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Research paper

Development of a highly-sensitive multi-plex assay using monoclonal antibodies for the simultaneous measurement of kappa and lambda immunoglobulin free light chains in serum and urine

John P. Campbell, Mark Cobbold, Yanyun Wang, Margaret Goodall, Sarah L. Bonney, Anita Chamba, Jane Birtwistle, Timothy Plant, Zaheer Afzal, Roy Jefferis, Mark T. Drayson *

Clinical Immunology Service, School of Immunity and Infection, College of Medical and Dental Sciences, University of Birmingham, Birmingham B15 2TT, United Kingdom

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ABSTRACT

Monoclonal κ and λ immunoglobulin free light chain (FLC) paraproteins in serum and urine are important markers in the diagnosis and monitoring of B cell dyscrasias. Current nephelometric and turbidimetric methods that use sheep polyclonal antisera to quantify serum FLC have a number of well-observed limitations. In this report, we describe an improved method using specific mouse anti-human FLC monoclonal antibodies (mAbs). Anti-κ and anti-λ FLC mAbs were, separately, covalently coupled to polystyrene Xmap® beads and assayed, simultaneously, in a multi-plex format by Luminex® (mAb assay). The mAbs displayed no cross-reactivity to bound LC, the alternate LC type, or other human proteins and had improved sensitivity and specificity over immunofixation electrophoresis (IFE) and Freelite™. The assay gives good linearity and sensitivity (<1 mg/L), and the competitive inhibition format gave a broad calibration curve up to 437.5 mg/L and prevented anomalous results for samples in antigen excess i.e. high FLC levels. The mAbs displayed good concordance with Freelite™ for the quantitation of normal polyclonal FLC in plasma from healthy donors (n = 249). The mAb assay identified all monoclonal FLC in serum from consecutive patient samples (n = 1000; 50.1% with monoclonal paraprotein by serum IFE), and all FLC in a large cohort of urine samples tested for Bence Jones proteins (n = 13090; 22.8% with monoclonal κ, 9.0% with monoclonal λ, and 0.8% with poly LC detected by urine IFE). Importantly this shows that the mAbs are at least close to the ideal of detecting FLC from all patients and neoplastic plasma cell clones. Given the overall effectiveness of the anti-FLC mAbs, further clinical validation is now warranted on serial samples from a range of patients with B cell disorders. Use of these mAbs on other assay platforms should also be investigated.

1. Introduction

The total of body plasma cells secretes about 1 g per day of kappa and lambda immunoglobulin free light chains (FLCs) into the extracellular fluids. These FLCs are cleared from the blood by glomerular filtration with a half-life of 2 to 6 h (Waldmann et al., 1972). A neoplastic clone of plasma cells must secrete up to 20 g of FLC per day to saturate FLC absorption in the proximal renal tubules of healthy kidneys and thus become detectable in urine (Drayson, 2012). Accordingly it would be preferable to detect and quantitate FLC in blood not urine but this is difficult because serum levels of FLC are mg/L compared to the one thousand-fold higher level of LC bound to whole immunoglobulin. Antibodies for routine clinical quantitation of serum FLC must have specificity for epitopes that are exposed on FLC and hidden on LC bound in whole immunoglobulin; further these antibodies must detect FLC from all patients and neoplastic plasma cell clones.

* Corresponding author.
E-mail address: m.t.drayson@bham.ac.uk (M.T. Drayson).

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Currently there is only one source of FDA approved serum FLC assays (Freelite™, the Binding Site Ltd., UK) (Bradwell et al., 2001). These immunoassays employ purified specific sheep polyclonal antisera adsorbed to render them specific for κ and λ FLCs, respectively, that are latex-enhanced for use in turbidimetric and nephelometric immunoassays. For the first time it has been possible to routinely measure serum FLCs from an array of patient groups that includes oligosecretory myeloma (Drayson et al., 2001), light chain only myeloma (Bradwell et al., 2003), light chain amyloidosis (Lachmann et al., 2003), monoclonal gammopathy of unknown significance (MGUS) (Rajkumar et al., 2004), healthy individuals (Katzmann et al., 2002), and others (Drayson, 2012). Dual measurement of serum κ and λ FLC levels has also highlighted the importance of the κ:λ ratio in the diagnosis and monitoring of B cell malignancies. The κ:λ ratio represents a sensitive balance between the two light chain types, whereby overproduction of one type by a malignant B cell clone leads to a perturbation of the normal κ:λ reference range (Freelite™ κ:λ ratio = 0.26–1.65 (Katzmann et al., 2002)). It is now possible to identify patients with a perturbed serum κ:λ ratio before disease has progressed to the extent that Bence Jones (BJ) protein appears in urine. The serum FLC ratio facilitates diagnosis and monitoring of oligosecretory myeloma and light chain amyloid where serum and urine immunofixation is negative, enables earlier diagnosis of active light chain only disease, reducing the risk of acute kidney damage (Hutchison et al., 2008) and gives prognostic information in all B cell dyscrasias and in healthy individuals (Dispenzieri et al., 2012).

These clinically significant developments are well established and international guidelines recommend the use of Freelite™ in diagnosis and management of a wide range of plasma cell dyscrasias (Dispenzieri et al., 2009). However, this first generation of serum FLC assays has technical limitations. A separate test for each κ and λ FLC measurement is required, introducing inter-test error and reducing the reliability of the κ:λ ratio result obtained. This variability is compounded further by the batch-to-batch differences observed in the polyclonal antisera produced from individual sheep (Tate et al., 2007, 2009). In clinical practise, it is important to detect both the elevation of one FLC type by secretion of malignant FLC and the reduction in levels of the alternate FLC by immunoparesis. Thus assays need to quantitate FLC levels ranging from 1 mg/L to >1000 mg/L. The latex-enhanced antisera have a calibration range of 3.7–56.2 mg/L for κ FLC and 5.6–74.8 mg/L for λ FLC, and are unreliable at the lower end. This can lead to an abnormal κ:λ ratio in healthy individuals and apparently significant changes in ratio between sequential samples from myeloma patients who are in fact still in remission. This problem is highlighted by 'gaps' above and below the working calibration range of the assay (Bradwell, 2008). The limited calibration range also requires that samples with high FLC be diluted several times. The assay is prone to antigen-excess (or "hook effect") which can cause false negative diagnoses in patients with grossly elevated FLC and false positive evidence of disease progression (Daval et al., 2007; Levinson, 2010a; Murata et al., 2010). Monoclonal FLC paraproteins tested on Freelite™ have been shown to be non-linear (Tate et al., 2007) meaning that dilutions could lead to inaccurate FLC quantitation. The polyclonal antisera in the assay are targeted against polyclonal FLC, as opposed to monoclonal FLC, potentiating the claim that the Freelite™ sensitivity to paraprotein levels slightly outside the normal reference range is negatively affected (Levinson, 2010b). Further, there are reports that the antisera are cross-reactive with bound κ and λ LC (Davern et al., 2008) leading to excessively high FLC results not representative of absolute FLC levels. A second generation of serum FLC tests is needed to overcome these problems. If monoclonal antibodies (mAbs) could be produced that specifically target human κ and λ FLCs, then they would provide a long term solution to the problems of the current polyclonal Freelite™ assay.

Development of FLC specific mAbs is difficult and complicated by the paucity of constant domain epitopes available for FLC recognition; which can be further reduced by polymerisation of FLC, particularly FLC λ, thus reducing the number of potential binding sites (Abraham et al., 2002; Bergen et al., 2004; Davern et al., 2008). Production of mAbs specific for FLC has been described previously (Abe et al., 1993, 1998; Nakano and Nagata, 2003; Davern et al., 2008) and these groups have demonstrated mAb specificity for epitopes that are exposed on FLC and hidden on LC bound in whole immunoglobulin. However these groups have either found that their mAbs did not detect FLC from all neoplastic plasma cell clones tested or have not tested sufficient clones to be confident that the mAbs would detect the FLC from at least 95% of neoplastic clones. Recently another group reported anti-FLC mAbs (te Velthuis et al., 2011; Hoedemakers et al., 2012) again, specificity with at least 95% of neoplastic FLC clones appears unlikely, especially for λ FLC (Drayson and Carr-Smith, 2012; Hutchison et al., 2012).

In the present study, we describe the development and initial validation of two anti-κ FLC and two anti-λ FLC mAbs in a competitive-inhibition multi-plex Luminex® assay (mAb assay). Whilst it is important that the new assay overcomes the problems with existing commercial assays, initial clinical validation must also demonstrate that the mAbs provide: (1) similar quantitation of polyclonal FLC from healthy donors to the Freelite™ assay; (2) appropriate sensitivity to reliably quantify low levels of FLC representative of immunosuppression or immunoparesis; and (3) by testing a large number of serum and urine samples it shows that the mAbs are at least close to the ideal of detecting FLC from all patients and neoplastic cell clones.

2. Materials and methods

2.1. Ethical approval

Ethical approval for development and validation of the FLC assay using residual, end-of-diagnostic use of patient serum and urine was granted by the Life and Health Sciences Ethical Review Committee of the University of Birmingham, UK. Financial support for the study was provided by the Clinical Immunology Service, University of Birmingham, UK.

2.2. Preparation of anti-FLC mAbs

Anti-FLC mAbs were prepared using standard methods (Galfre and Milstein, 1981). Briefly, BALB/c mice were immunised with κ or λ FLC purified from human urine