

Review

Free immunoglobulin light chains as target in the treatment of chronic inflammatory diseases

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Abstract

Immunoglobulin free light chains were long considered irrelevant bystander products of immunoglobulin synthesis by B lymphocytes. To date, different studies suggest that free light chains may have important functional activities. For instance, it has been shown that immunoglobulin free light chains can elicit mast cell-driven hypersensitivity responses leading to asthma and contact sensitivity. Free light chains also show other biologic actions such as anti-angiogenic and proteolytic activities or can be used as specific targeting vehicles. Levels of free light chain levels in body fluids increase markedly in diseases such as multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus. In this review, we will focus on the unexpected biological activities of immunoglobulin free light chains with special attention to its possible role in the induction of chronic inflammatory diseases. © 2006 Elsevier B.V. All rights reserved.

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1. Free immunoglobulin light chains

Immunoglobulins play an important role in host defence reactions. The immunoglobulins are built from two identical heavy chains and two identical light chains linked by disulfide bonds to form tetrameric structures. The C-terminal part of the heavy chain determines the class and subclass of the immunoglobulins and directs their biological activity. For instance, IgE is considered as the primary immunoglobulin in causing allergic inflammation. Recently, it was uncovered that free immunoglobulin light chains (Kraneveld et al., 2005; Redegeld and Nijkamp, 2003; Redegeld et al., 2002, 2004) can be involved in the induction of hypersensitivity reactions. In this review, we will focus on the features of free immunoglobulin light chains and their possible role in chronic inflammatory disorders.

1.1. Production/presence of free immunoglobulin light chains

Plasma cells produce five different classes of immunoglobulins: IgG, IgM, IgA, IgD, and IgE. In all classes of immunoglobulins either kappa or lambda light chains are incorporated. The N-terminal part of the heavy chains and the light chains contain hypervariable regions (complementarity determining regions), which are important for antigen binding.

Part of the immunoglobulin light chains is not incorporated in tetrameric immunoglobulins and is secreted as free light chains (Hannam-Harris and Smith, 1981; Hopper and Papiagianes, 1986; Shapiro et al., 1966; Skvortsov and Gurvich, 1968). These free light chains can exist as monomers (M.W. 22–27 kDa) and covalently and non-covalently bound dimers (M.W. 44–55 kDa). Polyclonal free light chains can normally be found in many biological fluids including serum, urine, cerebrospinal and synovial fluid (Abe et al., 1998; Bradwell et al., 2001; Cooper and Bluestone, 1968). It is estimated that a normal lymphoid system produces about 500 mg of free light chains per day. Free light chains are cleared by the kidneys through filtration and catabolism (Bradwell, 2003). Circulating kappa monomers are cleared in 2–4 h at 40% of the filtration rate, while dimeric (lambda) light chains are cleared in 3–6 h at 20% of the glomerular filtration rate (Bradwell, 2005). Normal excretion rates for free light chains range from 1–10 mg per 24 h for healthy persons. Abnormal levels of monoclonal free light chains (or Bence Jones proteins) are usually linked to a malignant clonal proliferation of plasma cells leading to a significant increase in free light chain production, serum levels and secretion in urine (> 1 g of Bence Jones proteins in urine per 24 h) (Bradwell, 2003).

1.2. Measurement of immunoglobulin free light chains

Several methods are described to measure free light chain levels in biological fluids (Levinson and Keren, 1994). Methods to determine presence and concentrations of free light chains include protein electrophoresis, immunofixation electrophoresis, capillary zone electrophoresis, enzyme-linked immunosorbent assays (ELISAs), and nephelometric assays (Abe et al.,

1993; Katzmann et al., 2005; Kleine and Damm, 2003; Lafitte et al., 2002; Nakano and Nagata, 2004; Peterson and Berggard, 1971; Tate et al., 2003; Tillyer, 1992). Some of these methods are rather laborious and time-consuming and not suitable for routine analysis. Also, caution must be taken when analyzing free light chain levels in serum or plasma using free light chain-specific antibodies. Measurement of free light chain levels may be inaccurate when the used 'specific' antibodies show (some) crossreactivity with light chains incorporated in intact antibodies (Nakano and Nagata, 2003). Because levels of free light chains in serum are much lower than these in whole immunoglobulins, even a small crossreactivity with whole antibodies may give an overestimation of the free light chain levels (Nakano and Nagata, 2003). Recently, a sensitive nephelometric free light chain assay suitable for routine analysis became commercially available (Bradwell et al., 2001).

The normal range levels of free light chains have been reported in many reports, but show considerable variation amongst different studies (Katzmann et al., 2002; Redegeld and Nijkamp, 2003). Values for serum kappa free light chain range from 1.2 to 21 mg/l and lambda free light chains from 3.0 to 42 mg/l.

1.3. Free light chains in disease

Increased levels of monoclonal free light chains have been measured as the consequence of clonal expansion of plasma cells. These diseases are indicated as monoclonal gammopathies and include multiple myeloma, light chain myeloma, Waldenstrom macroglobulinemia, nonsecretory myeloma, monoclonal gammopathy of undetermined significance, primary systemic amyloidosis, light chain-deposition disease and Sjögren syndrome (Bradwell, 2005; Katzmann et al., 2005; Levinson and Keren, 1994).

Many other (auto)immune/inflammatory diseases are accompanied with increased levels of oligoclonal free light chains

Table 1
Immune disorders associated with increased production of oligoclonal immunoglobulin free light chains

Disease	Immunoglobulin free light chains measured in	References
AIDS/HIV	Cerebrospinal fluid, serum	Contini et al. (2000), Elovaaara et al. (1991), Gallo et al. (1990)
Asthma	Plasma	Kraneveld et al. (2005)
Allergic disorders	Serum, urine	Cooper and Bluestone (1968)
Meningitis	Cerebrospinal fluid	Bollengier (1979), Fagnart et al. (1988)
Multiple sclerosis	Cerebrospinal fluid, serum, urine	Fagnart et al. (1988), Fischer et al. (2004), Goffette et al. (2004), Jenkins et al. (2001), Lamers et al. (1995), Lolli et al. (1991), Mehta et al. (1991)
Rheumatoid arthritis	Serum, urine, synovial fluid	Cooper and Bluestone (1968), Solling et al. (1981)
Sarcoidosis	Serum	Romer and Solling (1984)
Systemic lupus	Urine	Hopper et al. (2000), Hopper et al. (1989)

in different body fluids (Table 1). Several studies suggest that rises in free light chain levels appear to be associated with relapses of disease for example in case of systemic lupus erythematosus (Hopper et al., 2000, 1989), multiple sclerosis (Constantinescu et al., 1994; Rudick et al., 1995; Vakaet and Thompson, 1985), and rheumatoid arthritis (Solling et al., 1981). The increases in free light chain levels may indicate increased polyclonal B cell activity and hypersecretion of (auto)antibodies or increased local synthesis (e.g. intrathecal).

We have found that the concentration of kappa free light chains is significantly increased in sera of non-atopic and atopic asthma patients, as compared with nonasthmatic age-matched healthy subjects, and presumably reflects a polyclonal B cell response (Kraneveld et al., 2005). In future studies, it remains to be established whether these elevated levels of free light chains are causally related to the pathogenesis of disease. It will be important to determine the specificity of the κ and λ free light chains and to further investigate the relation between the increased production of free light chains and the development of asthma. It is tempting to speculate that (non)atopic asthma patients, where no clear-cut role for IgE can be ascribed, may be related to immunoglobulin free light chain-induced inflammation.

2. Biological activities of free immunoglobulin light chains

2.1. Immunoglobulin free light chains elicit immediate hypersensitivity-like responses

Recent work from our group indicated that immunoglobulin free light chains may not be a useless overspill of immunoglobulin production by B cells (Kraneveld et al., 2005; Redegeld and Nijkamp, 2003; Redegeld et al., 2002) (Fig. 1). We have shown that free light chains can dose-dependently transfer antigen sensitivity upon intravenous/local injection into

naïve mice. A second encounter with the cognate antigen induced mast cell activation and edema formation (ear swelling) in the sensitized animals. Mast cells were shown to be crucial in this hypersensitivity-like response elicited by light chains: no ear swelling could be induced in mast cell-deficient animals and mast cell reconstitution restored the response. Moreover, mast cells in ear tissue showed morphological signs of degranulation after challenge of free light chain-sensitized mice. Using in vitro cultured primary mast cells provided direct evidence that crosslinking of surface proteins with free light chains induced mast cell activation (see below).

2.2. Immunoglobulin free light chains seem crucial in contact sensitivity responses

Cutaneous sensitization of mice with low molecular weight compounds followed by a second contact with the appropriate antigen on the ear induces contact sensitivity reactions marked by a biphasic ear swelling response. Mast cells seem to be crucial for a full development of contact sensitivity responses, although it is unclear what mechanism of mast cell activation is involved. In addition, B cells were shown to be essential in the development of this typical T cell-mediated immune response. We have demonstrated that contact sensitization with dinitrofluorobenzene, picryl chloride, dinitrofluorobenzene and oxazolone results in production of immunoglobulin free light chains specific for the hapten used (Redegeld et al., 2002). Moreover, we were able to inhibit development of clinical signs of contact sensitivity by treating hapten-sensitized animals with F991, an Ig free light chain antagonist. F991 is a 9-mer peptide and is identical to the free light chain binding region identified in Tamm–Horsfall protein, a monomeric glycoprotein produced by cells in the ascending limb of Henle in the kidney (Huang and Sanders, 1997). This peptide has been demonstrated to have

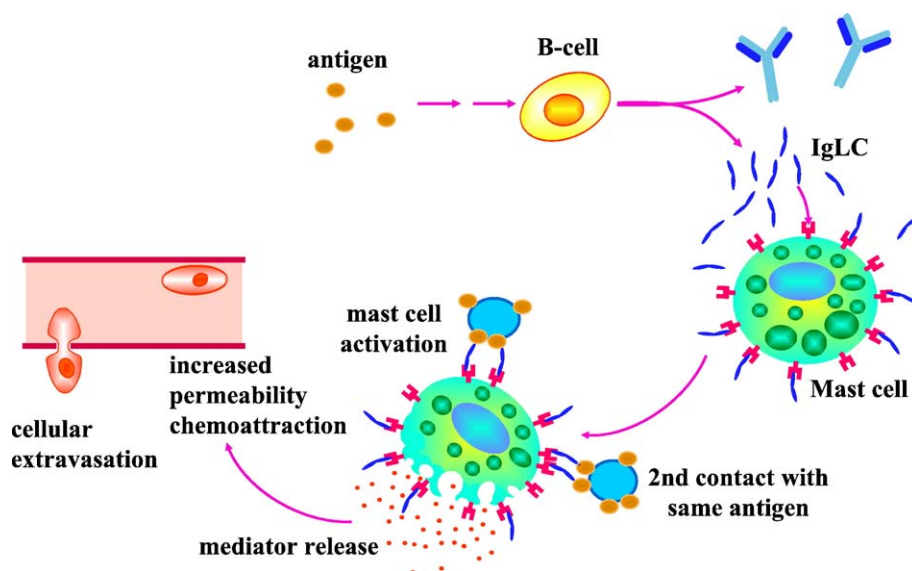


Fig. 1. Immune response leading to hypersensitivity reaction mediated by immunoglobulin free light chains and mast cells. After exposure to antigen, B lymphocytes will be activated to produce complete immunoglobulins. Besides producing, e.g. IgG and IgE, B cells will also release immunoglobulin free light chains. Mast cells can bind free light chains via a – yet unknown – surface receptor and become sensitized. A second encounter with the same antigen causes crosslinking of cell surface-bound free light chains, which results in mast cell activation. Mast cell mediators act as initiators of the inflammatory response observed at the site of antigen encounter.

specific binding affinity for free light chains and thereby inactivates the actions of the free light chain (Huang and Sanders, 1997; Redegeld et al., 2002).

2.3. Other biological activities

2.3.1. Targeting intracellular and extracellular proteins

Free immunoglobulin light chains or variable light chain domains have been used to target specific protein domains. A bacterially expressed lambda free light chain from a lung cancer-reacting human monoclonal antibody (HB4C5) showed binding to adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, but not to small cell carcinoma, which makes this free light chain a potentially useful lung cancer targeting vehicle (Nishimura et al., 1999). Also domain antibodies (VL) of free light chains could be useful bioactive drugs to target specific proteins (Holt et al., 2003). Intracellular expression of a light chain VL specific for huntingtin showed high-affinity binding to the target protein and rescued toxicity in a neuronal model of Huntington disease (Colby et al., 2004a,b). Other engineered VL domains from immunoglobulin light chains showed specific, high-affinity binding to selected targets such as B7.1 and B7.2 (van den Beucken et al., 2001).

2.3.2. Free light chains can stimulate anti-angiogenic activity

Recently, the anti-angiogenic activity of Neovastat, a shark-derived natural preparation currently tested for the treatment of non-resectable non-small cell lung cancer, was shown related to the presence of shark immunoglobulin light chains (Boivin et al., 2004). These free light chains appear to increase the affinity of tissue-type plasminogen activator for plasminogen and thereby stimulate the degradation of fibrin by the tissue-type plasminogen activator/plasminogen system (Boivin et al., 2004).

2.3.3. Proteolytic activity of free light chains

Certain human autoantibodies have demonstrated to catalyze the hydrolysis of peptide bonds and phosphodiester bonds. The catalytic activity of these antibodies resides in the light chain moiety. From a panel of human monoclonal free light chains (Bence Jones proteins) and their recombinant variable fragments the majority displayed proteolytic activity for different substrates (Paul et al., 1995; Thiagarajan et al., 2000). Some free light chains showed prothrombinase activity and could thereby promote the conversion of fibrinogen to fibrin, which could induce a procoagulant state. Coagulant disorders are well-known in multiple myeloma patients, but the clinical significance of prothrombolytic light chains is currently unknown. Free light chains could also display antigen-specific catalytic activity. The free light chains derived from an antibody against vasointestinal peptide hydrolyzed this peptide, while an irrelevant light chain was without activity (Sun et al., 1994). Interestingly, the heavy chain from the same antibody showed no proteolytic activity but inhibited the light chain-induced hydrolysis of vasointestinal peptide.

3. Mast cells

Mast cells are granular cells found in many species including humans in virtually all vascularized tissues, including skin, lymphoid tissue, and upper and lower respiratory airways. In these locations, mast cells are thought to function as sentinels in host defence, participating in both innate and acquired immunity and particularly beneficial in responding to bacterial or parasitic pathogens (Galli et al., 1999; Wedemeyer et al., 2000; Yong, 1997). Mast cells express many stimulatory and inhibiting receptors on their surface that allow them to respond to many different specific and nonspecific stimuli. Stimulation of mast cells leads to the release of a plethora of mediators with, e.g., vasoactive, constrictive, proteolytic, and inflammatory activity. By means of these mediators, mast cells participate in a wide variety of physiological and pathological processes (Hines, 2002).

Mast cells are important effector cells in immunoglobulin E (IgE)-mediated allergic diseases, such as asthma and food allergy, but also in other immunologic and nonimmunologic disorders. For a number of autoimmune disorders, including multiple sclerosis and rheumatoid arthritis, recent observations suggest a prominent role for mast cells in coordinating early phases, involving either IgE or IgG autoantibodies or other mechanisms (Benoist and Mathis, 2002; Rottem and Mekori, 2005).

3.1. Stimuli and receptors

Mast cells respond to many different stimuli, including physical stress, immunoglobulins, cytokines, chemokines, neuropeptides, complement components, proteases, bacterial compounds, and to a number of other compounds. Most stimuli are being recognized by specific receptors on the cell membrane. The best-studied mechanism for mast cell activation is through antigen-mediated crosslinking of IgE bound to its high-affinity receptor, FcεRI (Rivera et al., 2002; Turner and Kinet, 1999). However, many other stimulatory receptors have been identified, including the receptor for stem cell factor (c-kit), toll-like receptors, complement receptors, protease-activated receptors, and receptors for several cytokines, chemokines, and neurokinins (Juremalm and Nilsson, 2005; Marshall and Jawdat, 2004; Ott and Cambier, 2000; Vliagoftis and Befus, 2005). In addition to activating receptors, inhibitory receptors have been found that, upon engagement of a ligand, increase the threshold for activation through activating receptors (Ott and Cambier, 2000). For example, crosslinking of the inhibitory low-affinity receptor for IgG, FcγRIIB, together with FcεRI is negatively regulating degranulation induced by FcεRI (Daeron et al., 1995). For certain ligands both activating and inhibitory receptors are known to be expressed simultaneously on mast cells and the resulting effector response depends on the quantity and kinetics of each receptor. The recently identified activating and inhibiting receptors for CD200 are an example of such a balancing mechanism (Cherwinski et al., 2005; Varadaradjalou et al., 2003).

3.2. Mast cell mediators

Antigen-mediated crosslinking of the FcεRI–IgE complex triggers a phosphorylation cascade that induces release of intracellular calcium from the endoplasmic reticulum, leading to a relatively fast fusion of granules with the cell membrane and release of the content of the granules outside the mast cell. This content consists of preformed mediators, such as histamine, serotonin, proteoglycans, proteases, and peroxidase. In addition, the activated signalling cascade initiates the transcription of genes for mediators, such as lipid mediators (including leukotrienes and prostaglandins), a number of cytokines and chemokines, and adenosine (Galli et al., 2005). These newly synthesized mediators are released at a different speed, but usually more gradual and at a later time point than the preformed mediators. For example, leukotrienes and prostaglandins are produced more rapidly than the released cytokines (Lewis et al., 1982; Plaut et al., 1989; Razin et al., 1983).

However, this full-blown response of mast cells is only one way of reacting to external stimuli. The profile of released mediators depends largely on which receptor has been triggered and changes with the intensity of stimulation. For example, lipopolysaccharide (LPS) stimulated murine mast cells release interleukin (IL)-1β but not IL-4 or IL-5 in a toll-like receptor (TLR)-4 dependent manner, whereas peptidoglycan stimulates TLR-2-dependent release of IL-4 and IL-5, but not IL-1b (Supajatura et al., 2002). LPS stimulation is not giving a significant release of the preformed mediator histamine (Varadaradjalou et al., 2003). This implies that in addition to selectivity of mediator synthesis there must be selective mechanisms that restrain certain granules containing preformed mediators from fusing with the cell membrane. An incomplete degranulation has also been observed by immunoelectron microscopy in vivo and is referred to as “piecemeal” degranulation (Crivellato et al., 2003; Dvorak et al., 1994). The total effect of stimulating and inhibiting signals that come from the mast cell environment together determine the total activatory state and the quantity and spectrum of secreted mediators.

3.3. Mast cell activation by free light chains

Primary cultured murine mast cells can be activated after crosslinking of cell-surface proteins with immunoglobulin free light chains, resulting in release of granule contents (beta-hexosaminidase) and production of leukotrienes. Moreover, crosslinking of fluorochrome-labeled free light chains shifts the primary mast cell surface labeling to a punctated pattern, which is very similar to the pattern seen after crosslinking of IgE (Redegeld et al., 2002). The spots partially co-localize with a marker for lipid rafts, indicating that raft clustering may play an important role in light chain-mediated mast cell activation. (data not published). Taken together, these data suggest that, in free light chain-mediated hypersensitivity responses mast cells are activated through a receptor-mediated mechanism of sensitization and activation that shows homology with activation mediated by IgE–FcεRI clusters. However, despite these similarities, common gamma-chain knockout mice could still

be passively sensitized with antigen specific free light chain, indicating that receptors that signal via the common gamma-chain such as FcεRI or FcγRIII are not involved in light chain-mediated activation (Redegeld et al., 2002). Which receptor protein is involved in mast cell activation via immunoglobulin free light chains is currently under investigation.

4. Asthma

Asthma is a major and increasing global health problem, which is characterized by airway inflammation that leads to bronchoconstriction and respiratory hyperreactivity (Barnes, 2004; Tattersfield et al., 2002). Asthma is a highly complex disease that involves many inflammatory cells, mediators and proteins. Originally, manifestation of asthma was believed to correlate with the presence of antigen-specific IgE. Indeed about 60% of adult asthmatic patients have this form of atopic asthma, which starts during childhood. However, it is currently accepted that a profound group of asthmatics is not demonstrably atopic (nonallergic or intrinsic asthma) (Douwes et al., 2002; Pearce et al., 1999). In this population of asthmatics no signs of IgE-mediated hypersensitivity were found as evidenced by negative skin-prick test as well as normal serum IgE levels and eosinophil counts. This non-atopic form of asthma accounts for 40% of adult asthmatics and demonstrates histological and biochemical features that are similar to asthma that is associated with atopy (Humbert et al., 1999). In addition, standardized comparisons across populations or time show only a weak and inconsistent association between the prevalence of adult asthma and the prevalence of atopy (Pearce et al., 1999).

Atopic asthma has been extensively investigated. Considerable less information is available about the pathologic characteristics of non-atopic asthma. Both groups of asthmatics have respiratory symptoms, peak flow variability and bronchial hyperreactivity of similar severity. However, it was clearly demonstrated that in atopic asthmatics high numbers of eosinophils, mast cells and T lymphocytes characterized the airway inflammation, whereas non-atopic asthmatics mainly displayed high numbers of neutrophils and mast cells (Amin et al., 2000). These findings suggest that there are differences in the extent of the immunopathological response of these two types of asthma. Since mast cells have been implicated a role in the atopic as well as non-atopic asthma and the extent of degranulation of these cells is directly related to disease severity (Amin et al., 2000; Galli, 1997; Galli and Costa, 1995), focus on a novel non-IgE-mediated pathway of antigen-specific mast cell activation may be of importance in the understanding of asthma pathogenesis.

4.1. Animal models for non-atopic asthma

The mechanisms involved in non-atopic asthma are poorly defined. Several investigators have characterized hapten-induced pulmonary hypersensitivity (delayed type hypersensitivity or type IV hypersensitivity) reaction induced by sensitization followed by an intra-airway challenge of low molecular weight compounds such as picrylchloride/trinitrobenzene

sulphonic acid, toluene diisocyanate and dinitrofluorobenzene/dinitrobenzene sulphonic acid as models for non-atopic asthma (Buckley et al., 1997; Enander et al., 1983; Garssen et al., 1991, 1989; Scheerens et al., 1996). The features observed in these murine models resemble those found in non-atopic asthma and comprise hapten-induced acute bronchoconstriction, pulmonary edema, infiltration of neutrophils and mononuclear cells into the airways, and in vitro and in vivo airway hyperreactivity. The pulmonary hypersensitivity reactions were not associated with elevated serum levels of antigen-specific IgE (Dearman and Kimber, 1991; Garssen et al., 1991, 1989). Both early (<3 h) and late (24–48 h) phases were found to be dependent on the presence of T cells, because in athymic mice airway hyperreactivity and inflammation were suppressed, respectively (Garssen et al., 1991, 1989; Geba et al., 1997). In addition, there are several lines of evidence that demonstrate an important role for mast cells in the elicitation of hapten-induced T cell-mediated responses in the airways. First, the lung is a site enriched with mast cells (Garssen et al., 1989; Puxeddu and Levi-Schaffer, 2004). Various studies have reported the release of mast cell mediators such as serotonin, tumor necrosis factor- α and mast cell proteases shortly after challenge (Ameisen et al., 1989; Garssen et al., 1989; Kerdel et al., 1987; van Houwelingen et al., 2002; Van Loveren et al., 1984). Serotonin and tumor necrosis factor- α can act locally by increasing vascular permeability and inducing vasodilatation, thereby facilitating cellular infiltration (Ameisen et al., 1989; Kerdel et al., 1987; van Houwelingen et al., 2002). Treatment with mast cell stabilizers, tumor necrosis factor- α neutralizing antibodies or serotonin receptor antagonist suppressed hapten-induced hypersensitivity reactions in lung of mice (unpublished observations) (Ameisen et al., 1989; Garssen et al., 1989; Kerdel et al., 1987; van Houwelingen et al., 2002). Defective pulmonary hapten-induced hypersensitivity responses have been found in mast cell-deficient mice (Garssen et al., 1989; Kraneveld et al., 2002). Reconstitution of mast cells in these mice fully restored the airway responses, confirmatory for the hypothesis of the initiating role of mast cells in the cellular cascade leading to non-IgE-mediated pulmonary hypersensitivity responses (Kraneveld et al., 2002).

4.2. Free light chains and asthma

Our recent discovery that immunoglobulin free light chains mediate antigen-specific mast cell-dependent hypersensitivity-like responses in the skin (Redegeld et al., 2002), pointed to a possible role for these molecules in the pathophysiology of adult asthma. In a murine model for non-atopic asthma (see above), the role of immunoglobulin free light chains was investigated by the free light chain antagonist F991. Local and systemic administration of F991 completely inhibited both the early and late phase pulmonary responses in the hapten-induced hypersensitivity reaction in mice (Kraneveld et al., 2005). The therapeutic efficacy of F991 is attributed to its interaction with hapten-specific immunoglobulin free light chains, thus preventing these molecules to sensitize mast cells. The role of immunoglobulin free light chains in asthma pathogenesis was further evidenced by the demonstration that hapten-specific

immunoglobulin free light chains could passively transfer a pulmonary hypersensitivity response leading to key features of asthma such as mast cell activation and acute bronchoconstriction (Kraneveld et al., 2005). This response could also be abrogated by pretreatment with F991. Additional evidence for a requirement of mast cells was demonstrated by performing the same experiment in mast cell-deficient mice that did not manifest the immunoglobulin free light chain-induced pulmonary reaction after antigen challenge. Reconstitution of mast cells in this strain of mice via intravenous injection of in vitro cultured bone marrow-derived mast cells, confirmed the pivotal role of the cells in pulmonary immunoglobulin free light chain-mediated immune responses (Kraneveld et al., 2005). It is hypothesized that mast cell activation in the lung results from the interaction of receptor-bound immunoglobulin free light chains and antigen (Fig. 1).

5. Final conclusions

Current insights indicate that free light chains may play crucial roles in both immediate and delayed hypersensitivity responses. These findings reveal novel therapeutic opportunities in the treatment of chronic inflammatory (auto)immune diseases. Future (clinical) research should shed light on the role of free light chains in the pathogenesis of diseases such as asthma, contact dermatitis, and autoimmune disorders.

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