Methods 81 (2015) 56–65

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Detecting monoclonal immunoglobulins in human serum using mass spectrometry

John R. Mills *, David R. Barnidge, David L. Murray *

Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN 55905, United States

ARTICLE INFO

Article history:
Received 3 February 2015
Received in revised form 15 April 2015
Accepted 18 April 2015
Available online 24 April 2015

Keywords:
Monoclonal gammopathy
Immunoglobulin
Multiple myeloma
Mass spectrometry
Clinical diagnostics

ABSTRACT

Established guidelines from the International Myeloma Working Group recommend diagnostic screening for patients suspected of plasma cell proliferative disease using protein electrophoresis (PEL), free light chain measurements and immunofixation electrophoresis (IFE) of serum and urine in certain cases. Plasma cell proliferative disorders are generally classified as monoclonal gammopathies given most are associated with the excess secretion of a monoclonal immunoglobulin or M-protein. In clinical practice, the M-protein is detected in a patients' serum by the appearance of a distinct protein band migrating within regions typically occupied by immunoglobulins. Given each M-protein is comprised by a sequence of amino acids pre-defined by somatic recombination unique to each clonal plasma cell, the molecular mass of the M-protein can act as a surrogate marker. We established a mass spectrometry based method to assign molecular mass to the immunoglobulin light chain of the M-protein and used this to detect the presence of M-proteins. Our method first enriches serum for immunoglobulins, followed by reduction to separate light chains from heavy chains, followed by microflow LC-ESI-Q-TOF MS. The multiply charged light chain ions are converted to their molecular mass and reconstructed peak area calculations are used for quantification. Using this method, we term "monoclonal immunoglobulin Rapid Accurate Molecular Mass" or miRAMM, the presence of M-proteins can be reliably detected with superior sensitivity compared to current gel-based PEL and IFE techniques.

© 2015 Published by Elsevier Inc.

1. Introduction

B-cells play a central role in the adaptive immune system expressing a diverse repertoire of immunoglobulins (Igs). Secreted Igs protect against foreign antigens through several mechanisms including; binding to foreign antigens (i.e. bacteria, viruses), recruiting other immune cells, or directly neutralizing foreign substances [1]. Igs are homodimers composed of two heavy chains (HCs) and two identical light chains (LCs). Each LC pairs with a HC and each HC pairs with another HC through disulfide bonds (see Fig. 1). Both the HC and the LC have two distinct regions called the constant and variable region. The C-terminal half of each HC and LC encompasses the constant region which contains an epitope that defines the chain's isotype. The HC can have 5 different isotypes; IgG, IgA, IgM, IgD or IgE, while the LC can have 2; kappa or lambda. The N-terminal half of each HC and LC contains the variable region which includes the amino acid sequences that bind antigens. A single B-cell will create HC and LC variable regions by rearranging Ig specific gene segments [1]. If both heavy and light chain rearrangements generate functional sequences the expressed Ig is assembled and expressed on the cell surface of the B-cell. If this process is successful then the cell undergoes allelic exclusion to ensure each cell only expresses a single Ig [2]. Therefore, each clonal cell is uniquely defined by a particular gene rearrangement that encodes a specific Ig with a defined molecular mass. When a B-cell engages an antigen the cell is stimulated to proliferate which induces clonal expansion. B-cell clones that acquire mutations that improve antigen binding affinity will be selectively expanded in the germinal center. Those cells which differentiate into Memory B-cells may undergo class switching at the *IgH* locus which generates Igs encoding different constant regions prior to commitment to becoming long-lived plasma cells (PCs). These long-lived PCs reside in the bone marrow and secrete vast quantities of high-affinity antigen specific Igs. Each PC will express a defined Ig with a molecular mass which is identical to its original precursor cell. Humans produce a vast diversity of PCs – each with their own randomly generated Ig (upwards of 10^{12} possible unique

* Corresponding authors at: Department of Laboratory Medicine and Pathology, Mayo Clinic, 200 1st St SW, Rochester, MN 55905, United States. Fax: +1 (507) 284 1927.

E-mail addresses: mills.john2@mayo.edu (J.R. Mills), murray.david@mayo.edu (D.L. Murray).

http://dx.doi.org/10.1016/j.ymeth.2015.04.020
1046-2023/© 2015 Published by Elsevier Inc.

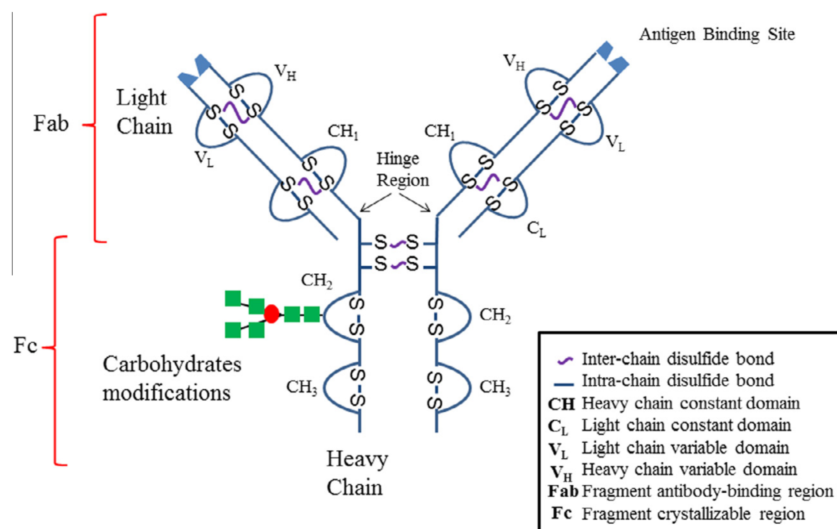

Fig. 1. Ig structure indicating domains and sites of covalent modification. Structure of a typical IgG immunoglobulin. Two identical HCs and LCs are connected by disulfide linkages. Furthermore, within each chain there are also disulfide bonds which are critical to maintain structure. The variable regions of both the HC and LC form the bivalent antigen binding sites. The class of the HC and LC are defined by the constant regions which have distinct amino acid residues depending on the class. Carbohydrate post-translation modifications on the HC generate different Ig glycoforms which can be functionally important.

sequences) creating what is often referred to as the normal polyclonal Ig repertoire [3].

However, PCs can become malignant resulting in an abnormal expansion of clonal cells as is the case for the plasma cell proliferative disorders, multiple myeloma (MM), AL amyloidosis, and Waldenström's macroglobulinemia. When this occurs a single monoclonal Ig can be detected above the normal polyclonal Ig repertoire. If there is clinical suspicion of a plasma cell proliferative disorder, the patient's serum and urine is typically tested for the presence of a monoclonal Ig or M-protein. M-proteins are typically detected using a combination of protein gel electrophoresis (PEL) and immunofixation (IFE) [4]. These techniques use the differential charge and size of Igs to separate them from other serum proteins on a gel media, usually agarose or cellulose acetate. Typically a low ionic strength buffer with an alkaline pH is used, which is sufficient to provide most proteins with a negative charge. After an electric field is applied the serum proteins migrate into distinct fractions knows as the albumin, α1 (composed predominantly of α1-antitrypsin), α2 (mostly α2-macroglobulin and haptoglobin), β (transferrin and C3) and γ (mostly Igs) regions. After separation, proteins in the gel are stained and the intensity of the staining is used to determine the relative contribution of a given fraction to the total serum protein content. In most healthy individuals the γ region contains the polyclonal Igs found in serum and is a broad and diffuse fraction driven by the vast diversity of Ig amino acid sequences. When an excessive amount of a specific monoclonal Ig is present a characteristic distinct monoclonal band or spike (M-spike) is typically observed in the γ fraction [5]. In instances where the presence of an M-protein is questionable, immunofixation electrophoresis (IFE) can be helpful. IFE is similar in principle to PEL with an additional in-gel immunoprecipitation step. Several identical aliquots of the patient's serum are separated by agarose gel electrophoresis in different wells. After separation one well serves as a reference well and is immediately fixed and whereas the other wells are incubated with antisera against the HC isotypes (IgA, IgG, IgM) and LC isotypes (κ or λ). The interaction of the antisera with the Ig in the gel induces a precipitate. Distinct bands are noted for each well and the relationship between HC and LC is established based upon similar monoclonal migration distances (see Fig. 5). IFE has a limit of detection that is ~10-fold lower than PEL; however, it is important to note IFE is not quantitative and is only useful for qualitative assessment. Capillary electrophoresis (CE) can be used as an alternative to gel electrophoresis [6]. CE uses small-bore (10–100 μM) fused silica capillary tubes and microfluidics to separate proteins which are then visualized using a UV detector. CE offers superior speed and automation capabilities. The equivalent to gel based IFE is immunosubtraction CE. In the case of the latter, serum is incubated with antisera for each of the HC and LC, then removing the complexes prior to running CE. The results are compared back to the serum sample without antisera to make a qualitative conclusion about the type of Ig present. Despite these technical improvements these methods have inherent limitations due to their laborious setup, poor resolution, limited sensitivities, and occasionally ambiguous results.

The most recent generation of mass spectrometers provide higher resolution, greater mass measurement accuracy, and greater sensitivity than previous generations. These new instruments (i.e. Orbitrap and Time-of-Flight (TOF) mass spectrometers) are also smaller and more robust and have begun to play a role in clinical diagnostics measuring the accurate molecular mass of intact proteins (i.e. IGF1, hemoglobin) [7–10]. Increases in the linear dynamic range, as well as increases in resolution and mass measurement accuracy, have made these instruments a sensible alternative to characterizing proteins as compared to traditional protein gel electrophoresis based methods. This was recently demonstrated for intact Igs where the molecular masses of therapeutic monoclonal Igs were determined with high accuracy [11–13]. Accordingly, manufacturers of therapeutic monoclonal Igs are using the newest generation of mass spectrometers to evaluate product quality via accurate molecular mass determinations of recombinant Igs.

Our group recently demonstrated how microflow liquid chromatography coupled with electrospray ionization (ESI) and Q-TOF MS (microLC-ESI-Q-TOF MS) could be used to identify and monitor a monoclonal immunoglobulin in a patient's serum and urine [14–16]. Instead of tracking the intact monoclonal Ig, the disulfide bonds holding the HC and LC together were reduced using DTT and the LC was the primary analyte monitored by MS. Once reduced, LCs were further separated from HCs using liquid chromatography and these LCs were then injected into the mass spectrometer where their masses are obtained. There are several analytical advantages to measuring LCs rather than the HC or the

Download English Version:

https://daneshyari.com/en/article/1993284

Download Persian Version:

https://daneshyari.com/article/1993284

Daneshyari.com