

Immunobiology of Antigen-Specific Immunoglobulin Free Light Chains in Chronic Inflammatory Diseases

Tom Groot Kormelink¹, Philip W. Askenase² and Frank A. Redegeld^{1,*}

¹*Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Universiteitsweg 99, 3584CG, Utrecht, The Netherlands;* ²*Section of Allergy and Clinical Immunology, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 06520-1013, USA*

Abstract: Mast cells are increasingly recognized as critical players in elicitation and maintenance of different inflammatory related disorders, like allergy, autoimmune diseases and cancer. Mast cells mature within the tissue and are able to adapt to microenvironmental changes. Together with the ability to produce multiple pro- and anti-inflammatory mediators upon activation, mast cells are highly capable of exerting immunomodulatory functions. Cross-linking of receptor bound IgE is the best known mechanism of antigen-specific mast cell activation. In this review we focus on a different route of activation, via immunoglobulin free light chains (FLCs). Here, we describe current knowledge and concepts on FLCs based on preclinical models and data on human subjects, after briefly recapitulating early research findings on FLCs. Furthermore, because FLC research mainly focuses on mast cells, several mast cell mediated pathologies and mouse models for mast cell research will be discussed. Whether targeting of mast cells is beneficial for the treatment of specific disorders has to be addressed in future studies. Specific inhibition of FLC-mediated mast cell activation may be an interesting avenue to treat chronic inflammatory diseases.

Keywords: Immunoglobulin free light chain, T cell factor, mast cells, inflammation, allergy, contact sensitivity, delayed type hypersensitivity.

GENERAL INTRODUCTION

Many of the pathological mechanisms that mediate inflammatory related disorders ranging from allergy to cancer are still poorly understood currently and subject to extensive research nowadays. Interestingly, the mast cells are increasingly recognized as critical players in the sensitization and effector phase of chronic inflammatory immune responses [1]. Mast cells are highly versatile cells, which are able to secrete a plethora of mediators upon activation and thereby exerting immunomodulatory functions [2-13]. Depending on the microenvironment and the activating signals, mast cells can direct the immune response into either pro- or anti-inflammatory directions. Binding of IgE to their high affinity receptor (FcεRI) and subsequent cross-linking of receptor bound IgE is the best and most powerful known mechanism of mast cell activation [14,15]. However, also a different route of antigen-specific activation have been described, namely via immunoglobulin free light chains (FLCs). About three decades ago the research was initiated when an unknown spleen supernatant factor showed to be able to induce an antigen-specific mast cell activation independent of IgE [16,17]. Initially, this factor thought to come from T cells was referred to as "T cell factor" (TCF) and later as the product of contact sensitization with picryl chloride (PCI) as PCI-Factor (PCI-F). The active proteins within these TCFs were later shown to be, at least partly, immunoglobulin free light chains, and consequently were a product of B cells/plasma cells instead of T cells [18,19]. In this review, we will outline the research concerning FLCs from the start to current findings and concepts. Preclinical models and data on human subjects will be addressed. Furthermore, the role of mast cells in mediating different pathologies and preclinical models involved in mast cell research will be addressed in more detail. Finally, data on FLC mediation of function in other cells will be described. Future research has to explore whether a general targeting of mast cells (e.g. stabilization) is functional for the treatment of specific disorders, or whether a more specialized mast cell

activation pathway, via FLCs for example, can be blocked to achieve disease improvement.

BIOLOGIC FUNCTIONS OF SUPERNATANT FACTORS LATER ASCRIBED TO FLC

A role for mast cells in contact sensitivity (CS) and delayed type hypersensitivity (DTH), in fact a required role, had never before been contemplated when work began on the mast cell activator T cell factor (TCF). The first studies suggesting this were pharmacological, and indicated for the first time that serotonin (5-HT) was required for the elicitation of CS and DTH [20]. In mice, 5-HT is a powerful vasoactive mediator, and compared with histamine that also is produced by mast cells and also stored in their granules, is 1000 more powerful in mediating vasoactivity [21]. Comparable vascular activation attempted with histamine results in severe acidosis and death. Fluorescent cytochemistry and uptake of ³H-5-HT were used to determine the possible sources of 5-HT in CS and DTH. These techniques indicated that mast cells were a possible source, as well as platelets [20,22]. In fact in mice, mast cells and platelets are the only source of 5-HT outside of the nervous system. Further, attempts to elicit DTH in immunized mice by challenge injection of antigen in the flank skin, the preferred site in guinea pigs and rats, failed. This correlated with a paucity of mast cells at that site. In contrast, elicitation of CS in the ears and DTH in the foot pads gave far superior results, and it was shown that these sites had particularly high numbers of 5-HT containing mast cells [20]. Further, mast cell-deficient mice have defective elicitation of CS and DTH and impaired recruitment of effector T cells locally to elicit these responses [23]. Finally, electron microscopic analysis indicated that mast cells in CS/DTH were undergoing partial piecemeal degranulation instead of the standard IgE mediated anaphylactic exocytosis [22-24], suggesting differential release of mediators. Functional studies showed preferential release of 5-HT compared to histamine; two mediators seemingly stored in the same granule, suggesting that indeed there was differential release [25-28] and thus the mechanism of release of mediators was different and specific in CS/DTH.

At this point it was still unclear how the mast cells were activated in CS/DTH. A mixture of four day PCI immune spleen and

*Address correspondence to this author at the Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Universiteitsweg 99, 3584CG, Utrecht, the Netherlands; Tel: +31-6-20252139; Fax: ??????????????; E-mail: f.a.m.redegeld@uu.nl

lymph node cells produced a supernatant factor thought to come from T cells, and thus was called T cell factor (TCF). This TCF readily adoptively transferred CS and DTH to naive recipients and showed to have mast cell activating properties [16,17,29-31]. However, since proof was lacking that TCF was the product of T cells, the name was changed to PCI-F as the product of PCI contact immunization. PCI-F was found to be remarkably antigen (Ag) specific compared to another similar acting heterologous specific factor from immune cells of oxazolone sensitized mice (OX-F) [29]. PCI-F had Ag-specificity in ear swelling [29] and activation of mast cells, but presence of IgE antibody was ruled out as the cause [16,31]. The fact that PCI-F had the specificity of an antibody led to examination of B cells as a source. It was subsequently demonstrated that the unusual B cell subset of B-1 B cells, that resides in the peritoneal cavity and are T cell independent in their activation and antibody production, were found to be activated very early (hours) after skin contact painting to immunize for CS. PCI-F is produced within hours of contact immunization by the activated B-1 B cells. Amongst other proteins, this PCL-F contained antigen-specific IgM [32-41]. In fact, monoclonal anti-TNP IgM myeloma proteins could substitute for PCI-F in reconstitution of CS in JH^{-/-} pan immunoglobulin deficient mice. Further research demonstrated that smaller components derived after fractionation of IgM were similarly able to reconstitute the initiation of CS. Thus, addition of an early-acting initiation component, like PCI-F or 1 day immune cells as a source of B-1 B cell IgM antibody, allowed the T cells to be recruited for full elicitation of CS. Not only pentameric IgM antibody was active in CS-elicitation, but also trimers, dimers, monomers, and heavy and light chain dimers that were not clearly known previously to be present in IgM preparations [19]. Although all of these fractions of IgM were active, it was shown that two mechanisms were involved. The pentamer down to the heavy chains activated the mast cells indirectly. They were found to be complement dependent by forming immune complexes with circulating hapten self protein complexes generating C5a to activate the mast cells via their receptor for C5a [42-44]. In contrast and surprisingly, the Ag-specific polyclonal light chains plus antigen activated the mast cells directly in a manner analogous to IgE antibody. These findings corresponded with finding that light chains can activate mast cells [18,19]. Indeed, an isolated immunoglobulin FLC of anti-TNP Ag specificity, could fully substitute for PCI-F in our system by reconstituting absent elicitation of CS in JH^{-/-} mice that were unable to elicit CS without a CS-initiator like PCI-F.

IMMUNOGLOBULIN FREE LIGHT CHAINS MEDIATE ANTIGEN-SPECIFIC MAST CELL ACTIVATION

As described above, the TCF consisted of a mix of several proteins. The presence of immunoglobulin light chains in this fraction was ultimately demonstrated after multiple steps of purification. Light chains are detectable with a size of 22-27 and 44-55 kDa indicating that they are not covalently linked to heavy chains, but present in free form in a monomeric and dimeric conformation, respectively (unpublished data). To demonstrate that these "TCF components" were critical for inducing the antigen-specific mast cell activation, hapten-specific FLCs were obtained by reducing and alkylating trinitrophenol (TNP)- and oxazolone-specific IgG₁. Subsequent intravenous injection of purified hapten-specific FLCs followed by a topical ear challenge with the appropriate hapten induced an increase in ear thickness as compared to unsensitized controls [18,19]. This FLC-induced ear swelling response is dose-dependent and comparable to the responses elicited after sensitization with hapten-specific IgE. In addition, passive sensitization with culture supernatant from *in vitro* cultures of spleen and lymph nodes from sensitized mice (PCL-F) resulted in the transfer of hypersensitivity only when FLCs were not depleted using immobilized antibodies specific for κ -light chains. These data showed that active epicutaneous sensitization results in the production of hapten-specific FLCs, as early as one day after sensitization. Passive

intravenous sensitization with antigen-specific FLC of pan B cell-deficient (μ MT) followed by local challenge resulted in a significant ear swelling response. This finding indicates that the FLC mediated reaction is not due to an interaction between FLCs and circulating immunoglobulins and therefore not dependent on intact immunoglobulins. Importantly, passive sensitization with hapten-specific heavy chains or non-specific light chains did not elicit an ear swelling response indicating that immunoglobulin heavy chains only weakly activate complement and do not activate mast cells.

Although controversial to some extent [45], it has been shown previously that the initiation of a profound delayed type contact hypersensitivity reaction is dependent on the activation of mast cells [23,46-49]. This activation results in the immediate release of various mediators like the vasoactive serotonin (5-HT) and histamine, proteases, lipid-derived mediators (leukotrienes etc.), chemokines and cytokines (particularly TNF- α and MIP-2 [46]) leading to local expression of vascular activation markers (through TNF- α for example), vasodilatation and an increased vascular permeability (leading to the early and then late phase response) and attraction of immune cells [50]. Initiated by the early response, T cells are recruited locally and then the Ag-specific minority is activated in the tissues by Ag-MHC complexes on a few local antigen presenting cells, like dendritic cells. There, activated T cells produce various cytokines that induce the local tissue cells to produce chemokines that recruit a non-specific inflammatory cell infiltrate (mainly monocytes and neutrophils) from the vasculature, leading to the late phase response [50].

To test whether the FLC mediated contact sensitivity was dependent on mast cells, mast cell-deficient (WBB6F₁-Kit^{W/W-v}) mice were passively sensitized with FLC. Subsequent challenge with the appropriate antigen did not result in a significant ear swelling response [18,19]. Local reconstitution of bone marrow derived mast cells at the site of challenge 6 weeks before active sensitization and challenge fully restored the ear swelling response. Moreover, histological and ultrastructural analysis of mast cells at sites of challenged tissue showed marked signs of degranulation. Comparable changes in mast cell ultrastructure were observed earlier when analyzing the effects of TCF on mast cells [51]. Direct binding of FLCs to the mast cell (BMMCs) membrane was also shown using FITC-labeled FLC and beads coated with FLCs to visualize binding to mast cells. These data indicate that FLC induced hypersensitivity is mediated via mast cells, yet the mechanism by which FLCs exert this function is still unclear and a specific FLC receptor has not yet been fully characterized. The involvement of the common γ chain, involved in signal transduction after cross-linking of the IgE and IgG₁ high affinity receptors Fc ϵ RI and Fc γ RIII, respectively, is excluded since normal ear swelling responses were elicited in common γ chain-deficient mice (Fc γ R^{-/-}) compared to wild type mice [18]. In addition, it was shown that the FLC-mediated contact sensitivity is independent of the action of complement [19].

F991, ANTAGONIST OF FLC

Tamm-Horsfall protein (THP; uromodulin) is a naturally occurring protein that is highly expressed in the kidney and has the capacity to specifically bind FLCs [52]. THP is a heavily glycosylated, monomeric protein produced and secreted by cells of the thick ascending limb of the loop of Henle. Analysis of the light chain-binding location site within THP revealed that a 9-mer peptide is completely responsible for the FLC-THP interaction [53]. The amino acid sequence of this 9-mer peptide was used to develop a peptide that binds FLC in the circulation thereby preventing FLC-binding to FLC targets, like a specific receptor on mast cells. Using this FLC-antagonist, F991, the ability of FLCs to initiate contact sensitivity was further demonstrated, since intravenous injection of F991 prior to challenge resulted in complete inhibition of the early ear swelling response after both passive and active sensitization. The late phase response was inhibited as well in the active sensi-

zation model [18]. F991 dose-dependently inhibited the contact antigen induced ear swelling responses mediated by FLCs but in contrast, it was ineffective in inhibiting IgE-, IgG- and IgM-mediated responses.

FLC PRODUCTION AND DISTRIBUTION

B cells are responsible for the production of immunoglobulins and are therefore critical in the humoral immune response. In addition, B cells play a critical role in host defense by initiating and/or modifying the immune response via antigen presentation to T cells and the production of multiple pro-inflammatory cytokines and chemokines. Intact immunoglobulins consist of two identical heavy chains and two identical light chains which are covalently linked in heavy and light chain pairs. Both heavy and light chains have a C-terminal constant region and an N-terminal variable region, the latter containing the antigen binding complementary determining regions. The variable regions of one disulfide bonded heavy and one light chain pair together constitute one of the antigen binding site. The diversity of variable regions is maintained by random rearrangement of a multitude of Ig variable (V), diversity (D), and joining (J) gene segments for heavy chains and V and J segments for light chains. Furthermore, somatic hypermutation of variable regions occurs following antigen activation [54]. The C-terminal Fc region of the constant part of the heavy chains determines the (sub) class of the immunoglobulin (IgG, IgE, IgM, IgA, IgD), and thereby its biological function. Two types of light chains are produced by mammalian B cells, either kappa (κ) or lambda (λ) [55]. The ratio in which both types are produced varies significantly among species. In human for instance, half to two-thirds of the light chains produced is kappa, whereas mice predominantly produce kappa FLCs (~95%) [56,57]. The production of heavy and light chains is independent of one another and normally there is a 10-40% overproduction of light chains compared to heavy chains [58-63]. The secretion of heavy chains is tightly regulated and will not occur as long as tetrameric antibodies are not formed. In this case, the free heavy chains are not transported to the Golgi complex and are degraded internally [64]. In contrast, light chains can be secreted by B cells without being incorporated into immunoglobulins, leading to the presence of unbound free light chains (FLCs) in the extracellular environment and in the serum. At this moment it is unclear whether all activated B cells randomly overproduce FLCs, or whether this is somehow regulated. Possibly, the AID induced mutations in the V region and consequent affinity for antigen, as well as the B cell type and/or strength of its activation signals together with their microenvironment, and/or maturation stage may influence FLC production [60,65-68].

FLCs can be detected in serum, but also many other body fluids like urine, sputum and saliva, nasal secretions, bronchial alveolar lavage fluid, and synovial and cerebrospinal fluid. In all body fluids kappa and lambda FLCs predominantly exist as monomers (22-27 kD) and covalently and non-covalently bound dimers (44-55 kD), but also polymeric forms of kappa and lambda FLC occur. Concentrations of FLCs are low at birth and gradually increase by age until adulthood [69-72]. The 'normal' range of FLC concentrations in serum of healthy adults is between 3.3-19.4 mg/L for kappa and 5.7-26.3 mg/L for lambda, but can be highly dependent on the detection assay employed and calibration standards [56]. The kidney is the main organ responsible for the removal of FLCs from the circulation. After glomerular filtration, FLCs are primarily removed by proximal tubular epithelial cells in which lysosomal proteolysis of FLCs takes place. Constituent peptides and amino acids from FLCs are subsequently returned to the circulation. When FLC concentrations exceed the reabsorptive capacity of the proximal tubular epithelial cells, high amounts of FLCs will be excreted in the urine. The precise clearance of FLCs by the kidney is unknown, but based on size and cationic charge serum half-lives of kappa and lambda FLC of 2-4 and 3-6 hours are calculated, respectively [52]. In

multiple myeloma, a tumor of antibody producing plasma cells, huge amounts of light chains are secreted in the urine and complex with Tamm-Horsfall protein to obstruct urine flow in the tubules to produce significant renal impairment; a condition known as myeloma kidney. In addition, patients suffering from other plasma cell dyscrasias which are associated with an increased FLC production show increased kidney injury [52].

ANTIGEN BINDING BY FLCs

In intact immunoglobulins the variable regions of both heavy and light chains together create two antigen binding sites per IgG and ten per IgM pentamer to mediate antigen binding. The overall binding of a complete single antibody is called affinity, which is determined by the variable regions in the immunoglobulin heavy and light chains. The presence of the variable region within light chains, together with the fact that many FLCs form dimers mimicking binding site of an intact antibody, indicates that FLCs may also have the capacity to bind antigen. However, studies concerning the binding of FLCs are contradicting and indicate that binding affinities of FLCs can be either negligible, equal, or even exceed those of parent immunoglobulins [73]. The *in vivo* data described above regarding the FLC-mediated mast cell activation indicate that FLCs are able to bind antigen by recognizing specific epitopes. Further, the fact that multiple FLCs on the surface of the mast cell are binding antigen means that their lower affinity still results, through multiple binding, into appreciable binding affinity resulting in significant Ag-specificity. Thus, CS-initiating ear swelling responses dependent on FLC bound to mast cells cannot be elicited by challenging the ears with an irrelevant hapten. Other preclinical models which will be discussed below, also suggest the presence of antigen-specific FLCs that mediate immunological responses by binding antigen specifically [74-77]. In line with these data, it is possible to prove antigen-specific binding of oxazolone (OX) and trinitrophenol (TNP) specific light chains using western blot and ELISA techniques Fig. (1). At present, assays for detecting antigen-specific human FLCs are lacking [78]. Only one paper described the presence of *Toxoplasma gondii* specific FLCs in cerebrospinal fluid from AIDS patients suffering from encephalitis [67]. Technical limitations which, amongst others, together with a possible lower binding affinity of FLCs and the lack of positive reference controls, might hamper detection of specific FLC in body fluids. Moreover, in case of analyzing serum, antigen-specific FLC concentrations may be too low to detect due to the short half-life of FLCs in combination with a considerable time of obtaining the sample after antigenic stimulation. Analysis of body fluids that contain FLCs produced locally in affected tissues might improve detection as demonstrated in cerebrospinal fluid [67]. Other interesting body fluids to analyze clinically are nasal secretions and synovial fluid, since highly increased FLC concentrations can be found in rhinitis and rheumatoid arthritis patients, respectively (see below) [79,80].

FLCs IN PRECLINICAL STUDIES OF ANIMAL MODELS AND IN HUMAN DISORDERS

The finding that FLCs are capable of mediating antigen-specific mast cell activation creates a new look at disorders in which B cells and mast cells are involved. In this respect, allergic diseases are of great interest. Allergy is an exaggerated immune reaction to usually harmless foreign proteins present in food and airborne pollens for example. Allergic hypersensitivity can be subdivided to IgE- and non-IgE-mediated allergies [81]. Allergic responses via IgE are intensively investigated since IgE is thought to play an important role in many allergic diseases [82-84]. IgE can bind antigen with high affinity and cross-linking of Fc ϵ RI-bound IgE on mast cells by multivalent antigen causes mast cell activation leading to the release of multiple mediators via exocytosis of the characteristic granules containing mediators such as histamine and 5-HT along with cytoplasmic stored and newly generated pro-inflammatory

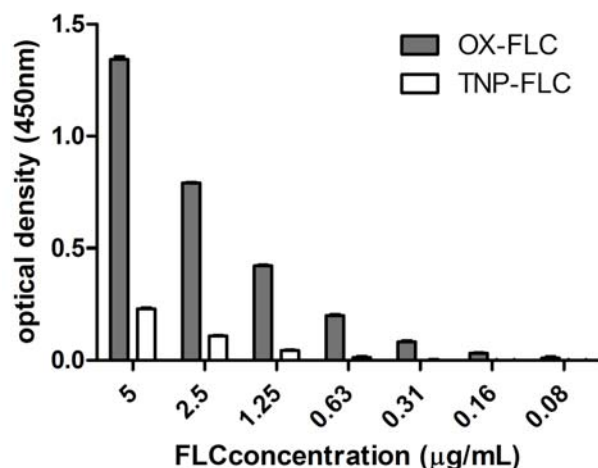


Fig. (1). Antigen specific binding of immunoglobulin free light chains (FLCs). Oxazolone-specific FLCs (OX-FLC) specifically bind to plate bound OX-conjugated albumin in a concentration dependent manner. Compared to OX-FLC, trinitrophenol-specific FLCs (TNP-FLC) hardly show binding to OX indicating an antigen specific binding capacity. Background staining of FLCs to albumin is negligible (not shown). Based on these binding data we calculated the binding affinity (Km) of OX-FLC to OX-conjugated albumin using a Lineweaver Burke plot of 0.12µM which is relatively high.

mediators including biogenic amines, cytokines and chemokines and certain growth factors [14,15,85]. Together these mediators mediate a local inflammatory response leading to an early and subsequent late phase response. Despite this important contribution of IgE to many allergies, a significant proportion (up to 40%) of patients suffers from a non-IgE mediated allergy. Examples of disorders displaying such a high percentage of patients in which IgE cannot be identified as the critical regulator of inflammation are food allergy, dermatitis, asthma, and rhinitis [86]. Nevertheless, mast cells still appear to play a key role in mediating these disorders. Despite some differences, many clinical and immunological similarities are recognized between IgE and non-IgE mediated allergies and treatment of each with corticosteroids is equally effective. The question arises what mechanism(s) are responsible for causing the various mast cell-mediated and allergen-specific, but non-IgE mediated, allergic diseases?

Besides IgE, several other factors can induce mast cell activation. These include IgG via FcγRI or FcγRIII (in mouse), complement components like C5a via C5aR, cytokines, toll-like receptor ligands, bacteria, viruses, and via interaction with T cells [2,87-89]. Moreover, a number of studies have indicated a possible functional role of antigen-specific FLCs in cow's milk allergy, asthma, inflammatory bowel disease (IBD), rhinitis and rheumatoid arthritis [74-77,79,80], which will be discussed in following paragraphs.

Cow's Milk Allergy

Food hypersensitivity is defined as reproducible adverse effects to foods, especially immediate responses, mainly to cow's milk and hen's egg, and is most common in the first years of life. Clinically, cow's milk allergy (CMA) causes various symptoms involving those in the skin, gastrointestinal and respiratory tract and even anaphylaxis. Although most children (>80%) outgrow CMA before three years of age, it is still one of the leading causes of food allergy in adults [90,91]. Recently, two preclinical CMA models were introduced involving oral sensitization to casein or whey, the major protein constituents of cow's milk. In both models, immediate ear swelling responses could be measured after intradermal challenge, while oral challenge with the appropriate cow's milk antigen

induced diarrhea-like symptoms and mast cell activation as demonstrated by increased systemic mMCP-1 concentrations [92]. Interestingly, whereas whey immunization clearly showed IgE mediated symptoms, casein induced a predominantly FLC-mediated allergy [76]. The early ear swelling response after challenging the ears of whey- or casein-sensitized mice with appropriate antigen could only be abrogated by F991 in the casein group. Furthermore, serum and especially in mesenteric lymph node (MLN) supernatant, FLC concentrations were clearly increased in casein-sensitized mice compared to whey-sensitized and controls mice, which is suggestive for excessive local production. In addition, antigen-specific FLCs are produced in the spleen, since casein sensitivity could be transferred to naïve mice by intravenous injection of spleen supernatants from casein-sensitized mice. The early phase responses in these recipient mice were also attenuated after F991 treatment, suggesting mediation by FLC. However, F991 treatment did not lead to a complete abrogation of the ear swelling responses in neither the active nor the passive casein model which may be explained by a small simultaneous effect of casein-specific IgG₁ or IgE (although not detected). In another study it was shown that the whey-induced CMA model, which is highly IgE dependent under normal conditions, can be switched to a FLC-mediated model after depletion of CD25⁺ cells (Tregs together with a small percentage of antigen-specific effector T cells) in the sensitization phase [77]. In this study, a single anti-CD25 treatment before whey-specific IgE was systemically detectable, completely abrogated the production of whey-specific IgE (possibly by deleting whey-specific effector T cells responsible for the induction of class switching in B cells to IgE/IgG₁), but did not influence early phase responses. A striking finding was that the anti-CD25 treated group showed increased FLC concentrations (especially in MLN) and the early phase response could be blocked by antagonizing FLCs. The dependence on FLCs of the allergic response in these treated mice also resulted in FLC-mediated gastrointestinal changes as indicated by a hyperreactivity of the colon upon exposure to carbachol, a muscarinic receptor agonist. It is known that mast cells can differentially release mediators dependent on the type of activating stimulus [4,85,93], that can induce end organ hyperreactivity to cholinergic agonists. Whether the FLC-induced colon hyperreactivity is also caused by a subset of mediators specifically released by FLC-activated mast cells, rather than released via IgE-mediated activation has to be proven. A mechanistic difference in mast cell activation by IgE and FLCs is supported by earlier work on TCF as described above [51]. Alternatively, the direct effect of FLCs on intestinal epithelial and/or smooth muscle cells cannot be excluded.

The cause of the differential immune responses initiated in the described whey and casein models is still speculative but may be a result of differences in physical, physiological and dietary properties of both classes of proteins. As a result, this can lead to different modes of immune cell activation (i.e. via dendritic cells, T cells, B cells), which could be further influenced by intestinal microenvironmental differences caused by the different proteins. The plasticity of these sensitization responses is supported by the finding that the IgE-mediated whey response is switched to FLC-dependent response after depletion of CD25⁺ cells. Based on this latter finding it is tempting to speculate that human CMA patients without detectable concentrations of cow's milk protein (CMP)-specific IgE comprise a distinctive intestinal microenvironment compared to those having cow's milk-specific IgE that shifts the balance from IgE (and/or IgG) to FLC production. This condition might be applicable to young infants with CMA as well since the majority is serum negative for CMP-specific IgE. In children 3-7 months of age who show a positive double blind placebo controlled food challenge, significantly increased kappa and lambda FLC concentrations were found in serum as compared to an age-matched control group of children who had a negative test. All these children had undetectable or very low CMP-specific IgE values [76].

Asthma

Asthma is a relatively common chronic disorder of the airways manifested by inflammation of the lower airways in particular, leading to respiratory symptoms and bronchoconstriction. The involvement of FLCs in asthma pathology was demonstrated using a murine experimental model showing clear similarities with adult asthma in humans [74]. In this model, topical cutaneous sensitization with DNFB followed by intranasal exposure to cognate antigen (DNBS) leads to a mast cell-dependent pulmonary hypersensitivity reaction, including early signs of bronchoconstriction [94]. Intranasal administration of F991, 30 minutes prior to challenge completely blocked DNBS induced early phase hypersensitivity responses as indicated by a reduced mast cell activation and bronchoconstriction. Late phase responses, manifested by mucosal leakage, neutrophilic cellular infiltration in BAL fluid and tracheal hyperreactivity 24 and 48 hours after challenge, could be blocked by F991 before airway challenge as well. In addition, passive sensitization with TNP-specific FLCs induced comparable early phase responses as compared with the active model. This response was also abrogated after F991 treatment and was shown to be mast cell dependent. Whether this mechanism also contributes to human asthma pathology in at least a subpopulation of patients is uncertain. Analysis of serum from atopic and non-atopic asthma patients indicated that kappa FLC concentrations are higher in both groups compared to non-asthmatic age-matched controls [74]. Total IgE concentrations were comparable between controls and non-atopic asthmatics.

Inflammatory Bowel Disease

Chronic, spontaneously relapsing inflammations of the gastrointestinal tract are collectively termed IBD (inflammatory bowel disease), and occurs in Crohn's disease or ulcerative colitis [95]. The precise mechanisms underlying the development of IBD are unknown but mast cells appear to be crucial by releasing mediators such as histamine and tryptase without a clear contribution of IgE [96,97]. The contribution of FLCs in IBD was analyzed using a mast cell-dependent murine IBD model [98]. Comparable to the asthma model described above, topical sensitization with DNFB followed by intrarectal exposure to cognate antigen (DNBS) leads to colonic hyperreactivity symptoms such as diarrhea, increased vascular permeability, and colonic mast cell and neutrophil infiltration. F991 treatment in this model prevented mast cell activation and inhibited diarrhea, cellular infiltration of the colon, and lymphoid follicular hyperplasia [75]. In line with these findings, intravenous injection of TNP-specific FLCs in non-sensitized mice resulted in local mast cell activation and increased permeability of colonic vasculature after intrarectal challenge with DNBS. Analysis of FLCs in IBD patients and control subjects revealed that FLC concentrations in serum, and FLC staining positivity in affected colon and ileum tissues, are increased in IBD patients.

Rhinitis

Allergy of the upper airways manifests as rhinitis and is characterized by inflammation of the nasal mucosa. Rhinitis can be classified into infectious, atopic (IgE-mediated), and non-atopic/non-infectious, with this latter group being highly heterogeneous but yet comprising a substantial number of rhinitis patients [86]. A subpopulation of patients with non-atopic rhinitis shows nasal mucosal inflammatory characteristics comparable to atopic rhinitis, including the increased number of mast cells [99-103]. Assessment of FLCs was performed in a group of patients with a non-atopic persistent rhinitis (non-allergic rhinitis with eosinophilic syndrome (NARES)), to delineate its putative contribution to the mast cell-mediated effects [79]. Serum and nasal secretions were analyzed to assess the systemic and local parameters of disease, respectively. The presence of tryptase and eosinophilic cationic protein in nasal secretions from both IgE-mediated rhinitis and NARES patients

clearly showed the involvement of mast cells and eosinophils in both disorders. Systemic and local IgE concentrations however, were at the level of healthy controls in NARES patients and no specific IgE to seasonal or perennial allergens was detected in this group. IgE-mediated rhinitis patients show high antigen-specific IgE concentrations in serum and nasal secretions as expected. Interestingly, local FLC concentrations are increased in both IgE-mediated rhinitis and NARES patients compared to control subjects, but this increase is significantly higher in the NARES group. Only a slight but significant increase of FLCs was detected in the serum from NARES patients, which is suggestive for a local immune response. Furthermore, higher numbers of FLC positive cells were detected within the nasal mucosa of both allergic groups. Laser microdissection and RNA analysis demonstrated that mast cells were amongst the FLC-positive cells. The discrepancy between IgE and FLC production in the two patient groups supports the hypothesis that a specific local environment or immune pathogenesis either directly or indirectly steers the B cell to predominantly produce IgE or FLC.

Rheumatoid Arthritis

In contrast to the allergic type disorders described above, rheumatoid arthritis (RA) is systemic autoimmune disorder featuring inflammation of synovial tissues mainly considered as a T cell and macrophage-dependent disease. Besides, autoantibody-producing B lymphocytes play a pivotal role in RA pathology which is greatly supported by effective treatment with B cell depletion therapy using rituximab [104-106]. Additionally, critical disease regulatory functions have also been ascribed to mast cells and mast cell mediators in RA disease pathology [107-110]. Increased serum FLC concentrations in RA patients, which correlate with disease activity, suggest that FLCs may be involved in mediating synovial mast cell activation [80,111,112]. Comparable to rhinitis patients, the highest FLC concentrations are detectable at the site of active inflammation, i.e. in the synovial fluid and tissue of affected joints. In RA patients, extremely high synovial fluid FLC concentrations were detected frequently exceeding those measured in serum. Probably as a consequence of this, in RA patients serum FLC concentrations reflect spill-over of FLC from local production in the inflamed joints [80]. Rituximab treatment in a cohort of 50 RA patients with active disease showed that FLC serum concentrations decrease after the start of treatment and more strikingly, relative changes in FLC concentrations correlated with changes in disease activity score and systemic markers of inflammation. Since anti-TNF treatment did not significantly change serum FLC concentrations although being clinically effective, changes in FLC concentrations are not a general feature of clinical response to treatment. This implies that the effect of rituximab may partly rely on reduction of FLC production by pathogenic B cells, or could be due to effects on antigen presenting capacity of the local B cells. These possibilities have to be further outlined in future studies.

Other Disorders Associated With Increased FLC

FLC analyses have been performed in clinical studies concerning several other disorders besides those described above. FLC analyses are performed to identify and monitor patients with B cell lineage tumours such as multiple myeloma and primary systemic amyloidosis, as many of them show highly increased monoclonal FLC concentrations and changes in kappa/lambda ratio's [113-115]. These patients do not present an increased incidence of allergic diseases or autoimmunity which can most likely be explained by the clonal nature of the secreted FLCs. The possibility that these FLCs will specifically bind an antigen to which the patient is exposed is probably negligible. Other disorders associated with increased serum, urine or cerebrospinal fluid FLC concentrations are multiple sclerosis, Sjögren disease, atopic dermatitis, sarcoidosis, systemic lupus erythematosus (SLE), neurological inflammations/disorders, and AIDS [111,116-125]. Also in multiple sclerosis

patients, high FLC concentrations correlated with an increased disease severity and clinical relapse in SLE patients was correlated to a preceded rise in urinary FLCs [119,126]. Current research in our laboratory also showed an increased FLC production and expression in two frequent and complex interstitial lung diseases idiopathic pulmonary fibrosis (IPF) and hypersensitivity pneumonitis (HP) (Groot Kormelink *et al.*, submitted) and tumour tissues of various aetiologies in multiple organs (Groot Kormelink, *et al.*, submitted).

MAST CELL ACTIVATION

Mast cells are dispersed throughout most tissues in the body but the majority of mast cells is located at anatomical sites that are in direct contact with the environment such as the skin, gastrointestinal tract, and the airways. This distribution provides them with the capability of initiating immune responses as soon as the body comes in contact with a pathogen [85,127]. The mechanisms of activation of mast cells, mediator release, the effector functions of mast cells in different immune responses and its immunoregulatory functions are extensively reviewed previously but will briefly be addressed below. Next to FLC, multiple other signals including IgE, IgG, complement factors, cytokines, toll-like receptor ligands, bacteria, and viruses are able to activate mast cells under general or more strictly defined conditions [2,87-89]. Three types of mediators released by activated mast cells are recognized: 1; pre-formed, granule associated mediators like histamine, neutral proteases and heparin. 2; *de novo* synthesized lipid mediators like LTC₄, LTB₄ and PGD₂. 3; various newly synthesized and secreted cytokines and chemokines [85,87,93]. Dependent on the types and strength of activating signals, mast cells can undergo complete or partial degranulation, also referred to as piecemeal degranulation. The former type of degranulation results in an excessive, more random release of prestored mediators, whereas piecemeal degranulation is the secretion of a selective subset of prestored mediators [89,93]. The differential release of mediators by partial degranulation is again highly dependent on the type and strength of the stimuli. For instance, partial mast cell degranulation was documented on the basis of electron microscopy and differential release of mediators in allergic cutaneous hypersensitivity due to PCI-F [22-28]. Ultimately, not only the type and strength of the stimuli but also the type of mast cell itself will determine the mediators that will be released. Mast cells start maturation once they entered the tissue in which they will ultimately reside during their lifespan. Consequently this means that the anatomical site of maturation together with its (local) microenvironmental conditions may largely influence maturation (or alter phenotype after maturation) leading to phenotypically distinct subpopulations of mast cells, a phenomenon called mast cell heterogeneity.

Physiologically mast cells are believed to be important for maintaining homeostasis. Mast cells essentially contribute to innate immune defences against parasites, bacteria, and to viral pathogens as well, and additionally mast cell are involved in modulating adaptive immune responses and inducing tolerance [128-130]. Moreover, mast cells are shown to be crucial to wound healing processes, at least partially by initiating immune responses after activation by endogenous danger signals, such as IL-33 released from necrotic structural cells [131-133]. Despite these beneficial actions for the host, mast cells are highly associated with allergic disorders, including non-IgE mediated allergies [50,86,134]. In addition, mast cells are increasingly being recognized as a critical cell type involved in the initiation, modulation and/or progression of autoimmune diseases and cardiovascular disorders [9,135-139]. The main autoimmune diseases that are currently being recognized to be significantly influenced by mast cell function are multiple sclerosis, rheumatoid arthritis, bullous pemphigoid, and insulin-dependent diabetes mellitus. Non-IgE mediated activation mechanisms appear

to be primarily important in autoimmune disorders since evidence of IgE-mediated mechanisms are limited, except for bullous pemphigoid, and some cases of Grave's ophthalmology and rheumatoid arthritis [140-143]. Tumourigenesis, the process of initiation and progression of tumours, is another disease type to be announced in which mast cells appear to have significant contribution to disease outcome [144-148]. In many human tumours the number of tumour infiltrating mast cells correlates with increased intratumour microvessel density, enhanced tumour growth and invasiveness, and poor clinical outcome. These clinical findings are supported by several rodent tumour models showing mast cells to be the first immune cell to enter the premalignant tissue and that mast cell stabilization or deficiency attenuated tumour growth [149-152]. However, a dual role is recognized for mast cells in tumour progression since its role in anti-tumoural immunity is also indicated in both clinical and preclinical studies [152-158]. Interestingly, the pro- or anti-tumourigenic effector function of mast cells seems to be determined by the microenvironment (i.e. peritumoral or intratumoral) and/or the presence of specific activating factors. This latter was shown by the tumour growth inhibitory effect of mast cell derived IL-6 released upon TLR2 activation [152].

As briefly discussed above, mast cells are clearly associated with different disease pathologies but their definite functions are far from straightforward and probably depend on multiple factors. Different outcomes between laboratories investigating similar preclinical disease models in wild-type and mast cell-deficient mice more or less subscribe this by a lack of consistent proof for mast cell dependency in some disorders. The reason for this discrepancy might be due to microenvironmental differences or variation in sensitization/immunization protocols. Another factor contributing to differences in preclinical experimental outcome is the use of different mast cell deficient mouse strains. Two commonly used mast cell deficient strains, WBB6F₁-Kit^{W/W-v} and C57/BL6-Kit^{W-sh/W-sh}, contain a naturally occurring mutation in the c-kit receptor. Another mast cell-deficient strain is the WCB6F₁-Kitt^{Sl}/Kitt^{Sl-d}, carrying spontaneous mutation in the c-kit ligand [159]. The drawback of these strains however, is that they are not only deficient for mast cells but also display other hematopoietic and non-hematopoietic abnormalities. Kit^{W-W-v} mice have abnormalities such as neutropenia, macrocytic anaemia, $\gamma\delta$ T cell deficiency, and deficiencies in melanocytes, germ cells and interstitial cells of Cajal. On the other hand, the more recently characterized Kit^{W-sh/W-sh} mice have normal numbers of neutrophils, and are not anaemic and infertile, which makes them more attractive candidates for mast cell research. Interesting examples of preclinical models showing discrepant results on the involvement of mast cells in mediating pathology are models regarding delayed hypersensitivity as described above, but also rheumatoid arthritis (RA) and experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis. The critical role of mast cells in RA was shown in different RA models using Kitt^{Sl}/Kitt^{Sl-d} and Kit^{W-W-v} mice [107,160,161]. On the other hand however, antibody-mediated arthritis and collagen-induced arthritis are not impaired in Kit^{W-sh/W-sh} mice [160,162]. In analogy to these findings, EAE models performed in wild-type and Kit^{W-W-v}, and also Kit^{W-sh/W-sh} mice suggested a detrimental role for mast cells in disease pathology [163,164]. Yet, using Kit^{W-sh/W-sh} mice in the same experimental model showed that mast cells are dispensable for EAE development or even exacerbate EAE responses [165,166]. This latter study also indicated an immunization dependent effect in the Kit^{W-W-v} strain [165].

A promising technique that might overcome at least some of the problems caused by the limitations encountered in naturally occurring mast cell deficient mice is the use of Cre/LoxP technology. This technology allows the generation of mast cell deficient mice without substantially affecting other cell types. In addition, mast cell specific gene expression of certain mediators can be knocked out which allows to investigate the contribution of mast cell-derived

cytokines or chemokines for example which can also be produced by other immune cells [1,167].

ROLE OF FLCS IN TOLERANCE AND IMMUNE SUPPRESSION

So far, the pro-inflammatory effects of FLC-mediated mast cell activation have been described extensively. However, several studies indicated the involvement of FLCs in suppressing immune responses. Similar to increased concentrations seen in patients with viral infections, FLCs were increased several days after initiation of a viral-induced experimental myocarditis model in mice. Daily subcutaneous injection of polyclonal FLC of unknown specificity starting one day before viral inoculation in this model resulted in a significant protective effect of FLCs as demonstrated by the lower degree of myocardial lesions and lower myocardial virus concentrations, and increased survival. FLC showed to inhibit viral replication directly, but lambda FLC supplementation also significantly induced IL-10 gene expression and tended to increase interferon- α and γ in heart tissue [168]. In a murine model for cow's milk allergy, FLCs are clearly involved as effector proteins (as described above). Surprisingly however, FLC concentrations increase not only after sensitization, tolerance induction prior to sensitization was accompanied with an increase in serum FLC concentrations as

well. This increased FLC production appeared to be essential for immune suppression after tolerance induction as it was demonstrated that antagonizing FLC by F991 after tolerance induction but before sensitization, attenuated the tolerizing effect of whey pre-treatment (Van Esch., PhD thesis 2011). In the same study, a single injection of FLCs of irrelevant specificity, one day before start of sensitization, mimicked the high dose tolerance induction. Similar observations were made in earlier studies, in which PCL-F, a crude spleen derived protein factor containing FLC, intravenously injected prior to sensitization inhibited the development of contact sensitivity response. These studies also indicated that the suppressor effects were not antigen-specific since intravenous injection of an irrelevant TCF before sensitization resulted in suppressed cutaneous delayed type hypersensitivity responses and inflammatory cell influx in gut, depending on the type of model used [169-172]. Finally, recent studies of suppressor T cells, distinct from regulatory T cells (Treg) and induced by high Ag dose tolerogenesis, suggest that suppression may be brought about via secretion of exosomes. These exosomes suppress specific CS effector cells by delivering inhibitory miRNA and are possibly coated with FLC to provide Ag-specificity to target the exosomes to specific cell types (manuscript in preparation).

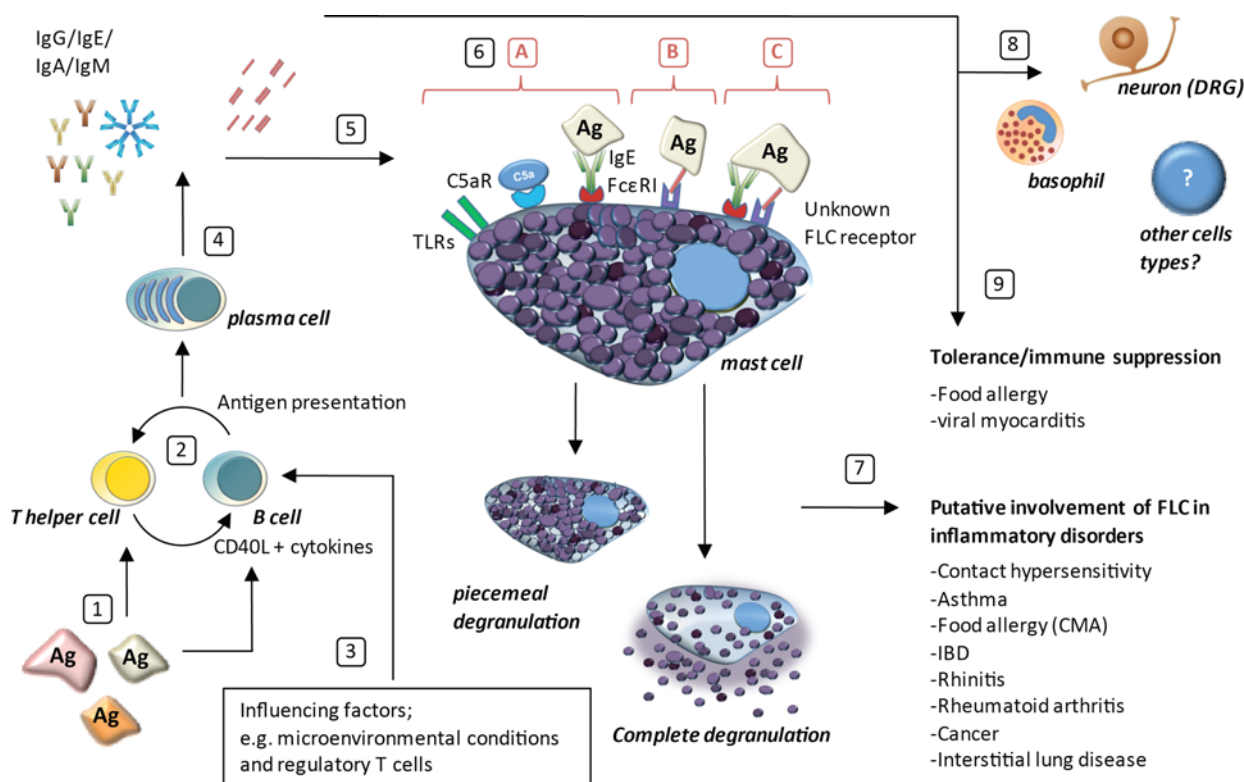


Fig. (2). Schematic overview summarizing current knowledge on FLCs and its involvement in immune responses based on the studies described in this review. 1. exogenous antigens, or endogenous antigens in case of autoimmunity, either directly activate B cells (B-1 B cells, that are T cell independent in their activation and antibody production), or indirectly activate B cells via activated, antigen-specific T helper cells (2). 3. Different factors such as environmental conditions and the presence of regulatory T cells (Tregs) can influence the reciprocal B cell-T cell activation. Depletion of CD25⁺ Tregs resulted in a shift from IgE to FLC producing B cells in Cow's milk allergy model. Activated B cells differentiate into plasma cells which produce immunoglobulins and/or FLCs (4). The immunoglobulin isotype and the amount of FLCs produced depend on the activating stimuli and B cell subsets involved (2 and 3), and IgE, IgG and FLC can bind to specific receptors located on the mast cell membrane (5). Mast cells can be activated via multiple mechanisms; 6A. Binding of IgE to their high affinity receptor (FcεRI) and subsequent cross-linking of receptor bound IgE is the best and most powerful known mechanism of mast cell activation. In addition, activation can be mediated via different Toll-like receptors (TLRs), and complement component C5a receptor (C5aR). 6B. Using different mouse models we demonstrated that FLCs are able to mediate antigen-specific mast cell activation. 6C. *In vitro* mast cell activation assays support these *in vivo* data, but FLC-mediated activation can only be detected when mast cells are somewhat pre-activated using IgE cross-linking for example. Mast cell activation leads to either complete or piecemeal degranulation leading to subsequent initiation or progression of several inflammatory disorders (7). 8. Next to the effect on mast cells, FLCs also affect dorsal root ganglion (DRG) function. Whether FLCs also bind to, and activate, other cell types like basophils is subject of ongoing research. 9. In contrast to the immune activating effects of FLCs described above, several studies indicated the involvement of FLCs in suppressing immune responses in models for cow's milk allergy and viral myocarditis. Mechanisms underlying these suppressive effects are still unclear.

OTHER EFFECTS OF FLCs

FLCs have been shown to display other biological activities. Proteolytic activity for different substrates has been shown for several monoclonal FLCs and for recombinant light chain variable fragments [173,174]. A study by Sun *et al.* demonstrated that the immune system can generate FLCs containing catalytic sites in response to polypeptide antigens enabling FLCs to hydrolyze proteins in an antigen-specific manner [175]. Another study showed that lambda FLC dimers, not control kappa or lambda light chains, contributed to the initiation of the alternative complement pathway activation in a patient suffering from membranoproliferative glomerulonephritis [176]. Yet, it is unknown whether this lambda FLC capacity can be found in other patients/conditions as well.

Other functions of FLCs are based on their ability to bind other cells than mast cells, such as neuronal cells and macrophages. Antigen-specific FLCs can bind to, and activate, murine dorsal root ganglion neurons upon application of appropriate antigen as measured by increases in intracellular Ca^{2+} [177]. This might indicate that FLCs are involved in local neurogenic inflammation and centrally mediated sensations such as pain. Binding of FLCs to macrophages is suggested by early studies on TCF. TCF was shown to bind to macrophages which subsequently the rapid release of TNF- α , IL-1, IL-6, PGE2, and nitric oxide (NO) [178-180]. In contrast to FLC-mediated mast cell mediator release, the TCF-primed macrophages produced these mediators in the absence of a cross-linking reagent. Whether FLCs were the actual factors present in the TCF fraction responsible for these effects on macrophages is unclear. Non-antigen specific FLCs also influence some essential neutrophil functions as it was demonstrated that FLCs significantly inhibited chemotactic neutrophil movement, reduced the activation of neutrophils, and attenuate neutrophil apoptosis [181,182]. To what extent these biological actions of FLCs contribute to the development of an immune response or pathological conditions warrants further study. Additionally, basophils are other interesting cells to investigate as putative target for FLC-mediated responses and their involvement in allergy and immunoregulation [183,184].

CONCLUDING REMARKS

Immunoglobulin free light chains are physiologically present in different human body fluids and increases in FLC concentrations are found in multiple disorders. The functional relevance of these increases is still not completely understood. As described in this review, our research implicates an important role for FLCs in mast cell-mediated, pro-inflammatory immune reactions particularly early life responses to CMA and adult allergic diseases like asthma, arthritis and IBD. Fig. (2) schematically summarizes current knowledge on FLCs and its involvement in immune responses based on the studies described in this review. Recent research by our group shows that FLCs may synergize with IgE-mediated mast cell activation [185]. The exact working mechanism of FLC-mediated mast cell activation is still unclear. Pivotal to a better understanding of FLC-mediated responses is the identification of a receptor for FLCs. Moreover, also the mechanism of FLC-mediated immunosuppressive actions needs further investigation.

In conclusion, current knowledge on the biological activity of FLCs obtained in *in vitro* research, and preclinical and clinical studies suggests that FLCs possibly affect multiple cell types, including mast cells in physiological and pathological conditions. Considering the pleiotropic biological effects FLCs, it would be interesting to investigate the therapeutic value of FLC antagonists such as F991 in further detail.

REFERENCES

- [1] Dudeck A, Dudeck J, Scholten J, *et al.* Mast Cells Are Key Promoters of Contact Allergy that Mediate the Adjuvant Effects of Haptens. *Immunity* 2011; 34(6): 973-84.
- [2] Galli SJ, Nakae S, Tsai M. Mast cells in the development of adaptive immune responses. *Nat Immunol* 2005; 6(2): 135-42.
- [3] Metz M, Maurer M. Mast cells - key effector cells in immune responses. *Trends Immunol* 2007; 28(5): 234-41.
- [4] Galli SJ, Grimaldeston M, Tsai M. Immunomodulatory mast cells: negative, as well as positive, regulators of immunity. *Nat Rev Immunol* 2008; 8(6): 478-86.
- [5] Kinet JP. The essential role of mast cells in orchestrating inflammation. *Immunol Rev* 2007; 217: 5-7.
- [6] Mazzoni A, Siraganian RP, Leifer CA, Segal DM. Dendritic cell modulation by mast cells controls the Th1/Th2 balance in responding T cells. *J Immunol* 2006; 177(6): 3577-81.
- [7] Metz M, Grimaldeston MA, Nakae S, Piliponsky AM, Tsai M, Galli SJ. Mast cells in the promotion and limitation of chronic inflammation. *Immunol Rev* 2007; 217: 304-28.
- [8] Sayed BA, Brown MA. Mast cells as modulators of T-cell responses. *Immunol Rev* 2007; 217: 53-64.
- [9] Sayed BA, Christy A, Quirion MR, Brown MA. The master switch: the role of mast cells in autoimmunity and tolerance. *Annu Rev Immunol* 2008; 26: 705-39.
- [10] Grimaldeston MA, Nakae S, Kalesnikoff J, Tsai M, Galli SJ. Mast cell-derived interleukin 10 limits skin pathology in contact dermatitis and chronic irradiation with ultraviolet B. *Nat Immunol* 2007; 8(10): 1095-104.
- [11] Hart PH, Grimaldeston MA, Swift GJ, Sedgwick JD, Korner H, Finlay-Jones JJ. TNF modulates susceptibility to UVB-induced systemic immunomodulation in mice by effects on dermal mast cell prevalence. *Eur J Immunol* 1998; 28(9): 2893-901.
- [12] Lu LF, Lind EF, Gondek DC, *et al.* Mast cells are essential intermediaries in regulatory T-cell tolerance. *Nature* 2006; 442(7106): 997-1002.
- [13] Ullrich SE, Nghiem DX, Khaskina P. Suppression of an established immune response by UVA--a critical role for mast cells. *Photochem Photobiol* 2007; 83(5): 1095-100.
- [14] Metcalfe DD, Baram D, Mekori YA. Mast cells. *Physiol Rev* 1997; 77(4): 1033-79.
- [15] Gould HJ, Sutton BJ, Beavil AJ, *et al.* The biology of IgE and the basis of allergic disease. *Annu Rev Immunol* 2003; 21: 579-628.
- [16] Ptak W, Askenase PW, Rosenstein RW, Gershon RK. Transfer of an antigen-specific immediate hypersensitivity-like reaction with an antigen-binding factor produced by T cells. *Proc Natl Acad Sci USA* 1982; 79(6): 1969-73.
- [17] Askenase PW, Rosenstein RW, Ptak W. T cells produce an antigen-binding factor with *in vivo* activity analogous to IgE antibody. *J Exp Med* 1983; 157(3): 862-73.
- [18] Redegeld FA, van der Heijden MW, Kool M, *et al.* Immunoglobulin-free light chains elicit immediate hypersensitivity-like responses. *Nat Med* 2002; 8(7): 694-701.
- [19] Paliwal V, Tsuji RF, Szczepanik M, *et al.* Subunits of IgM reconstitute defective contact sensitivity in B-1 cell-deficient mice: kappa light chains recruit T cells independent of complement. *J Immunol* 2002; 169(8): 4113-23.
- [20] Gershon RK, Askenase PW, Gershon MD. Requirement for vasoactive amines for production of delayed-type hypersensitivity skin reactions. *J Exp Med* 1975; 142(3): 732-47.
- [21] Schwartz A, Askenase PW, Gershon RK. The effect of locally injected vasoactive amines on the elicitation of delayed-type hypersensitivity. *J Immunol* 1977; 118(1): 159-65.
- [22] Tamir H, Theoharides TC, Gershon MD, Askenase PW. Serotonin storage pools in basophil leukemia and mast cells: characterization of two types of serotonin binding protein and radioautographic analysis of the intracellular distribution of [^3H]serotonin. *J Cell Biol* 1982; 93(3): 638-47.
- [23] Askenase PW, Bursztajn S, Gershon MD, Gershon RK. T cell-dependent mast cell degranulation and release of serotonin in murine delayed-type hypersensitivity. *J Exp Med* 1980; 152(5): 1358-74.
- [24] Kops SK, Van Loveren H, Rosenstein RW, Ptak W, Askenase PW. Mast cell activation and vascular alterations in immediate hypersensitivity-like reactions induced by a T cell-derived antigen-binding factor. *Lab Invest* 1984; 50(4): 421-34.
- [25] Van Loveren H, Kraeuter-Kops S, Askenase PW. Different mechanisms of release of vasoactive amines by mast cells occur in T cell-dependent compared to IgE-dependent cutaneous hypersensitivity responses. *Eur J Immunol* 1984; 14(1): 40-7.

- [26] Theoharides TC, Kops SK, Bondy PK, Askenase PW. Differential release of serotonin without comparable histamine under diverse conditions in the rat mast cell. *Biochem Pharmacol* 1985; 34(9): 1389-98.
- [27] Kraeuter Kops S, Theoharides TC, Cronin CT, Kashgarian MG, Askenase PW. Ultrastructural characteristics of rat peritoneal mast cells undergoing differential release of serotonin without histamine and without degranulation. *Cell Tissue Res* 1990; 262(3): 415-24.
- [28] Theoharides TC, Bondy PK, Tsakalos ND, Askenase PW. Differential release of serotonin and histamine from mast cells. *Nature* 1982; 297(5863): 229-31.
- [29] Askenase PW, Van Loveren H, Rosenstein RW, Ptak W. Immunologic specificity of antigen-binding T cell-derived factors that transfer mast cell-dependent, immediate hypersensitivity-like reactions. *Monogr Allergy* 1983; 18: 249-55.
- [30] Van Loveren H, Ratzlaff RE, Kato K, et al. Immune serum from mice contact-sensitized with picryl chloride contains an antigen-specific T cell factor that transfers immediate cutaneous reactivity. *Eur J Immunol* 1986; 16(10): 1203-8.
- [31] Meade R, Van Loveren H, Parmentier H, Iverson GM, Askenase PW. The antigen-binding T cell factor PCI-F sensitizes mast cells for *in vitro* release of serotonin. Comparison with monoclonal IgE antibody. *J Immunol* 1988; 141(8): 2704-13.
- [32] Van Loveren H, Kato K, Meade R, et al. Characterization of two different Ly-1+ T cell populations that mediate delayed-type hypersensitivity. *J Immunol* 1984; 133(5): 2402-11.
- [33] Ishii N, Takahashi K, Nakajima H, Tanaka S, Askenase PW. DNFB contact sensitivity (CS) in BALB/c and C3H/He mice: requirement for early-occurring, early-acting, antigen-specific, CS-initiating cells with an unusual phenotype (Thy-1+, CD5+, CD3-, CD4-, CD8-, sIg-, B220+, MHC class II-, CD23+, IL-2R-, IL-3R+, Mel-14-, Pgp-1+, J11d+, MAC-1+, LFA-1+, and Fc gamma RII+). *J Invest Dermatol* 1994; 102(3): 321-7.
- [34] Ishii N, Sugita Y, Nakajima H, Tanaka S, Askenase PW. Elicitation of nickel sulfate (NiSO₄)-specific delayed-type hypersensitivity requires early-occurring and early-acting, NiSO₄-specific DTH-initiating cells with an unusual mixed phenotype for an antigen-specific cell. *Cell Immunol* 1995; 161(2): 244-55.
- [35] Geba GP, Wegner CD, Wolyniec WW, Li Y, Askenase PW. Nonatopic asthma: *in vivo* airway hyperreactivity adoptively transferred to naive mice by THY-1(+) and B220(+) antigen-specific cells that lack surface expression of CD3. *J Clin Invest* 1997; 100(3): 629-38.
- [36] Szczepanik M, Akahira-Azuma M, Bryniarski K, et al. B-1 B cells mediate required early T cell recruitment to elicit protein-induced delayed-type hypersensitivity. *J Immunol* 2003; 171(11): 6225-35.
- [37] Campos RA, Szczepanik M, Itakura A, et al. Cutaneous immunization rapidly activates liver invariant Valpha14 NKT cells stimulating B-1 B cells to initiate T cell recruitment for elicitation of contact sensitivity. *J Exp Med* 2003; 198(12): 1785-96.
- [38] Itakura A, Szczepanik M, Campos RA, et al. An hour after immunization peritoneal B-1 cells are activated to migrate to lymphoid organs where within 1 day they produce IgM antibodies that initiate elicitation of contact sensitivity. *J Immunol* 2005; 175(11): 7170-8.
- [39] Campos RA, Szczepanik M, Itakura A, et al. Interleukin-4-dependent innate collaboration between iNKT cells and B-1 B cells controls adaptative contact sensitivity. *Immunology* 2006; 117(4): 536-47.
- [40] Campos RA, Szczepanik M, Lisbonne M, Itakura A, Leite-de-Moraes M, Askenase PW. Invariant NKT cells rapidly activated via immunization with diverse contact antigens collaborate *in vitro* with B-1 cells to initiate contact sensitivity. *J Immunol* 2006; 177(6): 3686-94.
- [41] Kerfoot SM, Szczepanik M, Tung JW, Askenase PW. Identification of initiator B cells, a novel subset of activation-induced deaminase-dependent B-1-like cells that mediate initiation of contact sensitivity. *J Immunol* 2008; 181(3): 1717-27.
- [42] Tsuji RF, Kikuchi M, Askenase PW. Possible involvement of C5/C5a in the efferent and elicitation phases of contact sensitivity. *J Immunol* 1996; 156(12): 4444-50.
- [43] Tsuji RF, Geba GP, Wang Y, Kawamoto K, Matis LA, Askenase PW. Required early complement activation in contact sensitivity with generation of local C5-dependent chemotactic activity, and late T cell interferon gamma: a possible initiating role of B cells. *J Exp Med* 1997; 186(7): 1015-26.
- [44] Tsuji RF, Kawikova I, Ramabhadran R, et al. Early local generation of C5a initiates the elicitation of contact sensitivity by leading to early T cell recruitment. *J Immunol* 2000; 165(3): 1588-98.
- [45] Galli SJ, Hammel I. Unequivocal delayed hypersensitivity in mast cell-deficient and beige mice. *Science* 1984; 226(4675): 710-3.
- [46] Biedermann T, Kneilling M, Mailhammer R, et al. 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* 2000; 192(10): 1441-52.
- [47] Askenase PW, Van Loveren H, Kraeuter-Kops S, et al. Defective elicitation of delayed-type hypersensitivity in W/W^v and SI/Sld mast cell-deficient mice. *J Immunol* 1983; 131(6): 2687-94.
- [48] van Loveren H, Meade R, Askenase PW. An early component of delayed-type hypersensitivity mediated by T cells and mast cells. *J Exp Med* 1983; 157(5): 1604-17.
- [49] Askenase PW, Metzler CM, Gershon RK. Localization of leucocytes in sites of delayed-type hypersensitivity and in lymph nodes: dependence on vasoactive amines. *Immunology* 1982; 47(2): 239-46.
- [50] Galli SJ, Tsai M, Piliponsky AM. The development of allergic inflammation. *Nature* 2008; 454(7203): 445-54.
- [51] Kops SK, Van Loveren H, Rosenstein RW, Ptak W, Askenase PW. Mast cell activation and vascular alterations in immediate hypersensitivity-like reactions induced by a T cell-derived antigen-binding factor. *Lab Invest* 1984; 50(4): 421-34.
- [52] Basnayake K, Stringer SJ, Hutchison CA, Cockwell P. The biology of immunoglobulin free light chains and kidney injury. *Kidney Int* 2011; 79(12): 1289-301.
- [53] Huang ZQ, Sanders PW. Localization of a single binding site for immunoglobulin light chains on human Tamm-Horsfall glycoprotein. *J Clin Invest* 1997; 99(4): 732-6.
- [54] Maizels N. Immunoglobulin gene diversification. *Annu Rev Genet* 2005; 39: 23-46.
- [55] Pilstrom L. The mysterious immunoglobulin light chain. *Dev Comp Immunol* 2002; 26(2): 207-15.
- [56] Katzmman JA, Clark RJ, Abraham RS, et al. Serum reference intervals and diagnostic ranges for free kappa and free lambda immunoglobulin light chains: relative sensitivity for detection of monoclonal light chains. *Clin Chem* 2002; 48(9): 1437-44.
- [57] Woloschak GE, Krco CJ. Regulation of kappa/lambda immunoglobulin light chain expression in normal murine lymphocytes. *Mol Immunol* 1987; 24(7): 751-7.
- [58] Hopper JE, Papagiannes E. Evidence by radioimmunoassay that mitogen-activated human blood mononuclear cells secrete significant amounts of light chain Ig unassociated with heavy chain. *Cell Immunol* 1986; 101(1): 122-31.
- [59] Shapiro AL, Scharff MD, Maizel JV, Uhr JW. Synthesis of excess light chains of gamma globulin by rabbit lymph node cells. *Nature* 1966; 211(5046): 243-5.
- [60] Hannam-Harris AC, Smith JL. Induction of balanced immunoglobulin chain synthesis in free light chain-producing lymphocytes by mitogen stimulation. *J Immunol* 1981; 126(5): 1848-51.
- [61] Waldmann TA, Strober W, Mogelnicki RP. The renal handling of low molecular weight proteins. II. Disorders of serum protein catabolism in patients with tubular proteinuria, the nephrotic syndrome, or uremia. *J Clin Invest* 1972; 51(8): 2162-74.
- [62] Skvortsov VT, Gurvich AE. Relative rates of synthesis of immunoglobulins and light chains in rabbit spleen cells during secondary response. *Nature* 1968; 218(5139): 377-8.
- [63] Hannam-Harris AC, Gordon J, Smith JL. Immunoglobulin synthesis by neoplastic B lymphocytes: free light chain synthesis as a marker of B cell differentiation. *J Immunol* 1980; 125(5): 2177-81.
- [64] Hendershot L, Bole D, Kohler G, Kearney JF. Assembly and secretion of heavy chains that do not associate posttranslationally with immunoglobulin heavy chain-binding protein. *J Cell Biol* 1987; 104(3): 761-7.
- [65] Askenase PW, Tsuji RF. B-1 B cell IgM antibody initiates T cell elicitation of contact sensitivity. *Curr Top Microbiol Immunol* 2000; 252: 171-7.
- [66] Vakaet A, Thompson EJ. Free light chains in the cerebrospinal fluid: an indicator of recent immunological stimulation. *J Neurol Neurosurg Psychiatry* 1985; 48(10): 995-998.
- [67] Contini C, Fainardi E, Cultrera R, et al. Evidence of cerebrospinal fluid free kappa light chains in AIDS patients with Toxoplasma gondii encephalitis. *J Neuroimmunol* 2000; 108(1-2): 221-6.

- [68] Tsuji RF, Szczepanik M, Kawikova I, *et al.* B cell-dependent T cell responses: IgM antibodies are required to elicit contact sensitivity. *J Exp Med* 2002; 196(10): 1277-90.
- [69] Solling K. Normal values for free light chains in serum different age groups. *Scand J Clin Lab Invest* 1977; 37(1): 21-5.
- [70] Cohen SB, Emery P, Greenwald MW, *et al.* Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: Results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks. *Arthritis Rheum* 2006; 54(9): 2793-806.
- [71] Cohen G, Rudnicki M, Schmaldienst S, Horl WH. Effect of dialysis on serum/plasma levels of free immunoglobulin light chains in end-stage renal disease patients. *Nephrol Dial Transplant* 2002; 17(5): 879-83.
- [72] Wolff F, Thiry C, Willems D. Assessment of the analytical performance and the sensitivity of serum free light chains immunoassay in patients with monoclonal gammopathy. *Clin Biochem* 2007; 40(5-6): 351-4.
- [73] Redegeld FA, Nijkamp FP. Immunoglobulin free light chains and mast cells: pivotal role in T-cell-mediated immune reactions? *Trends Immunol* 2003; 24(4): 181-5.
- [74] Kraneveld AD, Kool M, van Houwelingen AH, *et al.* Elicitation of allergic asthma by immunoglobulin free light chains. *Proc Natl Acad Sci USA* 2005; 102(5): 1578-83.
- [75] Rijnerse A, Redegeld FA, Blokhuis BR, *et al.* Ig-Free Light Chains Play a Crucial Role in Murine Mast Cell-Dependent Colitis and Are Associated with Human Inflammatory Bowel Diseases. *J Immunol* 2010; 185(1): 653-9.
- [76] Schouten B, van Esch BC, van Thuijl AO, *et al.* Contribution of IgE and immunoglobulin free light chain in the allergic reaction to cow's milk proteins. *J Allergy Clin Immunol* 2010; 125(6): 1308-14.
- [77] van Esch BC, Schouten B, Blokhuis BR, *et al.* Depletion of CD4(+)CD25(+) T cells switches the whey-allergic response from immunoglobulin E- to immunoglobulin free light chain-dependent. *Clin Exp Allergy* 2010; 40(9): 1414-21.
- [78] Nakano T, Takahashi H, Miyazaki S, *et al.* Antigen-specific free immunoglobulin light-chain antibodies: could it be a new diagnostic marker for patients with allergy? *Clin Biochem* 2006; 39(9): 955-9.
- [79] Powe DG, Groot Kormelink T, Sisson M, *et al.* Evidence for the involvement of free light chain immunoglobulins in allergic and nonallergic rhinitis. *J Allergy Clin Immunol* 2010; 125(1): 139-45 e1-3.
- [80] Groot Kormelink T, Tekstra J, Thurlings RM, *et al.* Decrease in immunoglobulin free light chains in patients with rheumatoid arthritis upon rituximab (anti-CD20) treatment correlates with decrease in disease activity. *Ann Rheum Dis* 2010; 69: 2137-44.
- [81] Redegeld FA, Wortel CH. IgE and immunoglobulin free light chains in allergic disease: new therapeutic opportunities. *Curr Opin Investig Drugs* 2008; 9(11): 1185-91.
- [82] Gould HJ, Sutton BJ. IgE in allergy and asthma today. *Nat Rev Immunol* 2008; 8(3): 205-17.
- [83] Von Bubnoff D, Novak N, Kraft S, Bieber T. The central role of Fc γ RI in allergy. *Clin Exp Dermatol* 2003; 28(2): 184-7.
- [84] Holgate S, Casale T, Wenzel S, Bousquet J, Deniz Y, Reisner C. The anti-inflammatory effects of omalizumab confirm the central role of IgE in allergic inflammation. *J Allergy Clin Immunol* 2005; 115(3): 459-65.
- [85] Galli SJ, Kalesnikoff J, Grimbaldston MA, Piliponsky AM, Williams CM, Tsai M. Mast cells as "tunable" effector and immunoregulatory cells: recent advances. *Annu Rev Immunol* 2005; 23: 749-86.
- [86] Groot Kormelink T, Thio M, Blokhuis BR, Nijkamp FP, Redegeld FA. Atopic and non-atopic allergic disorders: current insights into the possible involvement of free immunoglobulin light chains. *Clin Exp Allergy* 2009; 39(1): 33-42.
- [87] Marshall JS. Mast-cell responses to pathogens. *Nat Rev Immunol* 2004; 4(10): 787-99.
- [88] Mekori YA, Baram D. Heterotypic adhesion-induced mast cell activation: biologic relevance in the inflammatory context. *Mol Immunol* 2002; 38(16-18): 1363-7.
- [89] Theoharides TC, Kalogeromitros D. The critical role of mast cells in allergy and inflammation. *Ann N Y Acad Sci* 2006; 1088: 78-99.
- [90] Schafer T, Bohler E, Ruhdorfer S, *et al.* Epidemiology of food allergy/food intolerance in adults: associations with other manifestations of atopy. *Allergy* 2001; 56(12): 1172-9.
- [91] Host A, Halken S. Epidemiology and prevention of cow's milk allergy. *Allergy* 1998; 53(46 Suppl): 111-3.
- [92] Schouten B, van Esch BC, Hofman GA, van den Elsen LW, Willemssen LE, Garssen J. Acute Allergic Skin Reactions and Intestinal Contractility Changes in Mice Orally Sensitized against Casein or Whey. *Int Arch Allergy Immunol* 2008; 147(2): 125-34.
- [93] Theoharides TC, Kempuraj D, Tegen M, Conti P, Kalogeromitros D. Differential release of mast cell mediators and the pathogenesis of inflammation. *Immunol Rev* 2007; 217: 65-78.
- [94] Kraneveld AD, van der Kleij HP, Kool M, *et al.* Key role for mast cells in nonatopic asthma. *J Immunol* 2002; 169(4): 2044-53.
- [95] Neuman MG. Immune dysfunction in inflammatory bowel disease. *Translational Research* 2007 4; 149(4): 173-86.
- [96] He SH. Key role of mast cells and their major secretory products in inflammatory bowel disease. *World J Gastroenterol* 2004; 10(3): 309-18.
- [97] Collins SM. Similarities and dissimilarities between asthma and inflammatory bowel diseases. *Aliment Pharmacol Ther* 1996; 10 Suppl 2: 25-31.
- [98] Rijnerse A, Koster AS, Nijkamp FP, Kraneveld AD. Critical role for mast cells in the pathogenesis of 2,4-dinitrobenzene-induced murine colonic hypersensitivity reaction. *J Immunol* 2006; 176(7): 4375-84.
- [99] Amin K, Ludviksdottir D, Janson C, *et al.* Inflammation and structural changes in the airways of patients with atopic and nonatopic asthma. BHR Group. *Am J Respir Crit Care Med* 2000; 162(6): 2295-301.
- [100] Powe DG, Huskisson RS, Carney AS, Jenkins D, Jones NS. Evidence for an inflammatory pathophysiology in idiopathic rhinitis. *Clin Exp Allergy* 2001; 31(6): 864-72.
- [101] Amin K, Rinne J, Hahtela T, *et al.* Inflammatory cell and epithelial characteristics of perennial allergic and nonallergic rhinitis with a symptom history of 1 to 3 years' duration. *J Allergy Clin Immunol* 2001; 107(2): 249-57.
- [102] Jacobs RL, Freedman PM, Boswell RN. Nonallergic rhinitis with eosinophilia (NARES syndrome). Clinical and immunologic presentation. *J Allergy Clin Immunol* 1981; 67(4): 253-62.
- [103] Powe DG, Huskisson RS, Carney AS, *et al.* Mucosal T-cell phenotypes in persistent atopic and nonatopic rhinitis show an association with mast cells. *Allergy* 2004; 59(2): 204-12.
- [104] Looney RJ, Anolik J, Sanz I. B cells as therapeutic targets for rheumatic diseases. *Curr Opin Rheumatol* 2004; 16(3): 180-5.
- [105] Edwards JC, Cambridge G. B-cell targeting in rheumatoid arthritis and other autoimmune diseases. *Nat Rev Immunol* 2006; 6(5): 394-403.
- [106] Youinou P, Taher TE, Pers JO, Mageed RA, Renaudineau Y. B lymphocyte cytokines and rheumatic autoimmune disease. *Arthritis Rheum* 2009; 60(7): 1873-80.
- [107] Lee DM, Friend DS, Gurish MF, Benoist C, Mathis D, Brenner MB. Mast cells: a cellular link between autoantibodies and inflammatory arthritis. *Science* 2002; 297(5587): 1689-92.
- [108] Woolley DE. The mast cell in inflammatory arthritis. *N Engl J Med* 2003; 348(17): 1709-11.
- [109] Nigrovic PA, Lee DM. Synovial mast cells: role in acute and chronic arthritis. *Immunol Rev* 2007; 217: 19-37.
- [110] Nigrovic PA, Binstadt BA, Monach PA, *et al.* Mast cells contribute to initiation of autoantibody-mediated arthritis via IL-1. *Proc Natl Acad Sci USA* 2007; 104(7): 2325-30.
- [111] Gottenberg JE, Aucouturier F, Goetz J, *et al.* Serum immunoglobulin free light chain assessment in rheumatoid arthritis and primary Sjogren's syndrome. *Ann Rheum Dis* 2007; 66(1): 23-7.
- [112] Solling K, Solling J, Romer FK. Free light chains of immunoglobulins in serum from patients with rheumatoid arthritis, sarcoidosis, chronic infections and pulmonary cancer. *Acta Med Scand* 1981; 209(6): 473-7.
- [113] Bradwell AR, Carr-Smith HD, Mead GP, *et al.* Highly sensitive, automated immunoassay for immunoglobulin free light chains in serum and urine. *Clin Chem* 2001; 47(4): 673-80.
- [114] Dispenzieri A, Lacy MQ, Katzmann JA, *et al.* Absolute values of immunoglobulin free light chains are prognostic in patients with primary systemic amyloidosis undergoing peripheral blood stem cell transplantation. *Blood* 2006; 107(8): 3378-83.

- [115] Katzmman JA, Abraham RS, Dispenzieri A, Lust JA, Kyle RA. Diagnostic performance of quantitative kappa and lambda free light chain assays in clinical practice. *Clin Chem* 2005; 51(5): 878-81.
- [116] Fagnart OC, Sindic CJ, Laterre C. Free kappa and lambda light chain levels in the cerebrospinal fluid of patients with multiple sclerosis and other neurological diseases. *J Neuroimmunol* 1988; 19(1-2): 119-32.
- [117] Moutsopoulos HM, Steinberg AD, Fauci AS, Lane HC, Papadopoulos NM. High incidence of free monoclonal lambda light chains in the sera of patients with Sjogren's syndrome. *J Immunol* 1983; 130(6): 2663-5.
- [118] Mehta PD, Cook SD, Troiano RA, Coyle PK. Increased free light chains in the urine from patients with multiple sclerosis. *Neurology* 1991; 41(4): 540-4.
- [119] Hopper JE, Sequeira W, Martellotto J, Papagiannes E, Perna L, Skosey JL. Clinical relapse in systemic lupus erythematosus: correlation with antecedent elevation of urinary free light-chain immunoglobulin. *J Clin Immunol* 1989; 9(4): 338-50.
- [120] Goffette S, Schluep M, Henry H, Duprez T, Sindic CJ. Detection of oligoclonal free kappa chains in the absence of oligoclonal IgG in the CSF of patients with suspected multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2004; 75(2): 308-10.
- [121] Jenkins MA, Cheng L, Ratnaike S. Multiple sclerosis: use of light-chain typing to assist diagnosis. *Ann Clin Biochem* 2001; 38(Pt 3): 235-41.
- [122] Fischer C, Armeth B, Koehler J, Lotz J, Lackner KJ. Kappa free light chains in cerebrospinal fluid as markers of intrathecal immunoglobulin synthesis. *Clin Chem* 2004; 50(10): 1809-13.
- [123] Romer FK, Solling K. Repeated measurements of serum immunoglobulin-free light chains in early sarcoidosis. *Eur J Respir Dis* 1984; 65(4): 292-5.
- [124] Gallo P, Piccinno MG, De Rossi A, et al. Free light chains of immunoglobulins in the cerebrospinal fluid of human immunodeficiency virus type 1-infected patients. *J Neuroimmunol* 1990; 26(3): 229-38.
- [125] Kayserova J, Capkova S, Skalicka A, et al. Serum immunoglobulin free light chains in severe forms of atopic dermatitis. *Scand J Immunol* 2010; 71(4): 312-6.
- [126] Rinker JR, 2nd, Trinkaus K, Cross AH. Elevated CSF free kappa light chains correlate with disability prognosis in multiple sclerosis. *Neurology* 2006; 67(7): 1288-90.
- [127] Grimbaldston MA, Metz M, Yu M, Tsai M, Galli SJ. Effector and potential immunoregulatory roles of mast cells in IgE-associated acquired immune responses. *Curr Opin Immunol* 2006; 18(6): 751-60.
- [128] St John AL, Rathore AP, Yap H, et al. Immune surveillance by mast cells during dengue infection promotes natural killer (NK) and NKT-cell recruitment and viral clearance. *Proc Natl Acad Sci USA* 2011; 108(22): 9190-5.
- [129] Abraham SN, St John AL. Mast cell-orchestrated immunity to pathogens. *Nat Rev Immunol* 2010; 10(6): 440-52.
- [130] de Vries VC, Noelle RJ. Mast cell mediators in tolerance. *Curr Opin Immunol* 2010; 22(5): 643-8.
- [131] Enoksson M, Lyberg K, Moller-Westerberg C, Fallon PG, Nilsson G, Lunderius-Andersson C. Mast cells as sensors of cell injury through IL-33 recognition. *J Immunol* 2011; 186(4): 2523-8.
- [132] Nathan C. Points of control in inflammation. *Nature* 2002; 420(6917): 846-52.
- [133] Maurer M, Theoharides T, Granstein RD, et al. What is the physiological function of mast cells? *Exp Dermatol* 2003; 12(6): 886-910.
- [134] Brown JM, Wilson TM, Metcalfe DD. The mast cell and allergic diseases: role in pathogenesis and implications for therapy. *Clin Exp Allergy* 2008; 38(1): 4-18.
- [135] Christy AL, Brown MA. The multitasking mast cell: positive and negative roles in the progression of autoimmunity. *J Immunol* 2007; 179(5): 2673-9.
- [136] Benoist C, Mathis D. Mast cells in autoimmune disease. *Nature* 2002; 420(6917): 875-8.
- [137] Constantinides P. Infiltrates of activated mast cells at the site of coronary atheromatous erosion or rupture in myocardial infarction. *Circulation* 1995; 92(5): 1083.
- [138] Lindstedt KA, Kovanen PT. Mast cells in vulnerable coronary plaques: potential mechanisms linking mast cell activation to plaque erosion and rupture. *Curr Opin Lipidol* 2004; 15(5): 567-73.
- [139] Vanderlaan PA, Reardon CA. Thematic review series: the immune system and atherogenesis. The unusual suspects: an overview of the minor leukocyte populations in atherosclerosis. *J Lipid Res* 2005; 46(5): 829-38.
- [140] Fairley JA, Burnett CT, Fu CL, Larson DL, Fleming MG, Giudice GJ. A pathogenic role for IgE in autoimmunity: bullous pemphigoid IgE reproduces the early phase of lesion development in human skin grafted to nu/nu mice. *J Invest Dermatol* 2007; 127(11): 2605-11.
- [141] Dimson OG, Giudice GJ, Fu CL, et al. Identification of a potential effector function for IgE autoantibodies in the organ-specific autoimmune disease bullous pemphigoid. *J Invest Dermatol* 2003; 120(5): 784-8.
- [142] Schuerwegh AJ, Ioan-Facsinay A, Dorjee AL, et al. Evidence for a functional role of IgE anticitrullinated protein antibodies in rheumatoid arthritis. *Proc Natl Acad Sci USA* 2010; 107(6): 2586-91.
- [143] Ludgate M, Baker G. Unlocking the immunological mechanisms of orbital inflammation in thyroid eye disease. *Clin Exp Immunol* 2002; 127(2): 193-8.
- [144] Galinsky DS, Nechushtan H. Mast cells and cancer-No longer just basic science. *Crit Rev Oncol Hematol* 2008; 68(2): 115-30.
- [145] Murdoch C, Muthana M, Coffelt SB, Lewis CE. The role of myeloid cells in the promotion of tumour angiogenesis. *Nat Rev Cancer* 2008; 8(8): 618-31.
- [146] Ribatti D, Vacca A, Nico B, Crivellato E, Roncali L, Dammacco F. The role of mast cells in tumour angiogenesis. *Br J Haematol* 2001; 115(3): 514-21.
- [147] Theoharides TC, Conti P. Mast cells: the Jekyll and Hyde of tumor growth. *Trends Immunol* 2004; 25(5): 235-41.
- [148] Groot Kormelink T, Abudukelimu A, Redegeld FA. Mast cells as target in cancer therapy. *Curr Pharm Des* 2009; 15(16): 1868-78.
- [149] Starkey JR, Crowle PK, Taubenberger S. Mast-cell-deficient W/W^v mice exhibit a decreased rate of tumor angiogenesis. *Int J Cancer* 1988; 42(1): 48-52.
- [150] Coussens LM, Raymond WW, Bergers G, et al. Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. *Genes Dev* 1999; 13(11): 1382-97.
- [151] Soucek L, Lawlor ER, Soto D, Shchors K, Swigart LB, Evan GI. Mast cells are required for angiogenesis and macroscopic expansion of Myc-induced pancreatic islet tumors. *Nat Med* 2007; 13(10): 1211-8.
- [152] Oldford SA, Haidl ID, Howatt MA, Leiva CA, Johnston B, Marshall JS. A critical role for mast cells and mast cell-derived IL-6 in TLR2-mediated inhibition of tumor growth. *J Immunol* 2010; 185(11): 7067-76.
- [153] Johansson A, Rudolfsson S, Hammarsten P, et al. Mast cells are novel independent prognostic markers in prostate cancer and represent a target for therapy. *Am J Pathol* 2010; 177(2): 1031-41.
- [154] Welsh TJ, Green RH, Richardson D, Waller DA, O'Byrne KJ, Bradding P. Macrophage and mast-cell invasion of tumor cell islets confers a marked survival advantage in non-small-cell lung cancer. *J Clin Oncol* 2005; 23(35): 8959-67.
- [155] Maltby S, Freeman S, Gold MJ, et al. Opposing Roles for CD34 in B16 Melanoma Tumor Growth Alter Early Stage Vasculature and Late Stage Immune Cell Infiltration. *PLoS One* 2011; 6(4): e18160.
- [156] Rajput AB, Turbin DA, Cheang MC, et al. Stromal mast cells in invasive breast cancer are a marker of favourable prognosis: a study of 4,444 cases. *Breast Cancer Res Treat* 2008; 107(2): 249-57.
- [157] Nielsen HJ, Hansen U, Christensen IJ, Reimert CM, Brunner N, Moesgaard F. Independent prognostic value of eosinophil and mast cell infiltration in colorectal cancer tissue. *J Pathol* 1999; 189(4): 487-95.
- [158] Tomita M, Matsuzaki Y, Onitsuka T. Correlation between mast cells and survival rates in patients with pulmonary adenocarcinoma. *Lung Cancer* 1999; 26(2): 103-8.
- [159] Kawakami T. A crucial door to the mast cell mystery knocked in. *J Immunol* 2009; 183(11): 6861-2.
- [160] Zhou JS, Xing W, Friend DS, Austen KF, Katz HR. Mast cell deficiency in Kit(W^{sh}) mice does not impair antibody-mediated arthritis. *J Exp Med* 2007; 204(12): 2797-802.
- [161] van den Broek MF, van den Berg WB, van de Putte LB. The role of mast cells in antigen induced arthritis in mice. *J Rheumatol* 1988; 15(4): 544-51.
- [162] Pitman N, Asquith DL, Murphy G, Liew FY, McInnes IB. Collagen-induced arthritis is not impaired in mast cell-deficient mice. *Ann Rheum Dis* 2011; 70(6): 1170-1.

- [163] Secor VH, Secor WE, Gutekunst CA, Brown MA. Mast cells are essential for early onset and severe disease in a murine model of multiple sclerosis. *J Exp Med* 2000; 191(5): 813-22.
- [164] Stelekati E, Bahri R, D'Orlando O, *et al.* Mast cell-mediated antigen presentation regulates CD8⁺ T cell effector functions. *Immunity* 2009; 31(4): 665-76.
- [165] Piconese S, Costanza M, Musio S, *et al.* Exacerbated experimental autoimmune encephalomyelitis in mast-cell-deficient Kit W-sh/W-sh mice. *Lab Invest* 2011; 91(4): 627-41.
- [166] Bennett JL, Blanchet MR, Zhao L, *et al.* Bone marrow-derived mast cells accumulate in the central nervous system during inflammation but are dispensable for experimental autoimmune encephalomyelitis pathogenesis. *J Immunol* 2009; 182(9): 5507-14.
- [167] Scholten J, Hartmann K, Gerbaulet A, *et al.* Mast cell-specific Cre/loxP-mediated recombination *in vivo*. *Transgenic Res* 2008; 17(2): 307-15.
- [168] Matsumori A, Shimada M, Jie X, Higuchi H, Groot Kormelink T, Redegeld FA. Effects of free immunoglobulin light chains on viral myocarditis. *Circ Res* 2010; 106(9): 1533-40.
- [169] Ptak W, Bereta M, Ptak M, Askenase PW. Isotype-like suppression of T cell-mediated immunity *in vivo*. I. Delayed-type hypersensitivity specificity of T cell suppression induced by antigen-binding T cell factors that initiate contact sensitivity. *J Immunol* 1986; 136(5): 1554-63.
- [170] Garssen J, Nijkamp FP, Wagenaar SS, Zwart A, Askenase PW, Van Loveren H. Regulation of delayed-type hypersensitivity-like responses in the mouse lung, determined with histological procedures: serotonin, T-cell suppressor-inducer factor and high antigen dose tolerance regulate the magnitude of T-cell dependent inflammatory reactions. *Immunology* 1989; 68(1): 51-8.
- [171] Van Loveren H, De Weger RA, Garssen J, Los G, Askenase PW. Impairment of allograft tumor immunity by isotype-like suppression of antigen-specific T cell factors. *Transplantation* 1989; 47(3): 504-12.
- [172] Parmentier HK, Dijkstra JW, Wissink A, Ruitenber EJ, Askenase PW, van Loveren H. Antigen-specific T-cell factors induce isotype-like suppression of mast cell and eosinophil-rich T-cell-dependent inflammation in the intestine of mice infected with *Trichinella spiralis*. *Int Arch Allergy Appl Immunol* 1989; 90(2): 144-54.
- [173] Paul S, Li L, Kalaga R, Wilkins-Stevens P, Stevens FJ, Solomon A. Natural catalytic antibodies: peptide-hydrolyzing activities of Bence Jones proteins and VL fragment. *J Biol Chem* 1995; 270(25): 15257-61.
- [174] Thiagarajan P, Dannenbring R, Matsuura K, Tramontano A, Gololobov G, Paul S. Monoclonal antibody light chain with prothrombinase activity. *Biochemistry* 2000; 39(21): 6459-65.
- [175] Sun M, Gao QS, Li L, Paul S. Proteolytic activity of an antibody light chain. *J Immunol* 1994; 153(11): 5121-6.
- [176] Meri S, Koistinen V, Miettinen A, Tornroth T, Seppala IJ. Activation of the alternative pathway of complement by monoclonal lambda light chains in membranoproliferative glomerulonephritis. *J Exp Med* 1992; 175(4): 939-50.
- [177] Rijnierse A, Kroese AB, Redegeld FA, *et al.* Immunoglobulin-free light chains mediate antigen-specific responses of murine dorsal root ganglion neurons. *J Neuroimmunol* 2009; 208(1-2): 80-6.
- [178] Kops SK, Ratzlaff RE, Meade R, Iverson GM, Askenase PW. Interaction of antigen-specific T cell factors with unique "receptors" on the surface of mast cells: demonstration *in vitro* by an indirect rosetting technique. *J Immunol* 1986; 136(12): 4515-24.
- [179] Ferreri NR, Millet I, Paliwal V, *et al.* Induction of macrophage TNF alpha, IL-1, IL-6, and PGE2 production by DTH-initiating factors. *Cell Immunol* 1991; 137(2): 389-405.
- [180] Redegeld FA, Heijdra B, Knippels MC, Garssen J, Nijkamp FP. Nitric oxide production by macrophages stimulated by antigen-binding T-cell factors. *Immunol Lett* 1997; 57(1-3): 147-9.
- [181] Cohen G, Haag-Weber M, Mai B, Deicher R, Horl WH. Effect of immunoglobulin light chains from hemodialysis and continuous ambulatory peritoneal dialysis patients on polymorphonuclear leukocyte functions. *J Am Soc Nephrol* 1995; 6(6): 1592-9.
- [182] Cohen G. Immunoglobulin light chains in uremia. *Kidney Int Suppl* 2003; (84)(84): S15-8.
- [183] Falcone FH, Knol EF, Gibbs BF. The role of basophils in the pathogenesis of allergic disease. *Clin Exp Allergy* 2011; 41(7): 939-47.
- [184] Knol EF, Olszewski M. Basophils and mast cells: Underdog in immune regulation? *Immunol Lett* 2011; 138(1): 28-31.
- [185] Blokhuis BR, Thio M, Redegeld FA. Immunoglobulin Free Light Chains Synergistically Enhance IgE-Mediated Mast Cell Activation. *Allergy* 2011; 66(94): 498.