

Pathogenesis and Morbidity of Autoantibody Syndromes in Waldenstrom's Macroglobulinemia

Marvin J. Stone

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Abstract

A number of autoantibody syndromes have been identified that are mediated by monoclonal macroglobulins. Chronic cold agglutinin disease (CAD), mixed cryoglobulinemia (MC), and various polyneuropathies produce symptoms and signs such as cold sensitivity and sensorimotor neuropathies that bring the patients to attention ("the patients tell you"). Many of these patients would be classified as having monoclonal gammopathy of undetermined significance (MGUS) were it not for the consequences of antigen-antibody interaction. Other patients with autoantibody syndromes have overt Waldenström's macroglobulinemia (WM). These individuals present at an earlier stage than WM patients who do not have evident antibody activity. Thus the presence of autoreactive antibodies influences clinical presentation and natural history of the disorder. The frequency of monoclonal IgMs with functional antibody activity is unknown. The principal problem is detection. New methods have been described which may more precisely identify the antigens reacting with M-proteins. Some "autoimmune" disorders may represent cross-reactions to exogenous antigens. Elucidation of antigens reacting with monoclonal IgM proteins may provide important insights into the pathogenesis of WM.

Introduction

BIgM is a 925 kilodalton immunoglobulin that is the first antibody formed after primary immunization. It contains 10 antigen-combining sites per star-shaped pentamer.^{1,2} The term "macroglobulin" is apt since a mole of IgM weighs a ton. The study of myeloma proteins and Waldenström's macroglobulins from humans and mice has led to most of what we know about antibody structure, synthesis, genetics, and metabolism. Do these "paraproteins" function as true antibodies? If so, does identifica-

tion of the antigens tell us something about pathogenesis of these diseases?^{2,3} Such antigens could drive an immune response that results in monoclonal gammopathy or they may provide a clue to identification of cells which tend to become neoplastic. Another possibility is that the antigens reacting with Waldenström's macroglobulins are simply incidental.

A number of barriers exist in the detection of paraprotein antibodies. First, one usually doesn't know which antigen to look for. There appear to be more than 108 antigen specificities. By definition, a monoclonal antibody reacts with a single determinant unless that determinant is repeating. Second, the type of binding assays become pertinent. Primary binding assays are preferred because secondary assays, dependent on the formation of a three-dimensional structure, usually yield negative results. Occasionally, clinical clues are present, that is, the "patients tell you" what they have. Examples include cold sensitivity in cryoglobulinemia and chronic cold agglutinin disease or various sensory and motor peripheral neuropathies. Identification of antigens reacting with M-proteins may be aided considerably by epitope reconstruction (E-MAP) technology that has been described by Bogen et al.^{4,5} This method has the potential to open the "black box" of reactive antigens. It also may provide a handle in enumerating how many Waldenström's macroglobulins are antibodies and whether an immune response contributes to the development of WM.

IgM-Related Disorders

Symptoms attributable to the IgM monoclonal protein occur in some patients. Most have no overt evidence of lymphoma. Symptomatic patients have been classified as having "IgM-related" disorders.⁶ A revised classification shown in Table 1 reflects the fact that single-component IgM cryoglobulinemia is probably not an autoimmune disorder, but rather due to a concentration-dependent solubility state. By contrast, mixed IgM-IgG cryoglobulinemia (MC), chronic cold agglutinin disease (CAD), and peripheral neuropathy are autoimmune syndromes in which the monoclonal IgM is the antibody. Finally, deposit diseases such as amyloidosis occur. Most patients with cryoglobulinemia, chronic cold agglutinin disease, neuropathy, or amyloidosis would be classified as having IgM MGUS except for the consequences of immune complex formation or amyloid deposition in tissues.

Baylor Sammons Cancer Center, Baylor University Medical Center, Dallas, Texas

Address for correspondence: Marvin J. Stone, MD, Baylor Sammons Cancer Center, Baylor University Medical Center, Dallas, Texas 75246
Fax: 214-820-2780; e-mail: marvins@baylorhealth.edu

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Table 1 IgM-Related Disorders

Symptoms attributable to the IgM monoclonal protein, but without evidence of lymphoma:

- Single component IgM cryoglobulinemia
- Autoimmune disorders:
 - Mixed IgM-IgG cryoglobulinemia
 - Chronic cold agglutinin disease
 - Peripheral neuropathy
- Amyloidosis (AL)

2nd WM Workshop, 2002-revised 2010.

Mixed IgM-IgG Cryoglobulinemia (MC)

One of the two patients in Jan Waldenström's initial report had a cryoglobulin.⁷ Patients with MC have a variety of symptoms and signs related to cold sensitivity including purpura, arthralgias, and vasculitis. Some patients are asymptomatic.

Cryoglobulinemia has been a particular interest of ours at the Baylor Sammons Cancer Center in Dallas. Characterization of 182 cryoglobulins is shown in Table 2.⁸⁻¹¹ Once identified, the cryoglobulin was isolated and purified by reprecipitation or washing. Protein concentration was determined and identification of the components in the cryoglobulin was carried out by Ouchterlony analysis. Nearly 70% of the cryoglobulins were mixed IgM-IgG, whereas only about 20% were single component (see Table 2). The single-component cryoglobulins had a higher mean concentration of 4.4 mg/mL (range, 0.1-23.3) compared with the mixed IgM-IgG cryoglobulins (mean, 1.9 mg/mL, range 0.1-9.9). Thus, mixed cryoglobulins precipitate at a lower protein concentration than single-component cryoglobulins.

Mixed cryoglobulins are composed of monoclonal IgM autoantibody to the FC portion of polyclonal IgG.⁸⁻¹⁰ Rheumatoid factor assays are positive, often in strikingly high titer. The cryoprecipitating phenomenon is due to the immune complex as both reactants are soluble when isolated.^{1,8-11} Thermal amplitude is high in many mixed cryoglobulins, some of which precipitate or gel near 37°. The temperature at which the cryoprecipitate forms correlates better clinically than the cryocrit. In our experience, cryocrits are unreliable in quantifying cryoglobulins in either single or serial determinations.

During the last 15 years, several groups have reported that hepatitis C is associated with MC and that hepatitis RNA may be found in the cryoprecipitate.¹² Patients with MC and hepatitis C thus have monoclonal gammopathies. The gammopathies constitute a spectrum of clonal B-cell expansion that extends from preneoplastic to overt non-Hodgkin lymphoma.^{3,9,10} Approximately 10% of patients develop lymphoma, especially WM. Both the cryoglobulin and the lymphoma may decrease after interferon and rituximab treatment.¹³⁻¹⁵ Our experience in Dallas supports the strong association with hepatitis C.⁹ Eight of 9 patients with MC were rheumatoid factor-positive, some with very high titer (the highest being 1:327,680). Adequate material was available to test 6 of the rheumatoid factor-positive mixed cryos and all were positive for both hepatitis C antibody and RNA. Interestingly, the one rheumatoid factor-negative patient was also negative for hepatitis C antibody

Table 2 Cryoglobulin Types – Baylor Sammons Cancer Center/Dallas

Total	182
Mixed IgM-IgG	69.8% Mean = 1.9 mg/mL (Range = 0.1-9.9)
Single Component	19.2% Mean = 4.4 mg/mL (Range = 0.1-23.3)
Other	11.0%

Stone, *Clin Lymphoma & Myeloma* 9:97-99, 2009

Stone & Pascual, *Haematologica* 95:359-64, 2010

and RNA. Cryoglobulinemia has a marked effect on serum viscosity. This is especially true because of the high thermal amplitude of mixed cryoglobulins. As one approaches room temperature from 37°, the viscosity can become exponential.^{8,10,11}

Chronic Cold Agglutinin Disease (CAD)

Chronic cold agglutinin disease (CAD) is due to a monoclonal IgM antibody directed to the I or i antigen. Cold agglutinins are present in low titer in many normal individuals. These proteins were the first monoclonal antibodies recognized in patients with cold antibody immune hemolytic anemia.¹⁶ Patients with chronic CAD often have a disorder similar to IgM MGUS, but some have overt WM. The VH4-34 gene segment is required to encode anti-I and i specificities. The same segment encodes bacterial anti-lipopolysaccharide (LPS) activity and other specificities.^{3,9,11} Some of these IgMs are mutated; others not. B cells containing VH4-34 gene sequence may belong to the first line of defense against pathogens and thus constitute part of the innate immune system.

Monoclonal cold agglutinins clearly bind to autoantigens and cause disease, especially acrocyanosis, Raynaud's phenomenon and hemolytic anemia after cold exposure.^{9,11} The treatment of cold agglutinin disease can be difficult. The obvious maneuver of avoiding cold exposure is only variably effective. Nevertheless, it is advisable to warm blood or IV fluids to 37° in such patients. Plasmapheresis to reduce the monoclonal IgM has some effectiveness because IgM is 80% intravascular. The usual treatment approaches used with warm antibody immune hemolytic anemia, corticosteroids or cytotoxic agents, are generally of limited help in chronic CAD. Splenectomy is usually ineffective as well. The anti-CD20 monoclonal antibody, rituximab, has activity in some patients with chronic CAD (54% response rate with median response duration of 11 months). Berentsen et al have reported that the addition of fludarabine to rituximab produces a 76% response rate.¹⁷⁻¹⁹ Twenty-one percent of patients treated with rituximab and oral fludarabine according to the Berentsen regimen experienced complete remissions. These have not previously been seen in this disease. The median duration of response was more than 5 years. Moreover, some patients previously refractory to rituximab alone responded to rituximab plus fludarabine.¹⁹

IgM Neuropathies

A number of immune-mediated neuropathies have been described in patients with monoclonal IgMs^{20,21} and are considered elsewhere in this issue. Myelin-associated glycoprotein (MAG)

is the most common antigen and produces a demyelinating neuropathy which is mainly sensory. Other neural antigens, most of which are carbohydrates, have been described.

Significance of Paraprotein Antibodies

The three types of overt autoimmune syndromes: chronic cold agglutinin immune hemolytic anemia, MC and various peripheral neuropathies are well characterized. The autoantigens include the I and i red cell antigens in CAD, IgG FC for MC, and various neural-carbohydrate groups in the neuropathies. However, the putative antigens may be exogenous. Possible foreign antigens include bacterial LPS in CAD, hepatitis C virus in MC, and various bacteria or viruses in the neuropathies.^{3,11} It will be important to further define the role of these and other exogenous antigens in various autoimmune syndromes in WM. E-MAP technology may be of help in this regard.^{4,5}

Because of antigen-antibody interaction, patients with monoclonal autoimmune syndromes present at an earlier stage than those with typical WM who do not have evident antibody activity.²² The presence of such dangerous small clones with their monoclonal macroglobulin autoreactive antibodies influences clinical presentation and natural history in these patients.

Summary

- 1) Some monoclonal macroglobulins are antibodies. Identification depends on clinical clues and new techniques such as epitope reconstruction.
- 2) Autoantibody syndromes include chronic cold agglutinin hemolytic anemia, mixed cryoglobulinemia, and various peripheral neuropathies.
- 3) Exogenous antigens may be the putative antigens.
- 4) Patients with monoclonal macroglobulin autoreactive antibodies present at an earlier stage with distinct clinical presentation/natural history compared to other WM patients.

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