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Polyclonal free light chains: a biomarker of inflammatory disease or treatment target?

Judith A. Brebner* and Robert A. Stockley

Address: Lung Function and Sleep Department, Queen Elizabeth Hospital, Mindelsohn Way, Edgbaston, Birmingham, B15 2WB.

* Corresponding author: Judith A. Brebner (j.a.brebner@bham.ac.uk)

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Abstract

Free light chains are proteins produced by B lymphocytes during the process of antibody synthesis. Their production, as a reflection of B cell activation, can give insight into the activity of the adaptive immune system. In recent years, an automated immunoassay that provides quantitative measurement of free light chains in the serum has been developed. This assay has not only revolutionised the investigation of monoclonal light chain overproduction in plasma cell diseases, but has also allowed for the quantification of polyclonal free light chains in serum. The discovery of high levels of polyclonal free light chains in a number of inflammatory and auto-immune conditions has led to the examination of their value as a biomarker of disease activity. Research into their bio-activity has also highlighted their potential role in the pathogenesis of inflammatory disease, making them an attractive target for novel therapies.

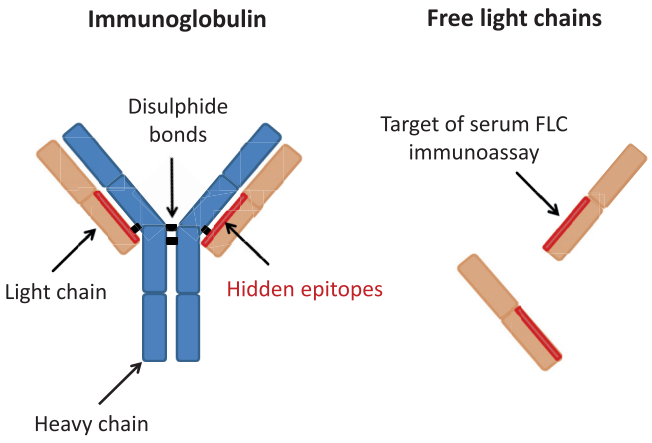
Introduction

Dr H. Bence Jones first described free light chains when he linked the presence of a urinary protein to the diagnosis of "mollities ossium" in 1847 [1]. Immunoglobulin free light chains are a by-product of antibody synthesis by terminally differentiated B lymphocytes, a key element of the adaptive immune system. Antibodies are immunoglobulins with a tetrameric structure composed of two identical heavy chains and two identical light chains linked by disulphide bonds (Figure 1). There are two light chain isotypes: Kappa (κ) and Lambda (λ). Heavy chain and light chain proteins are assembled in the endoplasmic reticulum during immunoglobulin synthesis. During this process there is an excess of light chain production in the region of 500 mg per day [2,3]. Excess free light chains are secreted into the circulation, where rapid renal clearance results in a short half-life of 2-6 hours. In recent years, our advancing knowledge of their diverse immunological functions has sparked new interest in their potential pathogenic role in chronic inflammatory and autoimmune diseases. In this article we describe the recent advances in our ability to measure free light chains and explore their utility as a novel biomarker and potential therapeutic target.

Measurement of free light chains

Commercial methods for identifying free light chains utilising serum and urine protein electrophoresis and immunofixation electrophoresis have been problematic due to their lack of sensitivity and cumbersome methodology [4]. The advent of a highly sensitive nephelometric immunoassay that uses antibodies that bind to epitopes of free light chains that are "hidden" in intact immunoglobulin molecules has had a significant impact on research in this field [5]. Using this assay, reference and diagnostic ranges for serum free light chains and the κ/λ ratio were determined by analysing the sera of healthy donors and patients with monoclonal gammopathies [6]. Some analytical performance limitations have been identified, such as variation in free light chain concentration from the same sample assayed using different batches of polyclonal free light chain antiserum, and non-linear dilution of some monoclonal free light chains [7,8]. If there are large quantities of free light chain

Figure 1. Intact immunoglobulin and free light chain structure



Each immunoglobulin is composed of two heavy chains and two light chains linked by disulphide bonds. The variability of the amino acid sequence of the "variable region" is responsible for the antigen binding specificity of the antibody. There are two types of light chain termed kappa (κ) and lambda (λ). The serum immunoassay targets "hidden epitopes" found on the interface between the light and heavy chains in the intact immunoglobulin molecule.

present in the serum, the phenomenon of "antigen excess", where non-precipitating immune complexes can form and result in falsely low free light chain concentrations, is also well recognised [9-11]. Awareness of these issues and close links between biologists and clinicians involved has been highlighted as crucial for the optimal interpretation of results.

Free light chains and disease

Concentrations of serum free light chains are dependent on the balance between production and renal clearance [12]. There is extensive knowledge of monoclonal free light chain overproduction in haematological disorders due to clonal plasma cell proliferation, which is beyond the scope of this article. Polyclonal free light chain overproduction can also occur when there is an excess production of multiple immunoglobulins, usually as a result of chronic immune stimulation. In the context of polyclonal hypergammaglobulinamia or renal impairment the κ/λ ratio should remain unchanged [12].

Polyclonal free light chains: a biomarker for disease activity?

Increased free light chain concentrations have been described in a variety of inflammatory and autoimmune diseases including systemic lupus erythematosus (SLE) [13,14], rheumatoid arthritis, Sjögren's syndrome [15], atopic dermatitis [16], asthma [17], rhinitis [18,19], food allergy [20], idiopathic pulmonary fibrosis, hypersensitivity pneumonitis [21], chronic obstructive pulmonary disease (COPD) [22], inflammatory bowel disease [23] and multiple sclerosis [24-26]. Evidence of the relationship of free light chain levels to disease activity in these conditions is emerging.

Gottenberg *et al.* were the first to demonstrate a relationship between free light chain concentrations and disease activity in patients with rheumatoid arthritis as measured by the Disease Activity Score 28 (DAS28) [15]. In this small study of 50 patients, they also demonstrated correlations between free light chains and other markers of B cell activation, such as total gammaglobulin, IgG and rheumatoid factor. Interestingly, total gammaglobulin and IgG levels did not correlate with the DAS28 in the same way as free light chains, which the authors felt may be accounted for by the short half-life of free light chains in comparison, making them a better marker of current disease activity. A larger prospective study of 710 patients with early arthritis also found elevated polyclonal free light chains in patients with early rheumatoid arthritis to correlate with DAS28 [27]. Correlation with disease activity has also been studied in patients with SLE. Hopper *et al.* demonstrated that clinical relapses can be associated with an antedescent elevation in urinary free light chains four to eight weeks prior to the onset of symptoms [14]. A larger study of 75 patients found serum free light chain levels (in addition to complement C3) correlated strongly with the SLE disease activity index (SLEDAI) and modified SLEDAI [13].

The use of free light chains as a biomarker of B cell activation has prompted evaluation of their potential role for monitoring the response to treatments, such as Rituximab (a monoclonal antibody that causes B cell depletion). In patients with rheumatoid arthritis treated with Rituximab, a significant reduction in serum free light chain concentrations has been seen only in patients that respond clinically. However, baseline levels have not been found to be a predictor of response [28]. A small study of 11 patients with SLE similarly found a significant reduction in free light chain concentrations following Rituximab therapy, which correlated with C3 consumption [29]. The growing enthusiasm for B cell-targeted therapies in inflammatory disease promotes the need for larger prospective studies to establish the importance of free light chain monitoring in these patients.

Serum free light chains have also been found to be raised in type 2 diabetes prior to the development of renal impairment, suggesting their possible role for predicting early diabetic nephropathy [30]. The fact that polyclonal free light chains are higher in patients with chronic kidney disease may seem unsurprising given their renal clearance but their pathogenic role in worsening tubular injury is also the subject of ongoing research [31].

Polyclonal free light chains and mortality

Dispenzieri *et al*. [32] have reported that a polyclonal increase in free light chains (measured by the combined sum of κ and λ concentrations) is a predictor of mortality in the general population. The study of 15,859 patients over the age of 50 without plasma cell dyscrasias found the excess risk of death was independent of age, sex and renal impairment [32]. Another study followed up 527 patients sent for routine haematological investigations (which had excluded a monoclonal gammopathy) and found the relative risk of death increased proportionally with combined free light chain concentrations. A combined concentration of >65 mg/l was found to be a risk factor for death with the highest prognostic value within the first 100 days. Forty one percent of patient deaths within this period were attributable to cardiovascular causes, suggesting that polyclonal free light chains might serve as a "cardiovascular risk factor" [33]. Given the established links between chronic inflammation and cardiovascular disease this certainly warrants further investigation.

Pathogenic role of free light chains: could they be a therapeutic target?

As evidence of the biological activity of free light chains is emerging, it has challenged the concept that their excess production is inconsequential (Figure 2) [34]. Hutchinson *et al*. have recently demonstrated that free light chains can bind to a broad range of cell membranes but have a high binding affinity with monocytes [35]. Given the important role that monocytes play in antigen presentation, this has prompted them to question whether one of the roles of free light chains is to assist antigen uptake by cells and hence promote the associated immune response. The ability of free light chains to bind to antigen independently is controversial [36,37], although a recent study by Thio *et al*. used a variety of techniques to show that free light chains could bind to antigen with significant affinity [38]. To date no specific free light chain receptor has been identified.

Free light chains and hypersensitivity reactions

In 2002, Redegeld *et al*. demonstrated that free light chains can elicit immediate hypersensitivity responses in sensitised mice and described how *in vitro* cross-linking of free light chains and mast cell surface proteins can induce degranulation [39]. F991 is a 9-mer peptide that acts as an immunoglobulin free light chain antagonist. It was derived using the amino acid sequence responsible for binding immunoglobulin light chains to Tamm-Horsfall glycoprotein, which is secreted from the ascending loop of Henle [40]. The potential role of free light chains in the pathogenesis of non-atopic asthma was subsequently raised by using a murine model to show that the use of F991 can abate the development of air flow obstruction, airway hyperresponsiveness and inflammation [17]. Similarly, the use of F991 in a murine model for inflammatory colitis has been shown to inhibit mast cell activation and prevent the development of diarrhoea [23].

Free light chain interaction with neutrophils

Free light chains have been shown to inhibit the apoptosis of neutrophils, which are key effector cells in the inflammatory process [41]. The increased concentration of free light chains in patients with end-stage renal disease has therefore been implicated as a cause for the pro-inflammatory state of these patients [42]. More recently, Braber *et al*. have described a link between free light chains and neutrophils in the pathogenesis of COPD. Increased serum free light chain concentrations were also found in three different murine models with emphysema. Antagonising the free light chain using F991 led to a reduction in neutrophil influx in the murine lungs [22]. The researchers also demonstrated that the binding of free light chains to human neutrophils results in *in vitro* production of interleukin-8, which can cause neutrophil activation, a key pathological feature in lung disease [22].

Protective role of free light chains in disease

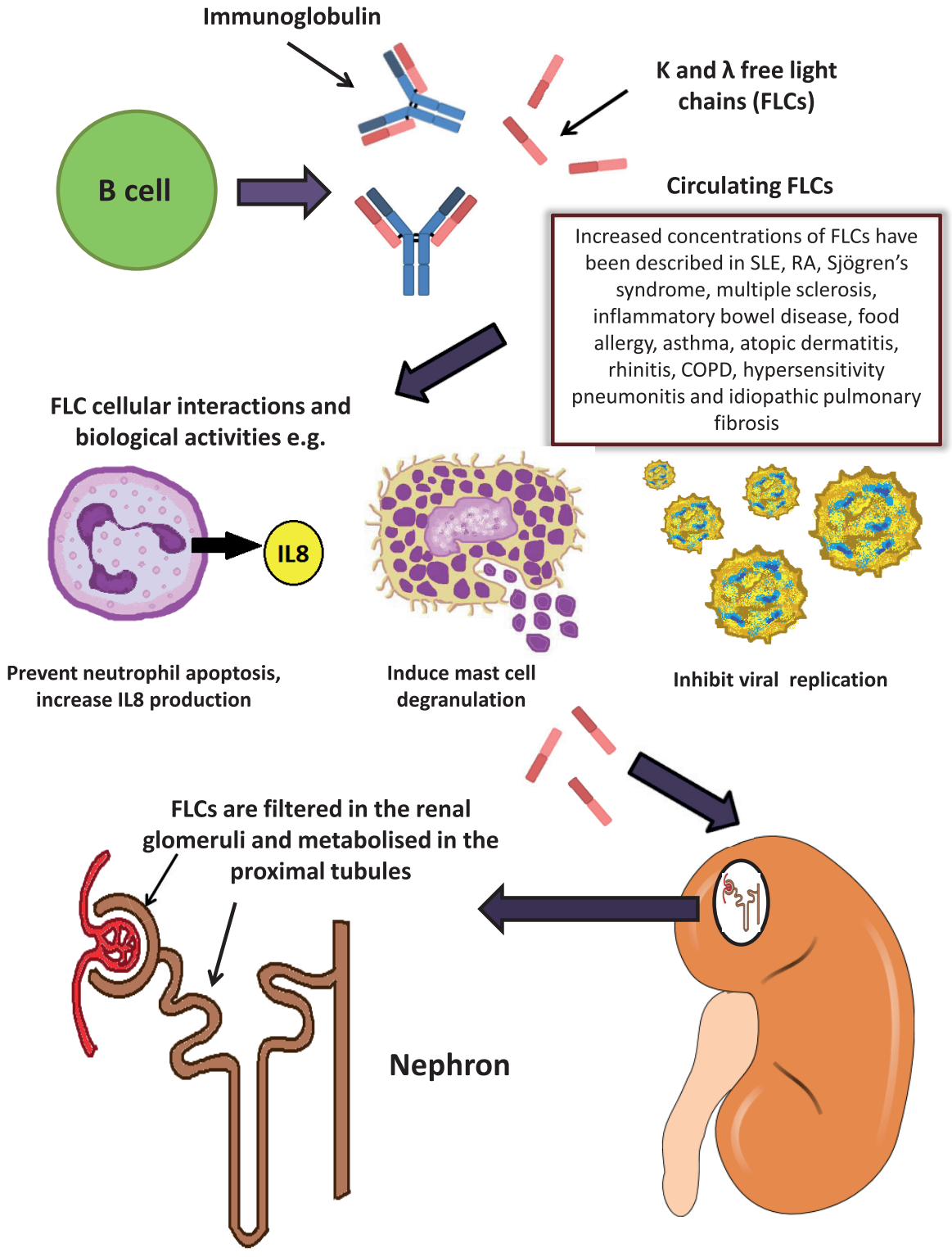
It is important to point out that free light chains have also been shown to have a potentially protective role. Matsumori *et al*. [43] demonstrated increased free light chain expression in mice infected with encephalomyocarditis virus. However, the application of F991 worsened the degree of myocarditis, whereas supplementation with free light chains led to reduced necrosis and improved outcome. Free light chains were shown to inhibit encephalomyocarditis viral replication *in vitro* [43].

Conclusion

Measuring polyclonal free light chains as a marker of B cell activation can give new insight into the activity of the adaptive immune system in a variety of inflammatory conditions [44]. However, further large prospective trials are needed to establish their role as a biomarker of disease activity and predictor of mortality in different patient populations. The increasing use of B-cell-targeted therapies in the treatment of autoimmune diseases means the potential utility of free light chains in risk-stratifying patients for treatment initiation, as well as monitoring response to therapy, is an exciting prospect for future research.

Free light chains have been shown to exert a variety of biological activities, and as our knowledge of their

Figure 2. Free light chain production, biological interactions and metabolism



Free light chains are produced by B cells, released into the circulation and have been shown to exert a variety of biological functions, including inhibition of neutrophil apoptosis and viral replication, and induction of mast cell degranulation. Free light chains are filtered in the renal glomeruli and metabolised in the proximal tubule of the kidney.

immunological function increases, the growing evidence suggesting their involvement in pathogenesis makes them an attractive target for novel therapies. Preclinical models using free light chain antagonists seem promising, but we do not yet know if this will translate clinically, and given the evidence of their protective effect to certain infections, their safety will need to be monitored closely. There are still many important questions that remain unanswered, such as whether, and where, free light chain receptors exist and their role, as well as antigen specificity of the light chains. Further investigation is needed to increase our understanding of the biological role of free light chains in inflammatory disease.

Abbreviations

COPD, chronic obstructive pulmonary disease; DAS28, disease activity score 28; SLE, systemic lupus erythematosus; SLEDAI, SLE disease activity index.

Disclosures

The authors declare that they have no disclosures.

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