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Understanding and Identifying Monoclonal Gammopathies

Mohammed Attaelmannan[1] and Stanley S. Levinson[1,2*]

Monoclonal gammopathies reflect conditions in which abnormal amounts of immunoglobulins are produced by a clone that developed from a single pro-B germ cell. The condition may reflect a disease process or be benign. The primary purpose of this review is to emphasize routine clinical laboratory techniques that currently are recommended for use in identifying monoclonal gammopathies from serum and urine. Selection of the preferred technique and correct interpretation often is dependent on an understanding of the immunological basis and clinical sequelae associated with these conditions. For this reason, we first briefly discuss the structure, production, and nature of immunoglobulins, and then describe important features of the associated diseases. Finally, we discuss strengths and weaknesses of the techniques and make reference to current recommendations to facilitate optimal testing. We discuss in detail high-resolution electrophoresis, methods for quantifying immunoglobulins, immunofixation electrophoresis, problems associated with analysis of urine immunoglobulins, and identification of cryoglobulins and immune complexes.

Monoclonal gammopathies result from an overproduction of a single abnormal clone of a plasma cell or B lymphocyte. The monoclonal immunoglobulin is recognized as a band of restricted migration on serum or urine electrophoresis (M-component).[3] When the band represents a monoclonal free light chain, it usually is called a Bence Jones protein (BJP). In some cases, more than one clone may produce monoclonal gammopathies (biclonal or, very rarely, triclonal) *(1)*. Usually, the production of a M-component does not seem to be a response by the immune system to an offending immunogen. Still, in some cases, M-components with activity against immunogens from infectious agents have been identified *(2)*. The exact meaning of this relationship remains obscure.

If a disease is caused by a monoclonal line of plasma cells, derived either from a malignant clone(s) or from a nonproliferative population of cells, the condition is called plasma cell dyscrasia. In some cases, monoclonal gammopathies may occur as a result of abnormal B cells, which have not yet developed into plasma cells. This type of gammopathy is seen in leukemia or lymphoma. It is important to note that many monoclonal gammopathies identified on serum electrophoresis are benign, so-called monoclonal gammopathy of undetermined significance (MGUS). Guidelines for laboratory evaluation of patients with monoclonal gammopathies recently have been described *(3)*.

Structure, Genetic Characteristics, Production of Immunoglobulins, and Nature of M-Components

There are five classes of antibodies: IgG, IgA, IgM, IgD, and IgE. Each basic unit is a monomeric antibody consisting of four chains: two heavy chains, providing class specificity, and two light chains, κ or λ. Each whole antibody consists of a constant, COOH-terminal end (Fc), and variable, NH_2-terminal end (Fab) *(4)*. Depending on the class, each intact monomeric unit has a molecular weight varying between ~150 000 and 200 000, whereas each light chain weighs ~22 000. The variable end of each antibody contains a unique antigen combining site, whereas the Fc portion, which contains common determinants, defines class and binds to plasma proteins and to cell Fc receptors. IgG usually exists as a monomer, IgA as a dimer in secretions but as a monomer in serum, and IgM as a pentamer.

The heavy chain variable region is coded by three separate gene segments, and the light chains by two

[1] Department of Pathology and Laboratory Medicine, University of Louisville, Louisville, KY 40292.
[2] Department of Veteran Affairs Medical Center, Louisville, KY 40206.
*Address correspondence to this author at: Laboratory Service, VAMC, 800 Zorn Ave., Louisville, KY 40206. Fax 502-894-6265; e-mail levinson@louisville.edu.

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[3] Nonstandard abbreviations: M-component, monoclonal component; BJP, Bence Jones protein; MGUS, monoclonal gammopathy of undetermined significance; HRE, high-resolution electrophoresis; IFE, immunofixation electrophoresis; and CE, capillary electrophoresis.

genes. Before transcription, the genes are randomly rearranged on the chromosome with a set of variable region genes being translocated to form a continuous gene product *(4, 5)*. The constant regions are then spliced.

It appears that there are nearly 200 functional heavy and light chain gene segments that give rise to combinations of gene products, allowing typical B-cell lines to produce $>5 \times 10^7$ antibodies with different unique variable end antigen combining sites *(4, 6–8)*. B cells that contain surface receptors that best fit antigens are encouraged to multiply (affinity maturation), providing a means whereby more specific antibodies with greater affinity can be produced *(4)*. This, together with nucleotide additions or deletions during combinational joining (junctional diversity), allows for $>10^9$ different antibodies *(4, 5, 9)*.

The immunoglobulins formed early in the normal response to an immunogen are IgM and IgD isotypes; these are located on the B-cell surface as recognition receptors. Many of the immature cells produce low-affinity early antibodies that bind to multiple antigens *(10, 11)*. The activated B cells begin to divide, and class switching from the IgD and IgM heavy chains produced earlier to the IgG, IgE, or IgA classes occurs *(4, 5)*. Much class switching and some somatic mutation occur in geminal centers of lymphoid organs under the influence of various cytokines *(5)*. As B cells mature into plasma cells, they home to the bone marrow.

In contrast to the great diversity of normal immunoglobulins, in monoclonal gammopathies a single abnormal cell line predominates (or in the rare case two or three). The abnormal cell(s) may produce an intact immunoglobulin, free light chains without heavy chains (often both intact and free), and rarely only heavy chains. The cells may produce immunoglobulins with peculiar or missing amino acids, or class switching may occur in some abnormal cells, causing lines that produce immunoglobulins with two or more heavy chain classes; each abnormal cell line produces only a κ chain or λ chain, never both.

Plasma Cell Dyscrasias

MULTIDISCIPLINE DIAGNOSIS

Diagnosis of plasma cell dyscrasia requires a multidisciplinary approach *(12, 13)*. Table 1 outlines the reasons for this.

Table 1. Plasma cell dyscrasias: Multidisciplinary diagnosis.

Discipline	Procedure
Biopsy	Bone marrow for myeloma
	Tissue for amyloidosis
	Solitary plasmacytoma
Clinical assessment	Active or smoldering myeloma
	MGUS
Radiology	Bone thinning or lytic lesions in myeloma
	Cardiac ultrasound in amyloidosis
Clinical laboratory	Hematology for cell counts and assessing anemia
	Clinical chemistry for hypercalcemia and kidney function
	Urinalysis for protein and kidney function
	Electrophoresis and immunoassay for confirming and classifying M-component type, assessing tumor burden, and assessing decreases in normal immunoglubulin concentrations
	Assessing cryoglobulinemia

MULTIPLE MYELOMA

Multiple myeloma is a plasma cell dyscrasia in which an increased number of monoclonal plasma cells (plasmacytoma) occurs in the bone marrow. The term multiple is used because plasmacytoma is found in multiple sites. Usually, there are <4% plasma cells in the bone marrow. Multiple myeloma is characterized by >30% plasma cells, or <30% and ≥10% plasma cells in bone marrow in the presence of other evidence, such as that outlined in Table 2 *(12–14)*. Patients with heavy BJP proteinuria (>1 g/24 h) usually have myeloma *(13)*. In the classical case, the M-component is abnormally increased in concentration but the normal immunoglobulins are decreased, giving rise to a profile in which one heavy chain class is increased but the others decreased. This pattern is shown in Fig. 1A. In ~5% of cases, the abnormal plasma cell does not secrete an M-component.

In multiple myeloma, IgG is the most common immunoglobulin, with IgA being the second most common. IgM is rare. Although in myeloma M-components typically appear to be low-affinity early antibodies with unknown antigen specificity, the fact that IgG and IgA are more common than IgM suggests that maturation of the abnormal cell has proceeded far enough along for antigen stimulation and class switching to occur *(15–19)*.

ASYMPTOMATIC OR SMOLDERING MULTIPLE MYELOMA

Some individuals with multiple myeloma show no symptoms. In this case, a M-component usually is inadvertently identified by serum electrophoresis. Various terms have been applied to this syndrome, including smoldering, indolent, and asymptomatic myeloma. Usually treatment is withheld until the M-component begins to rise or symptoms associated with myeloma appear *(14, 20)*.

SOLITARY PLASMACYTOMA

In some cases, only a single site of proliferation exists in bone *(21)*. Occasionally, the plasmacytoma is in tissue other than bone, often in the sinuses or nasopharynx. In these cases, the likelihood of remission or complete cure is

Table 2. Criteria for multiple myeloma when <30% plasma cells.

More than 10% plasma cells in bone marrow and:

(*a*) Significant M-component in serum (usually monoclonal IgG >20 g/L or IgA >10 g/L); or

(*b*) BJPs in urine; or

(*c*) Lytic bone lesions (~70%)

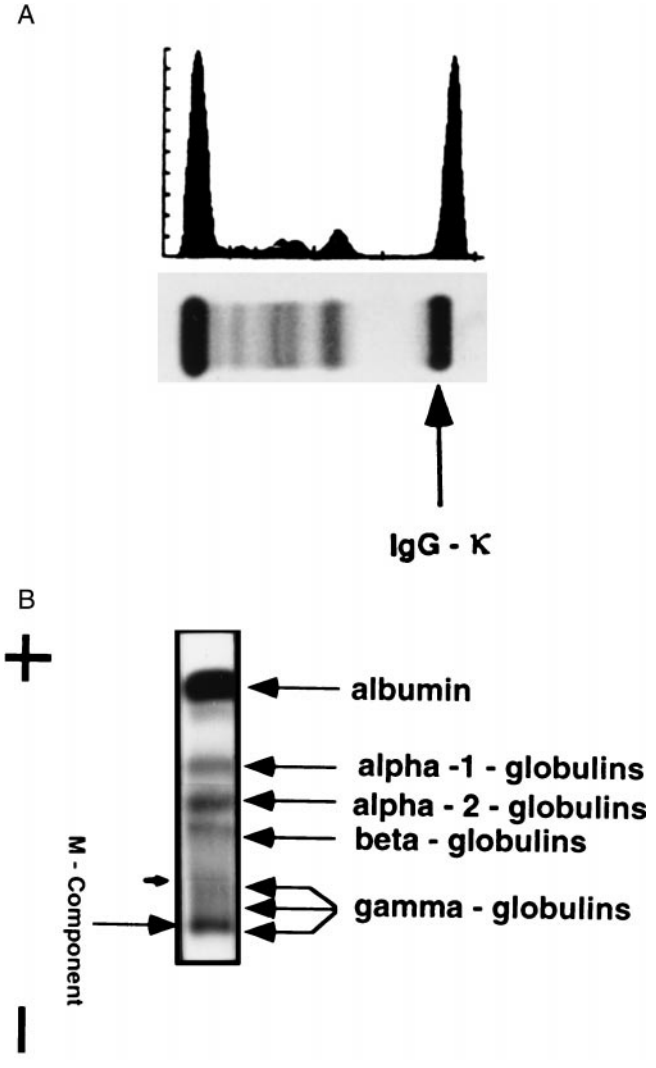

Fig. 1. Illustration of serum protein electrophoresis showing M-components.

Both M-components were identified as IgG-κ. *Panel A* shows a dense M-component (*arrow*) and densitometer tracing with an almost clear region surrounding the dense band, indicating reduced synthesis of other (normal) immunoglobulins. In this case, the best estimate of the M-component concentration can be made from the densitometer tracing of the γ area, which translated into 38 g/L (reference interval, 0.7–1.6 g/L). Immunonephelometric assay showed an IgG concentration of 49 g/L (7.2–16.8 g/L), IgA of 0.11 g/L (0.7–3.8 g/L), and IgM of 0.33 g/L (0.6–2.7 g/L), with a κ/λ ratio of 102 (1.2–2.6). *Panel B* shows a low-concentration M-component (*large arrow*) superimposed on a surrounding region of diffuse staining, indicating normal or increased synthesis of other immunoglobulins. Here the densitometer tracing translated into a γ-globulin of 22 g/L. IgG = 14 g/L, IgA = 4.7 g/L, and IgM = 2.5 g/L, with a κ/λ ratio of 2.6. In this case, the M-component can only be estimated from the staining density by visual inspection (the tracing is not shown). The M-component density is approximately one-third of the total staining in the γ region, or 22 g/L ÷ 3 = <10 g/L. A similar conclusion could be reached by inspection of the densitometer tracing if it were shown. Protein fractions are indicated in *B*. The *small arrow* indicates the origin. Polarities are indicated, and direction of electrophoresis is toward the positive electrode.

higher than for myeloma. Here, the M-component usually is of low concentration (<25 g/L) *(14)*.

POEMS SYNDROME

This refers to a rare disorder with peripheral neuropathy, organomegaly, endocrine deficiency, monoclonal gammopathy, and skin pigmentation *(22)*. The clinical course is more indolent than multiple myeloma *(22)*.

Lymphocytic Diseases

M-Components may be associated with B-cell lymphomas and leukemias. In these cases, IgM is more common than IgG or IgA, suggesting that the abnormal clone is more primitive than in myeloma. Waldenström syndrome is a low-grade small-cell lymphoma that produces monoclonal IgM *(23)*. Clinical features usually are related to growth of the tumor. Many patients produce monoclonal IgM, which may cause hyperviscosity syndrome, or type I or II cryoglobulinemia, which may lead to Raynaud syndrome, vasculitis, cold agglutinin hemolytic anemia, peripheral neuropathy, or immune complex disease *(23, 24)*. M-Components are also seen in leukemias, especially chronic lymphocytic leukemia where identification adds little to the diagnosis or predicted outcome.

Amyloidosis AL

Amyloidosis is a condition in which an abnormal proteinaceous material is deposited in tissues *(25)*. In immunoglobulin light chain-related amyloidosis (AL) the deposits are attributable to BJPs *(25)*. In amyloidosis, fibrils are formed that appear arranged in an antiparallel conformation with a β-pleated sheet structure when examined by x-ray diffraction. Under the polarizing microscope, amyloid appears as a Congo red-staining material that exhibits apple-green birefringence *(25)*. This appearance under polarized light is the most common method used to identify amyloid, although the electron microscope is more sensitive and is used for suspected cases when light microscopy is nondiagnostic *(25)*. Lambda chains appear to have a greater affinity to form amyloid than κ chains *(26, 27)*.

The most common organs affected in amyloidosis are the kidney, heart, liver, and gastrointestinal tract. Although the central nervous system usually is not affected in amyloidosis AL, peripheral nervous system complications are common, especially carpal tunnel syndrome *(25)*. These diseases may be associated with multiple myeloma or may occur in the absence of clear plasma cell malignancy. In the absence of myeloma, biopsy of the bone marrow is not helpful because it usually shows <5% plasma cells. Amyloid is best identified from biopsy of an involved organ, but because of the invasiveness associated with this approach, usually a rectal or more commonly an abdominal subcutaneous fat pad is biopsied. These are positive under polarized light in ~85% of cases *(25, 28)*. If the disease occurs in the absence of myeloma, a low-concentration monoclonal spike consisting of IgG or IgA, which often is small (<10 g/L), may be seen in serum, and a BJP, whose presence is very helpful in leading to the diagnosis, usually is seen in urine with or without a serum M-component.

Approximately 5% of patients with Waldenström macroglobulinemia develop amyloidosis, and this diagnosis

should be suspected in patients with IgM M-components and appropriate clinical presentation *(23)*.

Transplant Monoclonal Gammopathy

A transient monoclonal or oligoclonal gammopathy has been associated with bone marrow and solid tumor transplants. Generally, these gammopathies are of low concentration and short-lived *(14, 29, 30)*.

MGUS

A low-concentration M-component associated with no signs or symptoms of plasma cell dyscrasia or B-cell abnormality is seen in the serum of a small number of people under 70 years of age and ~3% of persons over 70 *(14)*. Usually, the concentration is <20 g/L IgG or 10 g/L IgA. As shown in Fig. 1B, on electrophoresis these low-concentration M-components usually are superimposed on a diffuse background of normal immunoglobulins. If the concentration is between 20 and 30 g/L or if a BJP is present, it may be difficult to differentiate from asymptomatic myeloma.

Idiopathic BJP proteinuria has also been described *(31)*. Some of these patients remain stable, but many develop myeloma. Low concentrations of BJPs (<0.2 g/24 h) may be seen in the urine of patients with MGUS and with chronic diseases other than myeloma *(31–33)*.

Identification of M-Components

Bone marrow biopsy and testing of serum and urine for a M-component are fundamental in the work-up of monoclonal gammopathies *(1, 12)*. In some cases, peculiar bands may be seen in serum that are not M-components. These include transferrin associated with iron deficiency anemia, highly increased β-lipoprotein, fibrinogen, or C-reactive protein *(34)*. For this reason, suspect bands identified by electrophoresis should be described as a paraprotein or possible M-component until characterized by immunoassay.

HIGH-RESOLUTION ELECTROPHORESIS

High-resolution agarose protein electrophoresis (HRE) is a technique that gives better resolution of serum, urine, and cerebrospinal fluid proteins than standard electrophoresis *(35)*.

As illustrated in Fig. 1B, traditionally agarose electrophoresis of serum proteins is divided into five fractions: albumin, α_1-globulins, α_2-globulins, β-globulins, and γ-globulins. HRE systems separate several other fractions, often totaling 10–12. Importantly, HRE permits better detection of low-concentration M-components migrating in the α_2, β, or γ regions of the gel *(36, 37)*.

On average, the maximal density of IgG is cathodal to the origin, IgM is coincident with the origin, and IgA is anodal to the origin, closest to the β region. Nevertheless, because of their great diversity, some of each immunoglobulin class exhibits charge characteristics that cause migration in the γ, β, and even pre-β regions. Likewise, intact M-components most commonly migrate in the γ region, but occasionally they are found in the β or even the α_2 region. Fig. 2 illustrates this effect. In some cases, the smaller BJPs migrate well into the α_2 region.

The most important function of HRE is as a screen for identifying possible M-components. Visual examination of the gel is the most sensitive means of detection because low-concentration M-components are readily missed by densitometry scanning.

QUANTIFICATION OF M-COMPONENTS

Quantification of M-components is desirable because it aids in assessing the tumor load and in determining whether the disease is progressing. Because of excellent reproducibility and ease of use, nephelometric and turbidimetric immunoassays of M-components currently are the most common methods for quantifying immunoglobulins. However, when assaying M-components, these methods are often inaccurate because the antibodies and calibrators used with the assay are developed using the vast variety of diverse normal immunoglobulins, whereas M-components exhibiting limited or incomplete antigenic determinants may react incompletely with the antiserum and behave peculiarly compared with the calibrator. Thus, the concentration of M-components is often under- or overestimated *(38)*. Quantification of the normal immunoglobulin classes not involved in the disease may be useful for determining the functional degree of hypogammaglobulinemia.

Because of these peculiar immunological properties, the best way to assess the concentration of a high-concentration M-component is by densitometry. If the M-component is migrating in the γ region of the electrophoretic gel and is increased, whereas the other immunoglobulins are decreased as seen in Fig. 1A, the concentration of M-component can be measured accurately by densitometry.

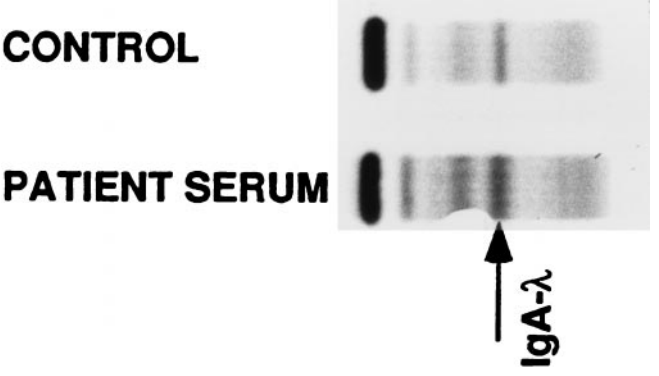

Fig. 2. Rapidly moving M-component.

In this case an IgA-λ (identified by IFE) is superimposed on β-globulins. Notice that the β-globulin fraction of the patient is approximately two- to threefold denser than the control. Immunonephelometric assay showed IgG = 9.6 g/L (reference interval, 7.2–16.8 g/L), IgA = 8 g/L (0.7–3.8 g/L), and IgM = 0.25 g/L (0.6–2.7 g/L), with a κ/λ ratio of 0.5 (1.2–2.6). IgA M-components often migrate in the β region, but IgG M-components migrate in the β region just as frequently. See legend of Fig. 1B for identification of bands.

Low-concentration M-components in serum or those migrating in the β region are usually superimposed on other proteins. Fig. 1B shows a low-concentration M-component superimposed on a diffuse background of polyclonal immunoglobulins. In such a case, the concentration can only be qualitatively estimated from the densitometer tracing by subtracting the approximate density of a small band from the approximate underlying staining.

IMMUNOFIXATION ELECTROPHORESIS

In immunofixation electrophoresis (IFE), proteins are fractionated on electrophoretic strips as with HRE but not stained. Each lane is overlaid with monospecific antisera, usually with activity against the three major immunoglobulin classes (IgG, IgA, and IgM), and against free and bound (intact) κ and λ light chains. Immunoglobulins are precipitated by the antisera in the gel. After a few hours, the gels are washed to remove unprecipitated proteins and then stained *(39, 40)*. The results of IFE are illustrated in Fig. 3. If a M-component is present, it appears as a band coincident with the paraprotein seen on HRE. It can be characterized as IgG, IgM, IgA, and κ or λ, depending on the pattern of precipitation.

With a detection limit down to ~0.25 g/L, IFE is more sensitive for detecting low-concentration M-components than HRE *(41)*. For this reason, IFE is recommended as the preferred technique for identification of low-concentration M-components *(3)*. This feature contributes very little to the identification of M-components in serum, where tiny components often are of little clinical significance and their identification adds expense to an unnecessary additional workup, but with urine greater sensitivity is important for identifying BJPs present in very low concentrations.

There is a problem associated with the reaction between antibodies and very high concentrations of M-components that may affect the interpretation of IFE patterns. With very high concentrations of M-component, the antiserum will be relatively dilute and complete precipitation may not occur. This phenomenon of antigen excess, illustrated in Fig. 3, is called the prozone effect. This effect usually is not a great problem with serum M-components because the concentration changes over only a limited range of ~10–60 g/L. In serum, a very dense band may appear as a donut with a clear spot in the center, but such patterns are still interpretable. In these cases, a solid band can be demonstrated by diluting the serum 2- to 10-fold and repeating the assay.

This problem is worse when analyzing BJPs in urine *(38)*. As described below, in urine much wider ranges of M-component concentrations are commonly encountered so that the prozone effect becomes a much greater problem. In addition, compared with intact immunoglobulins, free light chains more often react poorly with the antisera, producing indistinct bands *(38)*.

Quality control is an important feature for ensuring accurate testing. Antisera are produced against polyclonal immunoglobulins and may react poorly with M-components *(42)*. Antisera of sufficient quality to perform IFE are now available from several manufacturers. Each lot of antiserum should be tested against sera containing known M-components and especially against concentrated urine containing BJPs and urine containing general proteinuria without BJPs. Many laboratories keep a stock of at least two antibodies from different companies to reanalyze incomplete or peculiar precipitation patterns.

Problems Associated with Analysis of Urine

MONOCLONAL FREE LIGHT CHAINS

Free light chains usually are M_r 22 000 monomers or M_r 44 000 dimers *(43)*. As a result, BJPs readily pass through the glomerulus and are more concentrated in urine than in serum. In addition, because urine is relatively dilute in total protein compared with serum, urine can be concentrated further by differential filtration using mechanical

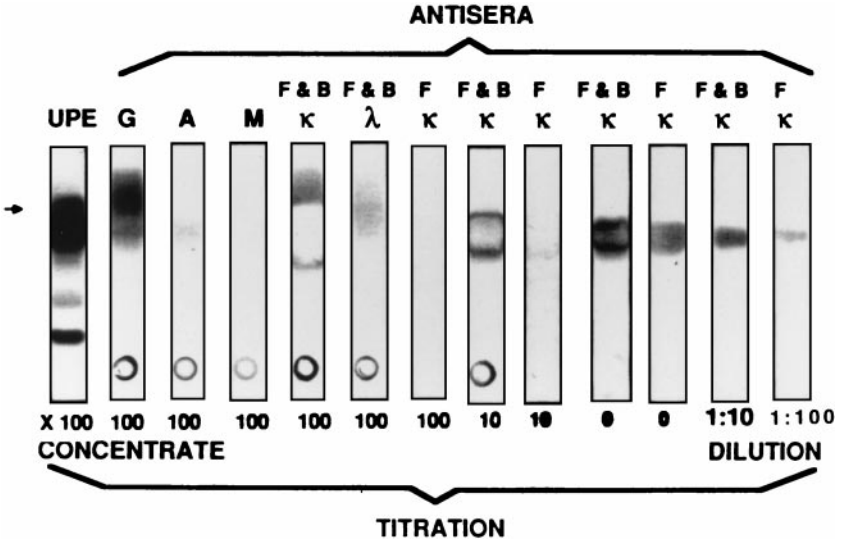

Fig. 3. Electrophoresis (*UPE*) and IFE of a urine sample containing a κ BJP.

Each monospecific antiserum used for fixation is indicated. *F & B* indicates those strips fixed with free and bound antisera; *F* indicates antisera against free chains only. The sample was mechanically concentrated and diluted over the range indicated. A very dense band in the γ region of the 100× UPE appears polyclonal because of its great density. The prozone effect is seen with the κ light chain antisera. More prozone effect is seen with the weaker *F* antisera. After dilution, a thin band representing a BJP is seen migrating just at the front of IgG. The direction of migration is toward the *bottom* (positive electrode). Of note is that, although a very dense band (which almost appears polyclonal) is seen with UPE in the γ region and although IFE of the 100× concentration with F & B antisera showed an extreme prozone effect, comparison of the two leaves little question that a κ-BJP is present. *G*, anti-IgG; *A*, anti-IgA; *M*, anti-IgM. *Arrow* indicates origin.

devices. To identify BJPs present in very low concentrations, it is recommended and important that urine samples be concentrated between 100- and 150-fold, regardless of the amount of total protein in the urine *(27, 44)*.

It seems to us that excessive concentration of urine above 150-fold is unwise because BJPs present in very low concentrations (<0.2 g/24 h), which are MGUS or associated with other chronic diseases, may be seen *(31–33)*, which would necessitate an unnecessary workup. Furthermore, excessive concentrations may cause an increase in background staining and, as will be discussed below, invariably give rise to polyclonal patterns that appear as multiple bands that can be confused with a monoclonal pattern *(27)*.

Because of its greater sensitivity, IFE should be performed along with HRE on all urine specimens suspected of having a BJP *(27, 45)*. Specimens should be 24-h collections. First morning collections may also be adequate, but random collections are unsuitable *(46)*.

Because of the wide range of protein concentrations achieved by mechanical concentration, ranging between ~0.1 and 20 g/L, high-concentration BJPs may appear overloaded on IFE or a prozone effect may occur, making interpretation more difficult. As shown in Fig. 3, more definitive patterns can be obtained by diluting the specimen and repeating the IFE. In practice, overloading rarely leads to a mistake in interpretation because the correspondence between the band seen on urine HRE and a distorted band on IFE provides a means by which correct interpretation can usually be achieved without repeat assay *(47)*.

BJPs react very idiosyncratically with antisera. As a result, even BJPs present in very high concentrations may react poorly, showing little or no banding. Thus, if the HRE pattern shows a suspect band, it is wise to test the sample with a second antiserum from a different source before concluding that a BJP is not present.

Another difficulty with IFE is that BJPs migrating coincidental with intact M-components cannot be distinguished using free and bound antiserum. This problem may be more hypothetical than real. As illustrated in Fig. 3, a comigrating BJP may be suspected when one observes a large antigen excess effect with the κ or λ free and bound light chain antiserum compared with a lesser effect with the heavy chain antiserum. Usually, if a BJP is migrating close to an intact monoclonal protein, dilution studies will distinguish it, showing that it is migrating close to but not exactly coincidental with the intact protein.

Nevertheless, it might be possible to miss a tiny BJP hidden directly behind a moderately sized intact immunoglobulin. Thus, to ensure that there is not a comigrating BJP, it may be necessary to fix specimens with an intact immunoglobulin in urine but without an obvious separate light chain band with free antisera (antisera that react only with free light chains). Fig. 3 illustrates IFE with the use of free and bound (intact), and free antisera. Because of lower activity titers in free antisera compared with free and bound antisera, the prozone effect attributable to antigen excess is greater. Thus, if suspect bands stain strongly with free and bound antisera, but not at all with free antisera, dilution studies should be performed. In our experience, because of its weak avidity, free antiserum has little usefulness other than for this purpose *(27, 44)*.

polyclonal free light chains

Polyclonal free light chains are secreted in excess of heavy chains *(48)*. Thus, normal urine that is sufficiently concentrated will exhibit polyclonal free light chains. As shown in Fig. 4, polyclonal free light chains may appear as multiple bands (ladder pattern) after IFE and isoelectric focusing. This pattern, observed as three to six equally spaced bands, is more often associated with κ light chains but may also be found after analysis of λ light chains, and can be uncovered in most urine specimens if sufficiently concentrated *(49–51)*. MacNamara et al. *(51)* demonstrated that this pattern is the property of normal free light chains by inducing the transient appearance of multiple bands with arginine infusion in healthy volunteers. The multiple banding pattern appears to be largely a product of charge differences *(50)*. The pattern may be related to homologies in the framework 1 region of the light chain variable region, which defines four serological subgroups of κ and six subgroups of λ light chains *(27, 52)*.

As a result of poorer resolving power, IFE devices from some manufacturers do not appear to have the ability to distinguish this pattern routinely. With some systems, electrophoresis of polyclonal free light chains in high concentrations will cause dense, protracted staining patterns that will evolve into a ladder band pattern with

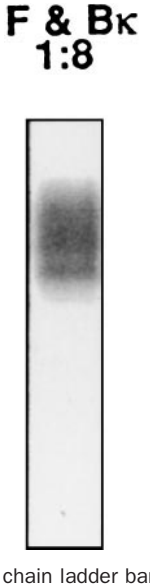

Fig. 4. Polyclonal free light chain ladder band pattern.

The gel was fixed with antisera against free and bound κ. Multiple bands are present but difficult to see. Some electrophoretic systems show these bands better than others. Notice that the band closest to the *bottom* could be mistaken for a BJP. The direction of migration is toward the bottom (positive electrode).

dilution, whereas patterns on other systems will evolve into diffuse staining zones. Although a ladder pattern, per se, does not reflect an abnormal condition, as illustrated in Fig. 4, the pattern may be mistakenly interpreted as a BJP or it may obscure a low-concentration BJP migrating coincidental with the pattern *(50, 51, 53)*. In the latter case, the presence of a low-concentration BJP may be overlooked. Because IFE is not a quantitative technique with a well-defined lower reference limit for detection, systems with sufficient separation power to discern these polyclonal patterns may offer little advantage over otherwise sensitive IFE systems that do not *(27)*.

Identification of IgD or IgE

If a κ or λ M-component is found in serum in the absence of a IgG, IgA, or IgM heavy chain, it is necessary to test the sample for IgD and IgE.

Identification of Cryoglobulinemia and Immune Complexes

Cryoglobulins are immunoglobulins that precipitate when cooled below body temperature. This occurs either because the structure of the immunoglobulin is such that it precipitates in the cold or because the immunoglobulin, usually IgM, contains rheumatoid factor activity, in which case the variable Fab of the rheumatoid factor binds to the Fc portion of polyclonal immunoglobulins, forming an immune complex that precipitates in the cold.

Three types of cryoglobulins have been described. Type I consists of a single monoclonal immunoglobulin; type II is mixed polyclonal-monoclonal, and type III is mixed polyclonal-polyclonal *(24)*. Types I and II may be attributable to M-components associated with multiple myeloma, Waldenström syndrome, or other lymphoproliferative diseases. As a result of precipitation in blood vessels close to the surface, these may cause several of the symptoms associated with monoclonal gammopathies, such as Raynaud syndrome, peripheral neuropathy, and gangrene in the absence of known vascular causes.

Thus, it has been recommended that cryoglobulins should be assessed in all patients with M-components and cold-sensitive complications *(3)*. Cryoprecipitates appear as gelling or precipitation. Because of the ambiguity associated with this endpoint, it is important to collect no less than 10 mL of blood. The blood should be kept at 37 °C during transport, clotting, and serum separation. The warm serum is placed in a 4 °C refrigerator and examined for up to 7 days. If a cryoprecipitate appears, it can be analyzed by IFE to confirm that it is a cryoglobulin and to type it *(24)*. To ensure the removal of adsorbed immunoglobulins before analysis, the cryoglobulin should be collected by centrifugation and then reprecipitated in saline by cooling. The pellet should be collected a second time and washed thoroughly with ice-cold saline. If no albumin is seen with HRE, it is likely that the precipitate has been adequately washed.

Soluble immune complex aggregates that may or may not be cryoglobulins may appear as a paraprotein band on HRE. In IFE, a band is seen in all lanes or in the IgG, IgM, κ, and λ lanes. Treatment for 4 h at 37 °C with a sulfhydryl reagent breaks the aggregates into component parts (we make a 1:10 dilution of 2-mercaptoethanol with isotonic saline and add 10 μL of the dilution to 100 μL of the patient's serum). After treatment, the IFE is repeated. If the repeat pattern appears as a monoclonal band in the κ or λ and the IgG or IgM lanes, this indicates a monoclonal IgG or IgM rheumatoid factor, respectively. If a polyclonal pattern is seen in all lanes, the rheumatoid factor was not monoclonal. If the pattern is ambiguous after treatment, the rule of thumb is to repeat the treatment for 12 or 24 h.

Turbidimetric/Nephelometric Analysis

Automated immunonephelometric or turbidimetric analysis has two uses in the workup of monoclonal gammopathies in serum: (*a*) They are useful for determining whether the immunoglobulin classes, other than the monoclonal, are decreased. When decreased, a diagnosis of myeloma is more certain. (*b*) Nephelometric and turbidimetric tests can be used for analyzing the κ/λ ratio. In some cases, this provides a means for identifying whether a paraprotein component in serum identified by HRE is monoclonal, as well as its immunoglobulin class, without the need for the more complex IFE. The typical κ/λ ratio is ~2, with a range of ~1–3. If the κ/λ ratio is abnormal and IgG, IgA, or IgM is increased, the class and type of paraprotein seen on HRE is identified without IFE *(54–56)*.

This technique works well when the abnormal immunoglobulin is very large, such as that shown in Fig. 1A. (It is clear from the electrophoretic pattern and immunonephelometric analysis that an IgG κ M-component is present.) In such a case, an additional assay is not needed. But the technique often does not show an abnormal κ/λ ratio when the abnormal immunoglobulin is of low concentration and the normal immunoglobulins are not decreased (Fig. 1B). This is attributable to a masking effect by the κ and λ activity of the normal immunoglobulins. In addition, this technique is not useful when biclonal or triclonal gammopathies are seen with HRE. In small laboratories where IFE is performed infrequently, this approach may effectively reduce the need for the more tedious IFE. On the other hand, in laboratories where IFE is performed frequently, κ/λ ratios serve little purpose.

Another advantage of having immunonephelometry or turbidimetry performed in the laboratory is that the various antisera used for the automated analysis can be used as a second source of antibody for reanalysis of peculiar IFE patterns.

Capillary Electrophoresis

High-performance capillary electrophoresis (CE) is a new technique in which proteins in a buffer can be separated in a narrow capillary (usually ~50 μm in diameter). Although no gel is used in the capillary, samples are

relatively small compared with the surface area of the capillaries so that separation of the proteins into components is facilitated by adsorption to the walls of the silica capillaries. Thus, the proteins separate on the basis of chromatographic partitioning to the capillary walls as well as charge. Very small sample sizes are made possible by very sensitive monitoring devices. As a result, similar to the fractions seen with HRE, the proteins are separated into ~12 identifiable fractions. A detector that records absorbance as the proteins pass by is located at the cathodal end of the capillary. CE may be able to detect paraprotein peaks as well as or even slightly better than HRE *(57, 58)*. The advantage of CE is that it is both automated and rapid, so that many samples can be analyzed in only a short time, making it less tedious than HRE, which usually is manual.

Suspected monoclonal proteins can be identified on CE by immunosubtraction. Here, CE is performed before and after exposing samples to solid particles coated with antibodies specific for immunoglobulin classes and κ and λ light chains. Removal of a peak by the coated beads provides a means for characterizing the nature of the M-component.

Although this technique has been widely studied for detection of serum M-components, it has not been perfected for analysis of urine proteins *(58)*. In addition, its effectiveness is still unclear for the detection of antigen excess, small M-components, and M-components migrating in the β region coincidental with other proteins. For these reasons, although some large laboratories may have validated its use and use it routinely, it is premature to recommend its use on a routine basis, although it may be the choice for the future *(58)*.

Conclusions

Monoclonal gammopathies may be indicative of diseases arising from B cells or plasma cells. The most common diseases are myeloma and amyloidosis AL. These diseases may be difficult to diagnose because they affect many tissues and exhibit nonspecific symptoms. Definitive diagnoses of these conditions are based on clinical criteria, laboratory testing, and biopsy. Identification and quantification of a M-component in serum or urine is helpful in leading to the diagnosis and evaluation of extent of disease. M-Components usually are identified and characterized by a combination of HRE and IFE, although in some cases immunonephelometry or turbidity may be used. The newer technique of CE may eventually replace these.

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