absence of antigen: a consideration of the biologic mechanisms and relevance. *J Immunol 175:4167*.
56. Ekoff, M., A. Strasser, and G. Nilsson. 2007. FcepsilonRI aggregation promotes survival of connective tissue-like mast cells but not mucosal-like mast cells. *J Immunol 178:4177*.
57. Wershil, B. K., Y. A. Mekori, T. Murakami, and S. J. Galli. 1987. 125I-fibrin deposition in IgE-dependent immediate hypersensitivity reactions in mouse skin. Demonstration of the role of mast cells using genetically mast cell-deficient mice locally reconstituted with cultured mast cells. *J Immunol 139:2605*.
58. Wershil, B. K., Z. S. Wang, J. R. Gordon, and S. J. Galli. 1991. Recruitment of neutrophils during IgE-dependent cutaneous late phase reactions in the mouse is mast cell-dependent. Partial inhibition of the reaction with antiserum against tumor necrosis factor-alpha. *J Clin Invest 87:446*.
59. Kashiwakura, J., H. Yokoi, H. Saito, and Y. Okayama. 2004. T cell proliferation by direct cross-talk between OX40 ligand on human mast cells and OX40 on human T cells: comparison of gene expression profiles between human tonsillar and lung-cultured mast cells. *J Immunol 173:5247*.
60. Nakae, S., H. Suto, M. Iikura, M. Kakurai, J. D. Sedgwick, M. Tsai, and S. J. Galli. 2006. Mast cells enhance T cell activation: importance of mast cell costimulatory molecules and secreted TNF. *J Immunol 176:2238*.
61. Williams, C. M., and S. J. Galli. 2000. The diverse potential effector and immunoregulatory roles of mast cells in allergic disease. *J Allergy Clin Immunol 105:847*.
62. Kay, A. B. 2001. Allergy and allergic diseases. First of two parts. *N Engl J Med 344:30*.
63. Klemm, S., J. Gutermuth, L. Hultner, T. Sparwasser, H. Behrendt, C. Peschel, T. W. Mak, T. Jakob, and J. Ruland. 2006. The Bcl10-Malt1 complex segregates Fc epsilon RI-mediated nuclear factor kappa B activation and cytokine production from mast cell degranulation. *J Exp Med 203:337*.
64. Furuta, G. T., A. Schmidt-Choudhury, M. Y. Wang, Z. S. Wang, L. Lu, R. I. Furlano, and B. K. Wershil. 1997. Mast cell-dependent tumor necrosis factor alpha production participates in allergic gastric inflammation in mice. *Gastroenterology 113:1560*.
65. Bradding, P., A. F. Walls, and S. T. Holgate. 2006. The role of the mast cell in the pathophysiology of asthma. *J Allergy Clin Immunol 117:1277*.
66. Galli, S. J., J. Kalesnikoff, M. A. Grimbaldeston, A. M. Piliponsky, C. M. Williams, and M. Tsai. 2005. Mast cells as "tunable" effector and immunoregulatory cells: recent advances. *Annu Rev Immunol 23:749*.
67. Yu, M., M. Tsai, S. Y. Tam, C. Jones, J. Zehnder, and S. J. Galli. 2006. Mast cells can promote the development of multiple features of chronic asthma in mice. *J Clin Invest 116:1633*.
68. Pedotti, R., J. J. De Voss, L. Steinman, and S. J. Galli. 2003. Involvement of both 'allergic' and 'autoimmune' mechanisms in EAE, MS and other autoimmune diseases. *Trends Immunol 24:479*.
69. Mican, J. M., and D. D. Metcalfe. 1990. Arthritis and mast cell activation. *J Allergy Clin Immunol 86:677*.
70. Secor, V. H., W. E. Secor, C. A. Gutekunst, and M. A. Brown. 2000. Mast cells are essential for early onset and severe disease in a murine model of multiple sclerosis. *J Exp Med 191:813*.
71. Lee, D. M., D. S. Friend, M. F. Gurish, C. Benoist, D. Mathis, and M. B. Brenner. 2002. Mast cells: a cellular link between autoantibodies and inflammatory arthritis. *Science 297:1689*.
72. Biedermann, T., M. Kneilling, R. Mailhammer, K. Maier, C. A. Sander, G. Kollias, S. L. Kunkel, L. Hultner, and M. Rocken. 2000. Mast cells control neutrophil recruitment during T cell-mediated delayed-type hypersensitivity reactions through tumor necrosis factor and macrophage inflammatory protein 2. *J Exp Med 192:1441*.
73. Kraneveld, A. D., H. P. van der Kleij, M. Kool, A. H. van Houwelingen, A. C. Weitenberg, F. A. Redegeld, and F. P. Nijkamp. 2002. Key role for mast cells in nonatopic asthma. *J Immunol 169:2044*.
74. Rijnierse, A., A. S. Koster, F. P. Nijkamp, and A. D. Kraneveld. 2006. Critical role for mast cells in the pathogenesis of 2,4-dinitrobenzene-induced murine colonic hypersensitivity reaction. *J Immunol 176:4375*.
75. Claman, H. N., K. L. Choi, W. Sujansky, and A. E. Vatter. 1986. Mast cell "disappearance" in chronic murine graft-vs-host disease (GVHD)-ultrastructural demonstration of "phantom mast cells". *J Immunol 137:2009*.
76. Yamamoto, T., Y. Takahashi, S. Takagawa, I. Katayama, and K. Nishioka. 1999. Animal model of sclerotic skin. II. Bleomycin induced scleroderma in genetically mast cell deficient WBB6F1-W/W(V) mice. *J Rheumatol 26:2628*.

77. Tanzola, M. B., M. Robbie-Ryan, C. A. Gutekunst, and M. A. Brown. 2003. Mast cells exert effects outside the central nervous system to influence experimental allergic encephalomyelitis disease course. *J Immunol 171:4385*.
78. Wang, H. W., N. Tedla, A. R. Lloyd, D. Wakefield, and P. H. McNeil. 1998. Mast cell activation and migration to lymph nodes during induction of an immune response in mice. *J Clin Invest 102:1617*.
79. Fujita, T., N. Kambe, T. Uchiyama, and T. Hori. 2006. Type I interferons attenuate T cell activating functions of human mast cells by decreasing TNF-alpha production and OX40 ligand expression while increasing IL-10 production. *J Clin Immunol 26:512*.
80. Fox, C. C., S. D. Jewell, and C. C. Whitacre. 1994. Rat peritoneal mast cells present antigen to a PPD-specific T cell line. *Cell Immunol 158:253*.
81. Meng, H., M. G. Tonnesen, M. J. Marchese, R. A. Clark, W. F. Bahou, and B. L. Gruber. 1995. Mast cells are potent regulators of endothelial cell adhesion molecule ICAM-1 and VCAM-1 expression. *J Cell Physiol 165:40*.
82. Schmidt-Weber, C. B., and K. Blaser. 2006. The role of TGF-beta in allergic inflammation. *Immunol Allergy Clin North Am 26:233*.
83. Steinke, J. W., J. Negri, R. Enelow, D. F. Baramki, and L. Borish. 2006. Proinflammatory effects of IL-4 antagonism. *J Allergy Clin Immunol 118:756*.
84. Akdis, C. A., and K. Blaser. 2001. Mechanisms of interleukin-10-mediated immune suppression. *Immunology 103:131*.
85. Hart, P. H., M. A. Grimbaldeston, and J. J. Finlay-Jones. 2001. Sunlight, immunosuppression and skin cancer: role of histamine and mast cells. *Clin Exp Pharmacol Physiol 28:1*.
86. Hart, P. H., M. A. Grimbaldeston, G. J. Swift, A. Jaksic, F. P. Noonan, and J. J. Finlay-Jones. 1998. Dermal mast cells determine susceptibility to ultraviolet B-induced systemic suppression of contact hypersensitivity responses in mice. *J Exp Med 187:2045*.
87. Depinay, N., F. Hacini, W. Beghdadi, R. Peronet, and S. Mecheri. 2006. Mast cell-dependent down-regulation of antigen-specific immune responses by mosquito bites. *J Immunol 176:4141*.
88. Theoharides, T. C., D. Kempuraj, M. Tagen, P. Conti, and D. Kalogeromitros. 2007. Differential release of mast cell mediators and the pathogenesis of inflammation. *Immunological Reviews 217:65*.