Southwestern Medical School

MEDICAL GRAND ROUNDS

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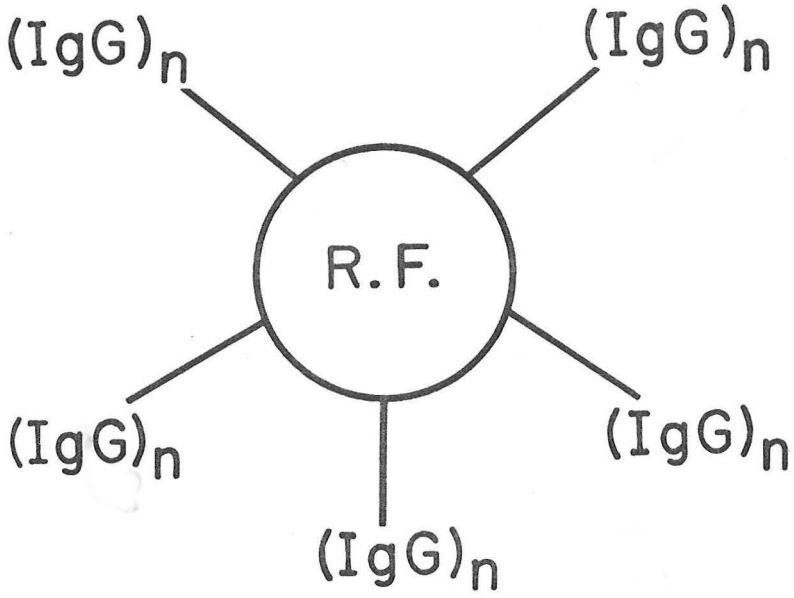
THE ROLE of RHEUMATOID FACTORS in HUMAN DISEASE

Hugo E. Jasin, M.D.



INTRODUCTION

Rheumatoid factors (RF) are antibodies with specificities directed to antigenic determinants on the Fc fragment of human or animal immunoglobulin G. They are part of a family of antibodies collectively known as "antiglobulins." This group of immunoglobulins include antibodies directed against antigenic determinants in native (1) or altered IgG (2) or against buried sites exposed by digestion with pepsin or other enzymes (3,4). In addition, antibodies to light chains (5,6), IgA (7), IgM (8) and IgE (9) have been described. However, these antibodies are not considered rheumatoid factors since they are not directed against the Fc fragment of IgG.

TABLE I.

THE FAMILY OF ANTIGLOBULINS

Rheumatoid Factors
Anti Gm Factors
Pepsin Agglutinators
Anti-IgA Antibodies
Anti-IgM Antibodies
Anti-IgE Antibodies
Anti-antibodies

The saga of the rheumatoid factors evolved from the initial observation by Cecil et al (10) that the sera of patients with rheumatoid arthritis agglutinated certain strains of group A streptococci. Further work (11-13) soon demonstrated that the agglutinating capacity of rheumatoid sera was not specific for the streptococcus; nonencapsulated pneumococci, enterococci and staphylococci were also agglutinated. These observations, coupled to the discovery that rheumatoid sera were able to agglutinate collodion particles (14) and sensitized red cells (15,16) suggested that the activity was not due to specific bacterial antibodies but to non specific agglutinating factors.

The work by Pike et al (17) showing that rheumatoid factor activity could not be absorbed out by streptococci or unsensitized erythrocytes and that the agglutinating ability of rheumatoid sera could be elicited with erythrocytes of several species paved the way for the formal

demonstration that the positive tests with rheumatoid sera involved an interaction between rheumatoid factor and IgG (18,19). (For comprehensive reviews see refs 20,21).

The antibody nature of RF has now been well established based on the demonstration that the activity against IgG has been detected in all three major immunoglobulin classes (22-25) directed against several antigenic determinants of the Fc fragment of IgG (26).

Assay Methods

Rheumatoid factors react not only with human IgG but also with IgG of several animal species (27,28). Moreover, this property may be peculiar to singular molecular subpopulations, some IgM rheumatoid factors react exclusively with human IgG (29,30) whereas others react with both (31). This pattern of crossreactivity has been shown to be of some clinical value, sera from patients with rheumatic diseases tend to react with human and rabbit IgG whereas sero positive sera from patients with other diseases react mostly with human IgG. This type of data is derived from assay tests commonly available which are based on aggregation or agglutination of particles coated with either human or rabbit IgG. IgM antibodies are multivalent, therefore they are much more efficient agglutinators of antigen-coated particles than the bivalent IgG or IgA antibodies. It is for this reason that the rheumatoid factor activity detected by these tests is mostly associated with IgM rheumatoid factor.

TABLE II.

COMPARISON OF COMMONLY USED TESTS FOR RHEUMATOID FACTORS

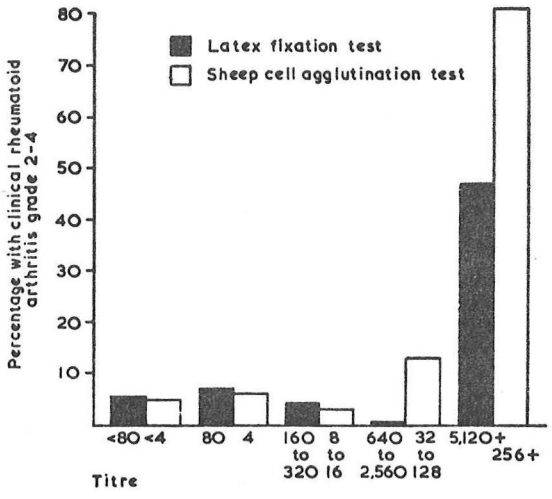
Test	Coating	Significant Titer (serum dilution)
RA - Slide latex fixation	Human IgG	1 to 2+ at 1:20
RA - Tube latex fixation	Human IgG	$\geq$ 1:160
Sensitized sheep cell agglutination (SSCA)	Rabbit IgG	$\geq$ 1:16

Modified from (32)

Starting with the early work of Rose et al (16) in 1948 a large body of literature has dealt with the diagnostic value, specificity and possible pathogenic role of the RF in human disease in general and in the rheumatic diseases in particular (20,21,33,34). In spite of the enormous amount of information accumulated in the last 30 years, the possible pathogenic role of the RF in the mediation of chronic inflammation, joint destruction and extraarticular manifestations in rheumatoid arthritis has not been clarified. I will therefore limit this discussion to the clinical correlations derived from the measurement of rheumatoid factors in the rheumatic diseases and to the consideration of three disease entities where there has accumulated more convincing evidence that these autoantibodies may have a direct pathogenic role in the generation of tissue injury.

CLINICAL CORRELATIONS IN THE RHEUMATIC DISEASES

Low titers of RF are found in a small fraction of the normal population and in a variety of diseases associated with infection or chronic inflammation. However, in most non-rheumatic diseases, the RF titers are low and tend to react only with human IgG yielding positive latex fixation tests and negative sensitized sheep cell agglutination.



Relationship of rheumatoid factor titers to rheumatoid arthritis (35).

The specificity of a positive RF test for rheumatoid arthritis increases with the agglutination titer particularly when measured by the SSCA (35). In the figure above, a population study carried on by Valkemburg et al depicts the distribution of positive RF tests in relation to rheumatoid arthritis. It is readily apparent that over 90 per cent of sera with a SSCA titer of 1:32 or more originated from patients with definite or classical rheumatoid arthritis. A variety of studies (20,21,23) have yielded an incidence of positive RF tests in over 80 per cent of patients with rheumatoid arthritis. The presence of positive tests is more likely to be associated with severe disease, however, no correlation has been found with disease activity (36,37) or laboratory indices of inflammation (16,36,38).

The titer of RF in rheumatoid patients has been recognized as having a useful prognostic value. In general, SSCA titers of 1:256 or greater tend to be associated with severe disease, unremitting course, rheumatoid nodules and higher incidence of extraarticular manifestations.

TABLE III.

CLINICAL FEATURES ASSOCIATED WITH HIGH TITERS OF RHEUMATOID FACTORS

Generalized, Severe Polyarthritis
Widespread Erosive Disease
Unremitting Course
Rheumatoid Nodules
Extraarticular Disease

Seropositivity is an early feature of the disease, a majority of patients will have positive RF tests in the first 6 months after the onset of arthritis (39,40). Thus, a negative test obtained from a patient seen soon after the initial symptoms should be considered a good prognostic sign since it is likely that the tests will remain negative thoughout the course of the disease.

The lack of correlation of RF titers with disease activity is also

reflected in the well established observation that RF titers remain remarkably constant throughout the course of the disease (20,37,41).

TABLE IV.

REVERSION TO SERONEGATIVITY IN

RHEUMATOID ARTHRITIS

Spontaneous Remission
Gold Salt Therapy
Penicillamine Therapy
Cytotoxic Therapy
Intercurrent Lymphoma

The few exceptions, listed in Table IV include patients undergoing a prolonged spontaneous or iatrogenic complete remission induced by gold salts (42) pencillamine (43,44) or cytotoxic therapy and the emergence of an intercurrent lymphoma, particulary in patients with Sjögren's syndrome (45).

As mentioned above, all the collagen diseases are associated with increased incidence of positive RF tests. Notice that in most instances listed in Table V, the SSCA parallels the results obtained with the latex fixation tests.

TABLE V.

INCIDENCE OF POSITIVE RHEUMATOID FACTOR TESTS IN THE RHEUMATIC DISEASES

Disease	Per cent Positive RA Latex	SSCA
Adult Rheumatoid Arthritis	70-85	60-70
Juvenile Rheumatoid Arthritis	10-20	6-25
Systemic Lupus Erythematosus	20-40	20-40
Systemic Sclerosis	15-35	15-35
Dermatomyositis	15-35	15-35
"Primary" Sjögren's Syndrome	52-96	39-74
"Secondary" Sjögren's Syndrome	100	72
Mixed Cryoglobulinemia	90-100	30
Hypergammaglobulinemic Purpura	100	62

Pathogenic Role of the Rheumatoid Factors

One of the major difficulties in trying to delineate a pathogenic role for the RF in rheumatoid arthritis is the common occurence of positive RF tests in a small segment of otherwise normal populations and in many diseases associated with chronic inflammation, polyclonal gammopathy and circulating immune complexes. Table VI shows a partial list of disease entities associated with RF. The it should be emphasized that in most non rheumatic conditions, the RF titers are lower that in rheumatoid arthritis.

TABLE VI

DISEASES COMMONLY ASSOCIATED WITH RHEUMATOID FACTORS

	Percent Positive	
	RA latex	SSCA
Infectious Diseases		
Bacterial Diseases	20	11
Subacute Bacterial Endocarditis	48	22
Viral Diseases	17	14
Syphilis	13	5
Leprosy	24	15
Liver Diseases		
Viral hepatitis	30-40	21
Chronic liver disease	30-80	15-25
Pulmonary Diseases		
Pulmonary Tuberculosis	11	6
Chronic Interstitial Fibrosis	20-50	5-45
Sarcoidosis	17	5
Miscellaneous		
Waldenström's Macroglobulinemia	30	22
Renal Homograft	74	8
Myocardial Infarction	20	12

(From reference 32)

There is considerable circumstantial evidence (46) to suggest that rheumatoid arthritis may represent an example of intraarticular immune complex disease. Rheumatoid factors are able to interact in vivo in the joint cavities with their "antigen" IgG resulting in the formation of immune complexes containing IgG-RF sometimes complexed to IgM RF (47).

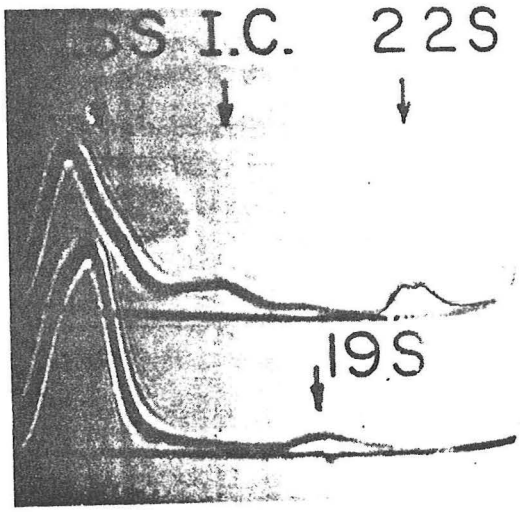
INTERACTIONS BETWEEN RHEUMATOID FACTORS AND IgG

$$IgM\text{-}RF + IgG \rightarrow IgM\text{-}RF\text{-}IgG \text{ (22S complex)}$$

$$n(IgG\text{-}RF_2 + IgG_n) \rightarrow IgG\text{-}RF_n\text{-}IgG_n \text{ (Intermediate complexes)}$$

$$IgG\text{-}RF_n\text{-}IgG_n + IgM\text{-}RF \rightarrow IgG\text{-}RF_n\text{-}IgG_n\text{-}IgM\text{-}RF$$

The 22S complex composed of IgM-RF-IgG_5 has been visualized directly by analytical ultracentrifugation of rheumatoid sera containing RF in high titer (48). Intermediate complexes composed of IgG and IgG-RF have also been described in the sera of patients with rheumatoid arthritis and other conditions listed below.



Ultracentrifugal patterns of a normal serum (bottom) and a rheumatoid serum (top) showing 22S and intermediate complexes.

TABLE VII.

DISEASES ASSOCIATED WITH INTERMEDIATE IgG COMPLEXES

Rheumatoid Synovitis
Rheumatoid Vasculitis
Felty's syndrome
Rheumatoid Hyperviscosity Syndrome
Hypergammaglobulinemic Purpura
Interstitial Pneumonitis

Direct evidence to implicate these aggregates in a cause-effect relationship to the rheumatoid process is lacking. However, there are certain disease entities in which the evidence for a direct pathogenic role of IgG and IgM-RF is more convincing. I would like to devote the rest of this talk to a clinical discussion of three diseases generated by the presence of large amounts of rheumatoid factors in blood and tissues.

"RHEUMATOID" HYPERVISCOSITY SYNDROME

Abnormal increases in blood viscosity may be due to elevated plasma or serum viscosity, to large numbers of circulating erythrocytes or white cells, or to an abnormal increase in cell rigidity leading to increased resistance to capillary flow (49,50).

TABLE VIII

ETIOLOGY OF THE HYPERVISCOSITY SYNDROME

Plasma or Serum Hyperviscosity	Waldenström's Macroglobulinemia
	Multiple Myeloma
	Connective Tissue Diseases
Increased Blood Cell Concentration	Polycythemia
	Leukemia
Increased Cell Rigidity	Sicklemia
	Spherocytosis

The association of elevated serum viscosity and hypergammaglobulinemia has been described over 45 years ago by Reimann (51). Waldenström documented serum hyperviscosity in his initial description of the first two patients with macroglobulinemia (52). In the following 25 years, hyperviscosity syndrome has been mostly described in patients with macroglobulinemia and less frequently in IgA (53) and IgG monoclonal gammopathies (54,55). It is likely that the most common mechanism responsible for the high serum viscosities in these conditions is the tendency of IgM, IgA and some IgG proteins to generate large protein aggregates at high concentrations. Additional factors that contribute to high blood viscosities are the interactions between these abnormal proteins and the blood cells (50).

Clinical Features of the Hyperviscosity Syndrome

The clinical manifestations of the hyperviscosity syndrome have been well described by Fahey (56) on the basis of 25 patients with Waldenström's macroglobulinemia admitted to the National Institutes of Health. The symptoms and signs listed below are due to a combination of phisiopathogenic mechanisms including 1) decreased perfusion, 2) endothelial damage, 3) increased plasma volume, 4) thromboembolism and 5) abnormal platelet function.

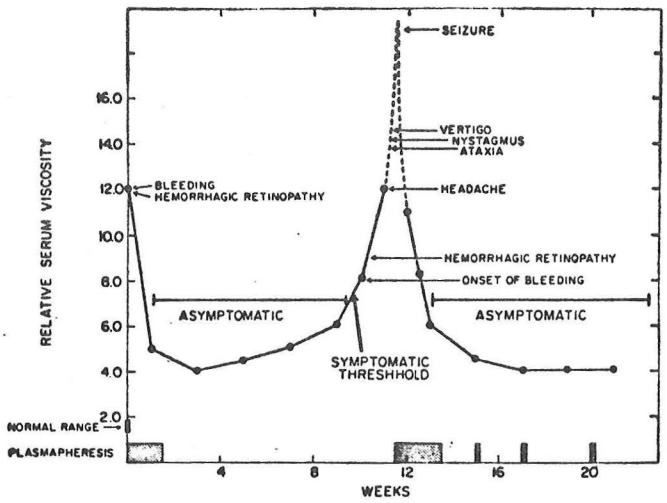
TABLE IX

CLINICAL FEATURES OF THE HYPERVISCOSITY SYNDROME

General	
	Weakness, fatigue, malaise, anorexia
Ocular	
	Partial or total loss of vision.
	"String of sausage" appearance of
	retinal veins, hemorrhages, papilledema
Hematologic	
	Mucosal bleeding
	Anemia
Neurologic	
	Headaches, dizziness, vertigo, nystagmus
	Somnolence, stupor, coma.
	Seizures, hearing loss.
Cardiovascular	
	Congestive heart failure
	Pulmonary hypertension

Subjective symptoms including fatigue, somnolence and vertigo may be prominent and quickly reversible by plasmapheresis. Visual disturbances are relatively uncommon and contrast with the usually impressive changes on fundoscopic examination. The most common symptoms are hematologic and neurologic. The diagnosis of hyperviscosity syndrome is frequently made on the basis of the patient's initial presentation with generalized mucosal bleeding which in Fahey's series was present in over 50 per cent of the patients. Vertigo, sommolence, stupor, generalized seizures and hearing loss are also commonly present. The cardiovascular manifestations are thought to be secondary to increased plasma volume, a feature present in most patients with serum viscosities greater than 4.0.

The diagnosis of serum hyperviscosity syndrome can be established with a simple device such as the Ostwald viscosimeter. The time required for a volume of serum to flow through a glass capillary is compared to that of water. Normal serum viscosities range from 1.4 to 1.8 relative to water. In view of the above mentioned interactions between the protein aggregates and erythrocytes, the measurement of relative viscosity in serum is not a true reflection of the conditions present at the capillary level. For this reason, the serum viscosity associated with clinical manifestations varies from one patient to another. For each individual patient, however, there is a fairly constant "symptomatic threshold" above which symptoms become manifest. In general, relative viscosities between 2 and 4 are rarely symptomatic, viscosities between 5 and 8 are commonly associated with symptoms, and practically all patients are symptomatic at viscosities of 10 or more. The figure below shows an example of a patients with a symptomatic threshold of 7.



Correlation of clinical findings and serum viscosity levels in a patient with macroglobulinemia (56).

Hyperviscosity Syndrome in the Connective Tissue Diseases

Prior to 1970, the association of overt hyperviscosity syndrome and rheumatoid arthritis or other connective tissue diseases had been described only once (57) in a patient with cryoglobulinemia and arthritis. Some years ago we were fortunate to be able to study two patients with rheumatoid arthritis, high serum viscosities and a clinical picture compatible with hyperviscosity syndrome (58).

CASE REPORT:

B.B. was a sixty two year old black woman with a history of chronic polyarthritis and subcutaneous nodules since 1962. She was first seen at Parkland Memorial Hospital in 1964 when the diagnosis of rheumatoid arthritis and essential hypertension were made. At that time, the SSCA was positive at 1:896. The patient was admitted to the hospital in 1968, with a history of anorexia, fatigue and malaise, gum bleeding and occasional epistaxis for 2 months, increasing dyspnea on exertion and weakness for the last month prior to admission. On physical examination there was gum bleeding and mild generalized lymphadenopathy. The liver was palpable 5 cm below the right costal margin and the spleen tip was also palpable. The extremities revealed mild to moderate changes of rheumatoid arthritis including rheumatoid nodules. There was a striking purplish red palmar erythema.

Laboratory findings: hemoglobin, 9.8 gm%; hematocrit 30%; WBC: 4600/mm^3, differential count: 50% neutrophils, 40% lymphocytes, 3% monocytes and 1% eosinophils. The erythrosedimentation rate was 140 mm per hour. Urinalysis was within normal limits; Bence-Jones protein absent; BUN: 13 mg%. VDRL nonreactive; LE preparation positive; tube latex fixation titer 1:650000, SSCA 1:57344. Cryoglobulins negative; serum C3:98 mg% (normal). Platelet count 127000/mm^3; clotting time: 30 minutes, PT and PTT normal.

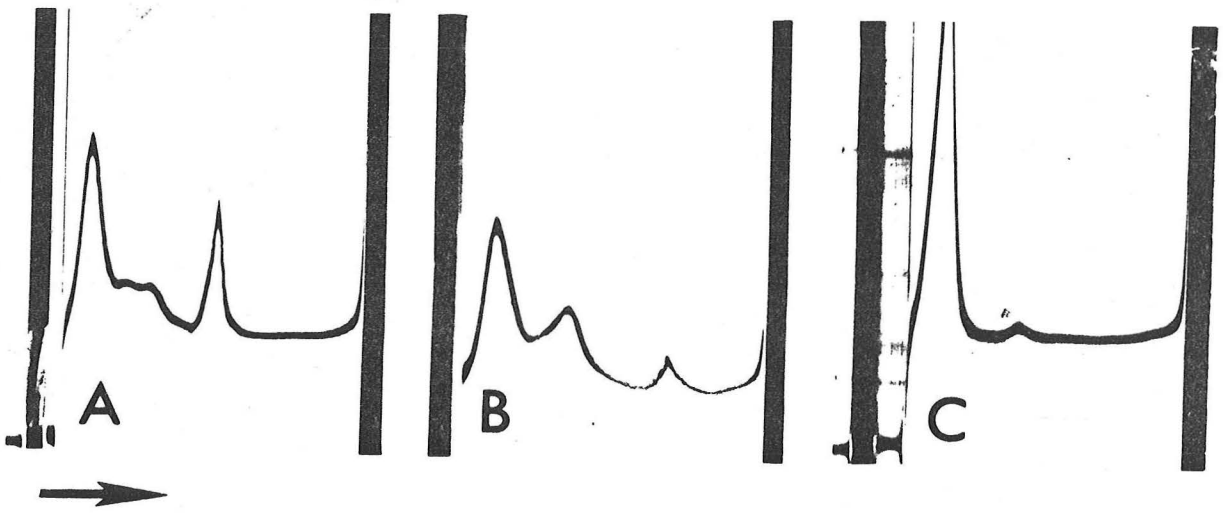
H.C. was a sixty four year old white man with a history of increasing malaise, weakness, dyspnea, epistaxis and easy bruising for 4 months. The patient had a ten year history of rheumatoid arthritis. On admission to the hospital, the changes of rheumatoid arthritis including subcutaneous nodules were recorded. In addition, a striking red palmar erythema was noted. The patient had physical findings compatible with congestive heart failure.

Laboratory findings included normal CBC and urinalysis. Bence-Jones protein were absent; cryoglobulins; absent. PT and PTT, bleeding and clotting times, normal.

TABLE X

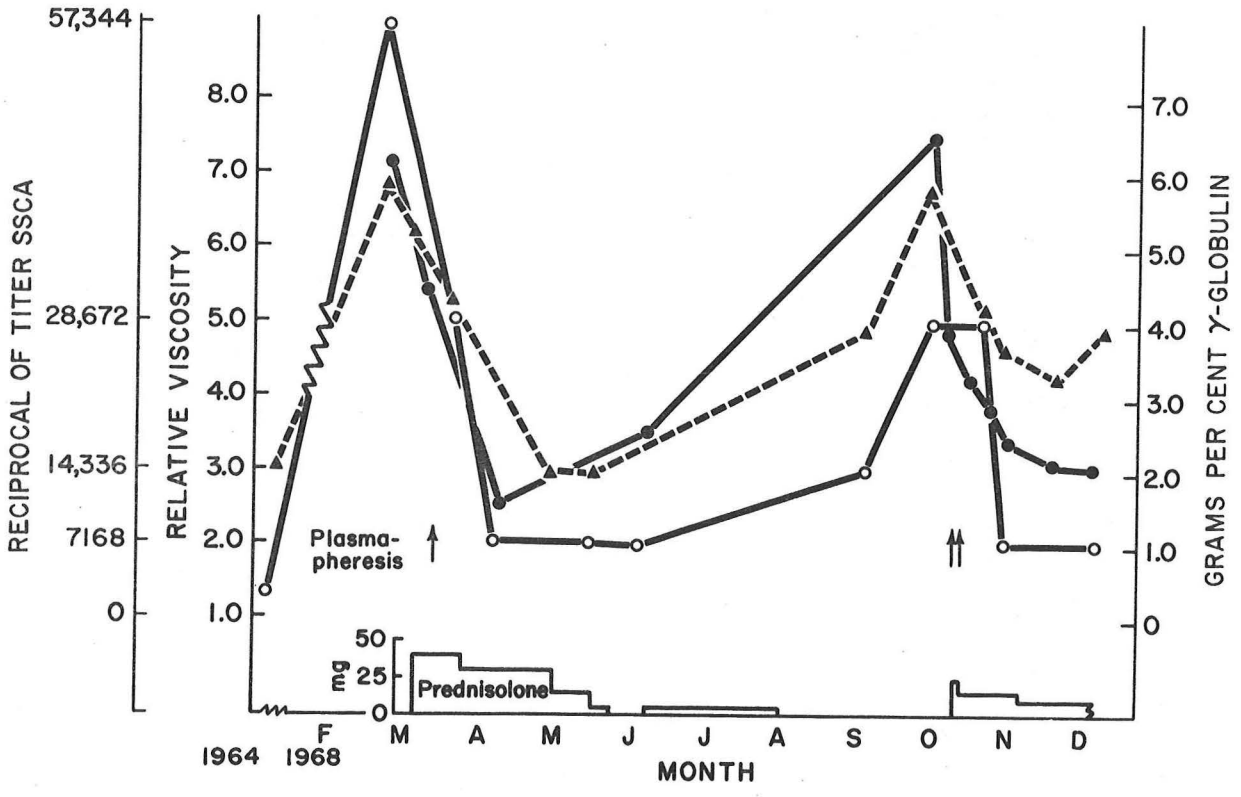
LABORATORY FEATURES IN RHEUMATOID HYPERVISCOSITY SYNDROME

	Grams %		
	BB	HC	Normal
Serum Protein	10.25	8.70	7.14
Gammaglobulin	5.75	5.70	1.53
Intermediate Complexes	2.65	3.50	0.53
IgM	0.93	0.21	0.10
SSCA	1:28672	7168	<1:14
Relative viscosity	7.5	5.1	1.6



Ultracentrifugal patterns of two patients with rheumatoid arthritis and hyperviscosity syndrome. A. B.B. - B. H.C. - C - normal serum.

B. B. was treated with of Prednisolone, 40 mg daily with subsequent gradual decrease to 5 mg. Plasma exchange of 2 units of blood was performed. Coinciding with therapy, the patient felt stronger, her dyspnea on exertion improved, and gum bleeding decreased considerably. There was a decrease in the size of the spleen an liver and the palmar erythema was also decreased. Prednisolone was eventually discontinued, and eight weeks later, weight loss and gum bleeding reappeared. In addition, there was a transient episode of diplopia. Fundoscopic examination revealed bilateral blurring of both optic discs and arteriolar tortuosity. Physical examination also showed diffuse gingival bleeding, marked palmar erythema and diffuse lymphadenopathy. Reinstitution of prednisolone therapy and plasmapheresis of 4 units of blood again resulted in striking clinical improvement with cessation of gingival bleeding, rapid increase in exercise tolerance, decrease in palmar erythema and optic disc blurring. Serial determinations of serum viscosity, γ-globulin and SSCA titers were carried out as depicted in the figure below.



Serial determinations of serum viscosity, γ-globulin concentration and SSCA titer in a patient with hyperviscosity syndrome and rheumatoid arthritis.

Serum viscosities of 7.1 and 7.5 were recorded during the two hospital admissions, levels compatible with the development of overt hyperviscosity syndrome. There was a marked rapid drop of all three parameters coinciding with institution of corticosteroid therapy. Plasmapheresis of 2 and 4 units of blood would not have been expected to decrease the serum protein concentrations appreciably. This remarkable response to corticosteroids has also been noticed in other patients subsequently described.

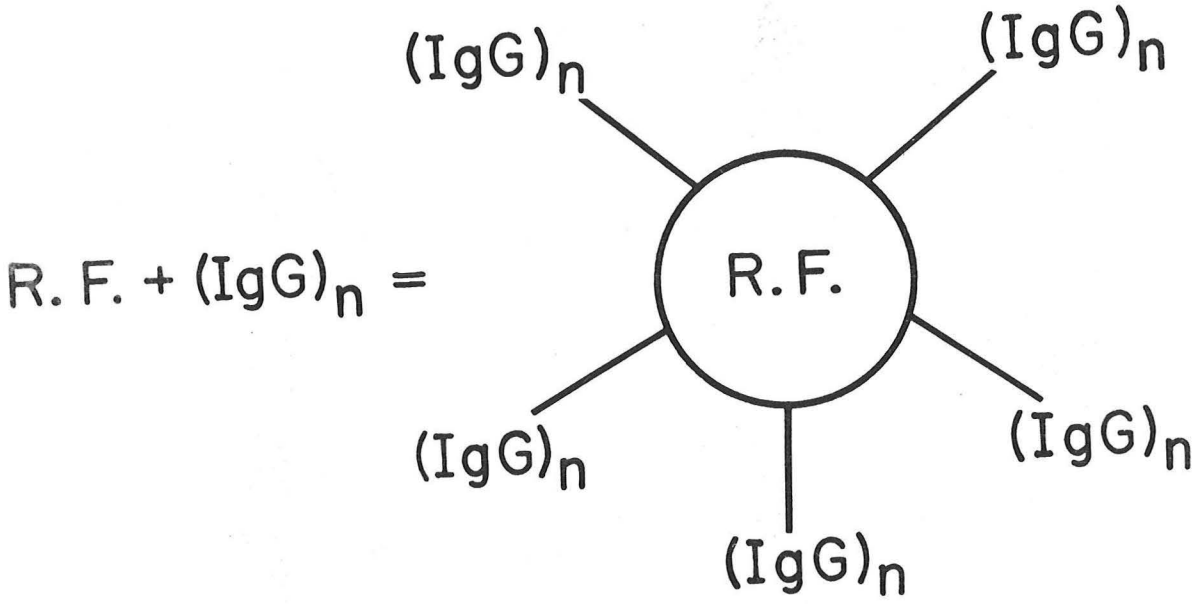
TABLE XI

RHEUMATOID HYPERVISCOSITY SYNDROME

1) Rheumatoid Arthritis
2) Bleeding Diathesis
3) Dyspnea and Weakness
4) Palmar Erythema
5) High Titer Rheumatoid Factor
6) Intermediate IgG Complexes
7) Serum Hyperviscosity

Investigation of the mechanisms involved in the generation of high serum viscosities in both patients showed that they were large part due to interaction of IgM-RF with intermediate IgG complexes resulting in the formation of large molecular conglomerates of high

intrinsic viscosities. This mechanism is depicted in the figure below.



Postulated configuration of the molecular conglomerates responsible for the generation of high serum viscosity.

Subsequent to 1970, a dozen cases with hyperviscosity syndrome and associated connective tissue disease have been reported. A summary of the salient features of this group of patients is shown in Table XII. Of note is the presence of intermediate complexes and IgM or IgA-RF in all the patients. Evidence for interaction between these molecular species was confirmed in the two patients described in reference 63.

TABLE XII
SERUM HYPERVISCOSITY SYNDROME AND CONNECTIVE TISSUE DISEASE

Age/Sex	Associated Disease	IgM-RF	I.C.[1]	Serum Viscosity	Reference
23/F	Rheumatoid Arthritis	+	+	20.0	57
62/F	Rheumatoid Arthritis	+	+	7.1	58
64/F	Rheumatoid Arthritis	+	+	5.0	58
44/M	Rheumatoid Arthritis	+	+	7.9	59
54/F	Rheumatoid Arthritis	+	+	4.6	59
69/F	Rheumatoid Arthritis	+	+	7.2	60
59/F	Sjögren's Syndrome	+	+	5.3	61
47/F	Sjögren's Syndrome	+	+	7.3	62
32/F	Sjögren's Syndrome	+	+	6.1	63
39/F	Sjögren's Syndrome	+	+	3.1	63
49/F	Sjögren's Syndrome	IgA	+	8.5	64
20/F	SLE	IgA	+	5.0	65

[1] I.C. = Intermediate complexes

The remarkable response to costicosteroid therapy in our patients has also been reported in over half the patients listed in Table XII. The reason for the susceptibility of this particular entity to steroid therapy is totally unknown.

Thus, this group of patients develop symptoms of serum hyperviscosity on the basis of concentration-dependent interactions between IgG and IgM-RF. It is likely that these immunoglobulin complexes are relatively inert in that they may not induce complement activation, and for this reason, vasculitis is not a prominent feature of the syndrome.

HYPERGAMMAGLOBULINEMIC PURPURA

CASE REPORT:

M. H. This 48 year old Latin-American female was first seen at Parkland Memorial Hospital, (Arthritis Clinic) in 1975. She offered a history of recurring petechial non blanching rash localized to the lower extremities since age 15. The rash recurrs after exercise, prolonged standing or wearing tight garments. Itching or burning may be present early after appearance of a new crop of lesions. The rash resolves in a few days leaving behind an area of hyperpigmentation. This patient developed dry eyes and dry mouth in 1968. Her vision deteriorated in spite of intensive treatment necessitating a corneal transplant in 1973. Over the last 5 years, her only complaints have been those associated

with sicca syndrome and recurring rash. Significant physical findings include severe changes of keratoconjuctivitis sicca with filamentous keratitis and corneal thinning. The patient shows extensive brownish hyperpigmentation over both legs and pretibial areas and scattered reddish confluent non palpable petechia overlying the hyperpigmented skin.

Laboratory findings: WBC: 5700/mm^3; hemoglobin 11.4 gm%; hematocrit 34%; platelets: 390,000/mm^3. ESR: 110 mm/hr. Urinalysis: pH5.5, specific gravity:1018, protein: negative; Hb; negative. SMA-12 normal except for a total protein of 9.8 g%; albumim; 3.9 g%, globulin 5.9 g%. RA latex fixation: positive 4+; SSCA; positive 1:64, ANA; positive 1:2560, speckled pattern. Anti-native DNA and anti-ENA: negative. Serum complement levels: within normal limits. Serum protein electrophoresis showed a large polyclonal increase in γ-globulin of 3.5 g%. Analytical ultracentrigugation revealed the presence of large amounts of intermediate complexes.

In 1943, Waldenström described three patients with long-standing recurring purpura of the lower extremities, elevated erythrosedimentation rate, mild anemia, and hypergammaglobulinemia (66).

TABLE XIII

CLINICAL FEATURES OF HYPERGAMMAGLOBULINEMIC PURPURA

Recurrent Leg Purpura
High ESR
Hypergammaglobulinemia
Benign Course

The disease has a remarkable female preponderance (9:1) with an age of onset between 18 and 40 years. Characteristically, the patients will have had symptoms from 3 to 5 years before seeking medical advise. The purpuric rash often appears after exercise, prolonged standing or wearing tight garments. Initially, small transient maculopapular lesions occur, often associated with pruritus or burning. Shortly after, petechia

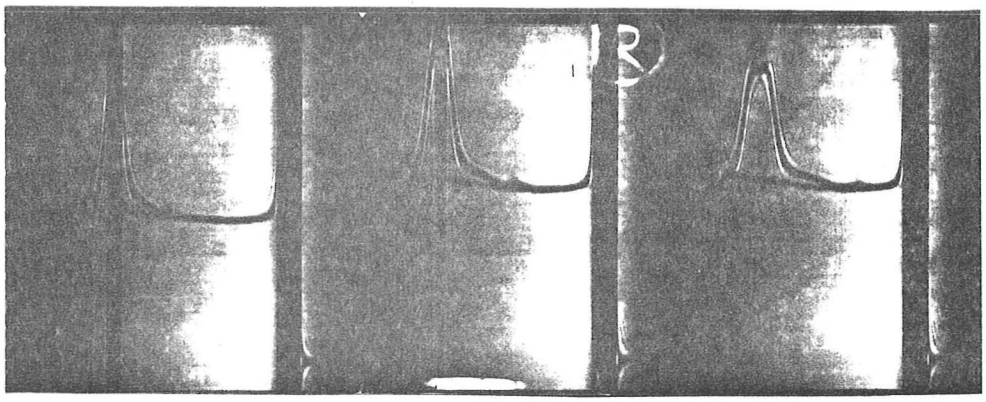
resembling punctate teleangectasiae appear. As these resolve, they may leave areas of brown pigmentation which eventually cover the pretibial area. The lesions are symmetrical and occasionally they may extend to the thighs, buttocks, and arms. The overlying skin is usually normal, skin biopsy shows perivascular infiltration localized to the small dermal vessels with a preponderant polymorphonuclear infiltrate in the early lesions. Vascular necrosis is spotty and infrequent.

A compilation of 81 cases from the literature (67-69) suggests two types of presentation. About 50 per cent of the cases are considered primary or idiopathic in that they do not seem to be associated with other clinical conditions. The remaining 50 per cent show, as it was the case in our patient, an association with Sjögren syndrome, systemic lupus erythematosus or undifferentiated connective tissue disease. Most patients follow a benign course with recurrent rashes and no evidence of visceral involvement. Many patients have been followed for 20 or 30 years and this monotonous clinical course has been recorded frequently. However, there is some evidence that these patients may have a higher incidence of multiple myeloma which in the three instances documented in references (70-72) developed many years after the diagnosis of hypergammaglobulinemic purpura had been made.

TABLE XIV

LABORATORY FEATURES OF HYPERGAMMAGLOBULINEMIC PURPURA

Polyclonal Gammopathy
Positive Rheumatoid Factor Tests
Intermediate Complexes



Ultracentrifugal pattern of the serum from a patient with hypergammaglobulinemic purpura.

The cardinal laboratory finding in patients with HP is the presence of abnormal amounts of gamma globulins by serum protein electrophoresis (66-69) in a pattern suggesting polyclonal gammopathy. Intermediate complexes with sedimentation rates between 9 and 17S were demonstrated in three patients by Kunkel et al in 1961 (73) on analytical ultracentrifugation. Subsequently, the work by Capra et al (69) and Kyle et al (68) established the constancy of this finding in a group of 40 patients. Capra also demonstrated that the complexes contained large amounts of IgG-RF associated with IgG "antigen". Subsequent work by Clark et al (73) showed the frequent occurrence of IgA-RF as a constituent of the intermediate complexes. Although the sera of these patients contain small amounts of IgM-RF, analytical ultracentrifugation fails to reveal significant interaction with the intermediate complexes, thus, most of the complexes are smaller than 19S, the size of IgM itself. Moreover, when IgM-RF is inactivated by sulphydryl reagents, the sera still demonstrate significant RF activity in contrast to rheumatoid sera, suggesting that most of the RF resides in the intermediate complexes in the form of IgG and IgA.

It should be pointed out that these patients do not show significant depression of serum complement levels, suggesting that these intermediate size complexes are rather inert in that they may not be able to mediate tissue injury through the well known pathway of complement activation and attraction of inflammatory cells. The groups of patients

with hyperviscosity syndrome and HP have large concentrations of intermediate complexes. Why should serum hyperviscosity develop in one group of patients and not the other is not known. It is possible that the high serum viscosity is due to the tendency of the complexes to form large molecular aggregates brought together by the multivalent IgM-RF. In HP, IgM-RF is absent or present in small concentrations so that the intermediate complexes are not able to aggregate further.

The third group of patients to be described next, present a strikingly different picture. In this group, the RF complexed to IgG behave as a typical immune complex consuming complement and inducing vascular injury.

ESSENTIAL MIXED CRYOGLOBULINEMIA

CASE REPORT:

E.B. this 49 year old black female was admitted to Parkland Memorial Hospital on December 4, 1969 with symptoms of congestive heart failure which became symptomatic one week prior to admission. She denied previous hypertension, heart or renal disease. She had no history of arthritis or arthralgia, Raynaud's phenomenon purpura or febrile episodes. Her review of systems was non-contributory.

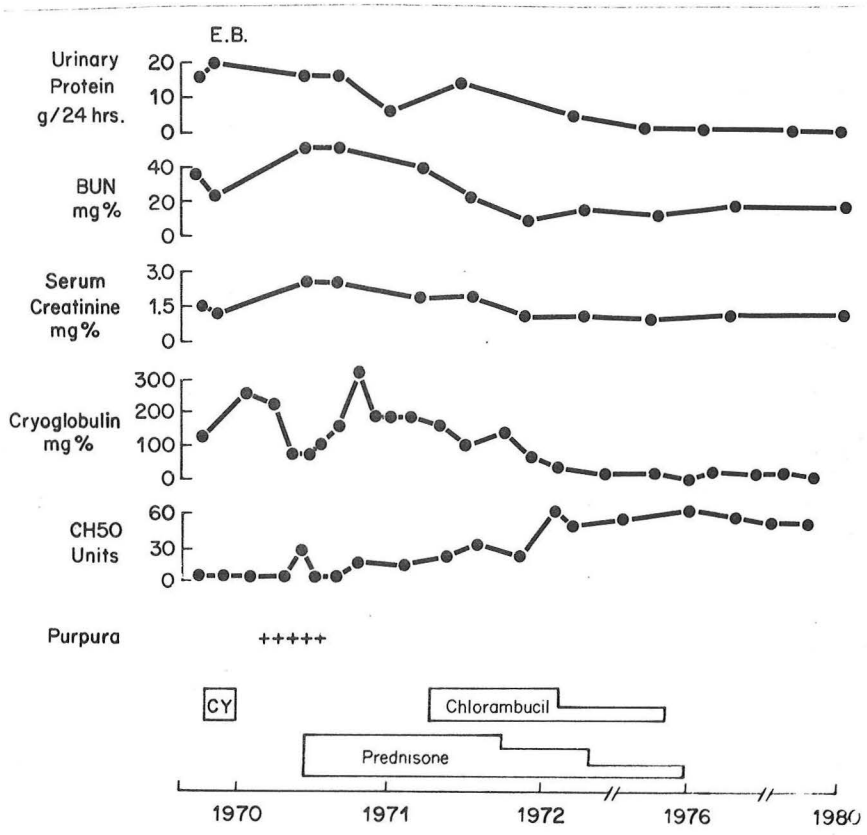
On admission, her physical examination revealed BP 210/110, P 90 and regular, afebrile. Her fundi revealed an exudate in OD without hemorrhages. JVP was increased at 45°. There was bilateral dullness and rales at both bases. She had cardiomegaly with S_3 gallop and II/VI systolic murmur. Abdominal exam revealed a large liver, palpable 15 cm below the RCM. The extremities showed 2+ pitting edema with intact peripheral pulses. She had no urticaria, purpura or lymphadenopathy. Her neurological exam was normal.

Laboratory findings: Hb 9.9 g%, HcT 28.6%. WBC 5900/mm^3, ESR: 85 mm/hr. Platelets: 198000/mm^3. Peripheral smear revealed mild polychromasia, schistocytes, burr cells and helmet cells. Urinalysis: pH 5.0, sp. gr. 1021, Protein 300 mg%. No sugar or acetone. Microscopic: Many RBC, WBC, hyaline and RBC casts. Urine culture: sterile. BUN: 35 mg%, creatinine: 1.5 mg%. Glomerular filtration rate: 50cc/min. Urinary protein 2.1 g/24 hours; serum protein electrophoresis: albumin 2.2 g%, γ-globulin 0.7 g% . RA latex: positive 4+, SSCA: 1:3584; ANA: negative. Cryoglobulins: 250 mg%, Immuno-chemical analysis: mixed cryoglobulin containing IgM and IgG. IgM has rheumatoid factor activity. Serum C3: 60 mg% (normal > 100), CH50: 10U (normal 60-110U).

Renal biopsy: Increased mesangial cellularity with focal endothelial cell swelling. Scattered glomerular capillary fibrinoid necrosis with subendothelial deposits by trichrome stain. Basement membrane appeared normal. Immunofluorecence microscopy

studies showed glomerular deposits of IgG, IgM, C3 and rheumatoid factor activity. Diagnosis: Diffuse glomerulonephritis.

Course: The patient was initally treated with cyclophosphamide, 100 mg daily, but leukopenia forced withdrawal of this drug. Four months after her discharge, she developed a purpuric rash of the lower extremities. Serum cryoglobulins which had decreased to 75mg% shortly after discharge, increased to 313 mg% and her renal function deteriorated. BUN rose to 54 mg%,serum creative to 2.6 mg% and urinary protein remained at 1.7 g per 24 hours. She was treated with Prednisone, 25 mg daily for one year. While receiving corticosteroids her renal function did not seen to deteriorate further, proteinuria decreased to 600 mg per 24 hours, serum cryoglobulins ranged from 185 to 125 mg% and serum complements remained low. Chlorambucil 4 mg daily was began in June 1971 in an attempt to reduce corticosteroid dose. Four months after iniciation of immunosuppressive therapy BUN fell to 16 mg%, serum creatinine to 1.2 mg%, cryglobulins to 20-40 mg% and hemolytic complements rose to low normal levels. In 1973 her urinary protein was within normal limits. Both Prednisone and Chlorambucil were eventually discontinued in 1976, since that time the patient has remained symptom free with no recurrence of purpura and stable renal function.



Clinical course of patient E.B. with essential mixed cryoglobulinemia.

The appearance of protein precipitates following cooling of serum or plasma was described in 1933 by Wintrobe and Buell (74) in a patient with multiple myeloma, Raynaud's phenomenon and purpura. Most of the cases reported before 1958 were associated with multiple myeloma or lymphoid malignancies (75-77). These cold precipitable proteins are found in large concentrations in serum and are composed of one immunoglobulin class, usually a monoclonal component. They are not uncommonly reported first to the unsuspecting attending physician by the pathology laboratory since, as shown in Table XV, they may not cause any cold associated symptoms (78).

TABLE XV

SINGLE COMPONENT CRYOGLOBULINEMIA

Diagnosis	Cryoglobulin		Symptoms
	Type	Concentration gm%	
Multiple Myeloma	IgG	1.5	0
Multiple Myeloma	IgG	1.7	0
Multiple Myeloma	IgG	1.1	0
Multiple Myeloma	IgG	0.3	0
Essential	IgG	2.8	3+
Essential	IgG	1.5	3+
Liver Cirrhosis	IgG	0.04	1+
Purpura	IgG	0.03	1+
Lymphocytic Leukemia	IgM	1.7	0
Lymphosarcoma	IgM	2.0	2+
Lymphosarcoma	IgM	0.8	0
Lymphosarcoma	IgM	1.4	0
Lymphosarcoma	IgM	1.2	0
Lymphosarcoma	IgM	0.5	0
Lymphosarcoma	IgM	2.5	0

*From Meltzer and Franklin (78)

Cryoglobulins composed of more than one immunoglobulin component were first described by Dr. J. LoSpalluto (79) in a patient with petechial rash and renal tubular acidosis. The cryoglobulin contained IgG and IgM with rheumatoid factor activity. Cryoprecipitability depended on the interaction of the cryoglobulin IgM-RF with IgG obtained from the patient or from normal subjects or even other species of animals.

TABLE XVI

COMPOSITION OF CRYOGLOBULINS

A.	Pure or Single Component
	1. IgG
	2. IgM
	3. Bence-Jones protein (L chains)
A.	Mixed
	1. IgM + IgG
	2. IgA + IgG
	3. IgM + IgA + IgG

Subsequent studies have established the fact that the mixed cryoglobulins are the most common type found in an ever increasing number of disease entities (80,81).

TABLE XVII
DISEASES ASSOCIATED WITH CRYOGLOBULINEMIA

A.	INFECTIOUS
	Viral
	Infectious Mononucleosis
	Cytomegalovirus
	Acute Hepatitis B
	Chronic HBV Infection
	Lyme arthritis
	Bacterial
	Subacute Bacterial Endocarditis
	Lepromatous Leprosy
	Acute Poststreptococcal Glomerulonephritis
	Lymphogranuloma Venereum
	Postintestinal Bypass with Arthritis
	Syphilis
	Fungal
	Coccidiodomycosis
	Parasitic
	Kala-Azar
	Toxoplasmosis
	Malaria
	Echinococcosis
	Schistosomiasis
B.	AUTOIMMUNE DISEASES
	Systemic Lupus Erythematosus
	Rheumatoid Arthritis
	Polyarteritis Nodosa
	Sjögren's syndrome
	Scleroderma
	Sarcoidosis
	Henoch-Schoenlein Purpura
	Behcet's Syndrome
	Polymyositis
	Pulmonary Fibrosis
	Pemphigus Vulgaris
C.	LYMPHOPROLIFERATIVE DISEASES
	Macroglobulinemia
	Lymphoma
	Chronic Lymphocytic Leukemia
	Immunoblastic Lymphadenopathy
D.	RENAL DISEASES
	Proliferative Glomerulonephritis
E.	LIVER DISEASES
	Laennec's Cirrhosis
	Primary Biliary Cirrhosis
	Chronic Hepatitis
F.	FAMILIAL
G.	ESSENTIAL

(Modified from ref. 80.)

It is readily apparent from the group of diseases listed above, that there is a remarkable degree of overlap with the group of diseases associated with RF (Table VI). This is not unexpected, since one of the components of the mixed cryoglobulins is indeed a rheumatoid factor. In two large series (80,81) with 212 cryoglobulins analyzed, 68 per cent were found to contain more than one immunoglobulin. In the studies of 126 sera, recently reported by Gorevic et al (80), almost 50 per cent of the mixed cryoglobulins occurred in patient with lymphoproliferative or autoimmune diseases whereas in the other half, comprising 40 patients, no underlying etiology was apparent.

In 1966, Meltzer and Franklin (82) described a group of 9 patients with a distinct clinical syndrome characterized by leg purpura, weakness arthralgias and a mixed cryoglobulin containing IgM-RF similar to the patient E.B described in this protocol.

Clinical Features of Essential Mixed Cryoglobulinemia

Over two thirds of the patients with this syndrome are women. The average age at onset is 51 years with a range of 21 to 72 years.

TABLE XVIII

CLINICAL FEATURES OF ESSENTIAL MIXED CRYOGLOBULINEMIA

	Percent
Purpura	100
Arthralgia	72
Hepatomegaly	70
Splenomegaly	52
Renal disease	55
Leg Ulcers	30
Raynaud's Phenomenon	25
Lymphadenopathy	18
Sjögren's syndrome	15

(Modified from ref. 80).

The cardinal clinical feature is the presence of intermittent non-thrombocytopenic purpura invariably involving the lower extremities. In about 25 per cent of the cases the lower trunk is also involved. In most cases, the rash occurs initially, so that many cases are diagnosed by dermatologists. The onset of rash is very capricious,

symptom free periods may vary from days to years. The purpuric lesions occur in "crops" lasting from 3 to 10 days; recurrent involvement leave extensive hyperpigmentation which persist for many years. Occasionally, severe involvement around the malleoli may precede the appearance of leg ulcers which are slow healing.

Skin biopsies of purpuric lesions reveal dermal vasculitis with various degrees of involvement of the subcutaneous tissue. The vasculitic lesions are non-specific, most show endothelial swelling, perivascular infiltrates of polymorphonuclear or mononuclear leukocytes, hyaline thrombi and varying degrees of leukocytoclasis. Immunofluorescence studies show the presence of IgM, IgG and/or C3 in 50 per cent of the biopsies.

The arthralgias occurring in over 70 per cent of the patients are intermittent involving hands and knees simmetrically, usually without evidence of objective arthritis. Clinically apparent renal involvement is seen in 55 per cent of the patients. Although it may be the presenting feature, as was the case in our patient, in most cases it follows the onset of purpura with an average delay of 4 years. The nephritis is commonly associated with hypertension, edema, proteinuria and a urinary sediment compatible with glomerulonephritis. Nephrotic syndrome is present in less that 25 per cent of the patients. The pathologic pictures is most compatible with diffuse or focal proliferative glomerulonephritis, only one third of the biopsy specimens may show evidence of vasculitis. Immunofluorescence studies invariably show granular deposits of IgM, IgG and/or C3.

Hepatomegaly is present in 70 per cent of the patients reported by the NYU group. The involvement is usually subclinical, laboratory abnormalities are detected in most patients with large livers, with elevation of serum alkaline phosphatase as the most common aberration. The liver biopsies may be normal in 40 per cent of the cases, in the remainder, the pathologic picture may range from mild triaditis to frank cirrhosis. Serologic evidence of previous hepatitis B virus infection was found in close to 60 per cent of the patients studied by the group at N.Y. University. This very high incidence of HBV infection may reflect in part the cultural make-up of the population studied. The true incidence of previous HBV infection in patients with essential cryoglobulinemia may be closer to 30 percent (C. Christian, Personal Communication).

Laboratory Features

In contadistinction to the large concentrations reported in patients with single component cryoglobulins, the sera of patients with mixed cryoglobulin disease contain between 20 to 400 mg%. Only in few patients the cryoglobulin levels correlate with disease activity, in most, no correlation has been found. A distinctive feature of this disease is the invariable presence of rheumatoid factor both in the isolated cryoglobulin itself and in the cryoglobulin free serum. Thus, a negative test for rheumatoid factor usually rules out the diagnosis of mixed cryoglobulinemia.

TABLE XIX

LABORATORY FEATURES OF ESSENTIAL MIXED CRYOGLOBULINEMIA

	Percent
Cryoglobulins	100
Positive Rheumatoid Factor Tests	100
Low Serum Complements	85
HBV Antigen or Antibody	30-60

The immune complex nature of mixed cryoglobulins is strongly supported by the almost invariable finding of low serum complement levels in most patients. This is the result of increased catabolic rate (83,84) presumably on the basis of in vivo complement activation, and decreased synthesis of the early complement components (84). Moreover, there is ample evidence that the isolated cryoglobulins interact with complement components (85) in vivo and in vitro. Although there are isolated well studied cases such as the patient E.B. described here where the serum complement levels seem to correlate with skin or renal disease activity, the available data does not allow generalization, for there are cases in which the complement levels have remained low in the face of a marked decrease or disappearance of the cryoglobulins.

Prognosis and Treatment

The prognosis in mixed cryoglobulinemia is dictated by the presence of renal involvement. In the series of 40 patients of Gorevic et al (80), 14 of 20 patients with renal involvement had died, compared to 4 of 13 without renal disease. Average survival time after onset of renal involvement was 23.5 months. The survival of patients with nephritis did not correlate with any the therapeutc modalities employed.

TABLE XX

PROGNOSIS OF ESSENTIAL MIXED CRYOGLOBULINEMIA

	No of Patients
RENAL DISEASE	22
Deceased	14
Alive	6
Lost to follow-up	2
NO RENAL DISEASE	18
Deceased	4
Alive	9
Lost to follow-up	5

(From ref. 80)

The disease may pursue a capricious course, spontaneous remissions, onset of renal disease and wide spread vasculitis may ensue without correlation with cryoglobulin levels or any other clinical or laboratory feature.

Thus, no definite conclusion can be drawn regarding the efficacy of the therapeutic modalities employed since the available data is mostly anecdotal. Moreover, the relative rarity of the disease may preclude the institution of prospective studies. In general, aggressive therapy with high dose corticosteroids and/or immunosuppressive agents should be employed in patients with progressive renal disease or widespread vasculitis. Our case, and a handful of reports in the literature (86,87) suggest that chemotherapy may be effective in controlling the severe manifestations of the disease. Intensive plasmapheresis is another therapeutic maneuver which may be useful in the management of the disease without the high incidence of severe complications (88).

PATHOGENIC CONSIDERATIONS

The pathogenicity of circulating immune complexes and the resulting clinical picture is dictated by multiple factors such as type and quantity of antigen, magnitude of the immue response, class and subclass of immunoglobulin produced, ability to activate complement, antibody avidity, etc. This potential for an almost infinite number of combinations and permutations of these factors is reflected in the protean nature of human immune complex disease. The entities we have discussed today are a good

example of the many possible clinical presentations that a single antigen-antibody system can generate through variation of some of the physico-chemical parameters mentioned above. In hyperviscosity syndrome, the main feature seems to be the tendency of the complexes to form large aggregates; in HP, similar complexes do not aggregate further at the concentations found in serum; in mixed cryoglobulinemia, the RF complexes behave like "classical"immune complexes inducing vascular damage.
The pathogenic mechanisms operative in these diseases have been clarified because they represent pure examples of a gross exageration of one single physico-chemical abnormality. In rheumatoid arthritis, the pathogenic potential of the RF may result from subtle combinations of known and unknown factors. Thus, it is not surprising that despite the intense and continuous research efforts of the last 30 years, the role of this autoantibody in rheumatoid arthritis has not yet been clarified.

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