Cryoimmunoglobulins

Properties, prevalence in disease, and removal[1]

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Cryoglobulins are immunoglobulins or immunoglobulin-containing complexes that spontaneously precipitate and form a gel at low temperatures and become soluble again when the temperature is raised. There are distinct types: single monoclonal proteins (type I), mixed cryoglobulins with a monoclonal component (type II), and mixed cryoglobulins containing only polyclonal components (type III). Chemically, these proteins are not significantly different from their noncryoprecipitating counterparts; their cryoprecipitability is related more to electrostatic interactions and solubility than to structure or nonprotein composition. Many cryoglobulins are immune complexes. In addition to serum, they can be found in other physiological fluids as well as tissue and have been shown to occur in a wide variety of diseases. Their presence in tissues such as the kidney, vascular structures, and synovial fluid may be related to the pathogenesis of that disease. Serum concentrations of cryoglobulins can be reduced through the use of drugs, plasma exchange, and plasma filtration.

Index term: Cryoglobulins

Cleve Clin Q 52:175–192, Summer 1985

Cryoglobulins are a group of serum proteins that spontaneously precipitate and form a gel at low temperatures and become soluble again when the temperature is raised. Some cryoprecipitates and cryoglobulins occur naturally in low concentrations and contain a high concentration of factor VIII, while others are associated with a particular disease: they contain components which are potentially pathogenic and have immunologic importance. In this paper, we intend to review studies dealing with the chemical, biological, and immunological properties of cryoimmuno-

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0009-8787/85/02/0175/18/$5.50/0

globulins; analysis of cryoproteins; cryoprecipitates and their composition; production and generation of cryoimmunoglobulins in disease; and occurrence in physiological fluids and tissue. We will also discuss their occurrence in various diseases, with emphasis on renal, hepatic, rheumatic, and neurological disorders, their relationship to the immune system in disease, and methods of removing or controlling their production. (For the purpose of this discussion, the terms *cryoproteins*, *cryoglobulins*, and *cryoimmunoglobulins* will be used synonymously.)

In 1929, Heidelberger and Kendall[1] studied complexes of pneumococcal polysaccharide and its antibody and found that at certain intermediate concentrations, the precipitate was insoluble at 0° C but redissolved at room temperature or 37° C and could be precipitated again on cooling; moreover, this process was reproducible. Four years later, cold-induced precipitation of serum proteins in a patient with multiple myeloma was reported by Wintrobe and Buell.[2] Frequently, 1,000–10,000-fold changes in solubility occur over a temperature range of less than 10° C.

The fact that serum cryoprecipitates contain immunoglobulins has aroused the interest of many investigators, who have hypothesized that cryoprecipitates are immune complexes. In 1947, Lerner et al[3] systematically studied cold-induced precipitation and employed the term *cryoglobulins* to denote a group of serum proteins which could form a reversible precipitate or gel in the cold, further characterizing the cryoproteins as gamma globulins. In addition to immunoglobulins, which are cryoprecipitable, other cold-precipitable plasma proteins include cryofibrinogens, C-reactive protein-albumin complexes, a heparin-precipitable protein related to fibrinogen, and a nonclotting component of Cohn fraction I-1 from pooled normal serum. Cold-insoluble Bence Jones proteins (immunoglobulin light chains) have also been described.

Collection of cryoproteins

Cryoproteins can be observed in the serum after storing it in the cold for several days. However, if blood is not handled correctly, measurements of cryoglobulins may be in error. Storage of clotted blood in the refrigerator is one of the major problems which may occur during routine processing in clinical laboratories. There is no generally accepted method of collecting, washing, and redissolving serum cryoglobulins and expressing protein concentrations, so that comparisons between different laboratories are difficult. Generally, blood is collected from fasting patients—usually in warm syringes—or from subjects in a room which is kept at 37° C, and it is maintained at 37° C while clotting proceeds (a process which may take several hours). Following centrifugation, the serum is separated at 37° C. In serum with high levels of monoclonal cryoimmunoglobulins, a precipitate or gel may form immediately on withdrawal into 37°C syringes; in such cases, a chelating anticoagulant [EDTA or ACD] is sometimes added and one volume of $CaCl_2$ (16 g/100 ml of distilled water) is added to each 100 volumes of plasma so that the serum can be extracted from the cell-free fibrin clot at 37° C without significant loss of cryoprecipitate. Finally, the serum is stored at 4° C for 72 hours or more.

Analysis of cryoglobulins and physiological fluids

Cryoprecipitates are generally washed several times in cold buffered or nonbuffered aqueous saline, sometimes accompanied by resuspension of the precipitate at 37° C followed again by precipitation at 4° C. Any materials that do not dissolve are usually discarded. The cryoglobulin concentration is either measured directly by centrifugation (cryocrit) and radial immunodiffusion or indirectly by comparing the serum protein concentration before and after cryoprecipitation. Protein concentrations are estimated by measuring ultraviolet absorption at 280 nm of an aliquot dissolved in acid or by the method of Lowry, Biuret with modifications, or Folin and Lowry. The various components may be separated and purified by gel filtration. Sedimentation coefficients are measured by ultracentrifugation. The purity of the samples is tested by ultracentrifugal analysis, electrophoresis, immunoelectrophoresis, and special immunologic techniques. Proteins may be typed with various subclass-specific antisera from several species of animals. Immunoglobulins are quantified by radial immunodiffusion or nephelometry and categorized as monoclonal or polyclonal by typing of light chains using either immunoelectrophoresis or double-diffusion gel analysis. In the case of mixed cryoglobulins, depolymerization by thiol reagents is sometimes employed for identification of the light chains comprising the IgM component. If little cryoglobulin is present, the component im-

munoglobulins can be purified by ultracentrifugation in a sucrose-density gradient at an acid pH. Specific antibody activity within cryoglobulins (e.g., anti-DNA or rheumatoid factor) may also be determined.

Occurrence of cryoglobulins

Cryoimmunoglobulinemia is not as unusual as is commonly thought. Brouet et al[4,5] found no cryoglobulins in 100 normal controls under 60 years of age and only in 4% of healthy older subjects. Using different isolation procedures, Cream[6] noted that normal persons have low levels of cryoglobulins (<80 μg/ml), although 20% have more than 100 μg/ml. While cryoglobulins are only infrequently measured, determination may be made if tests for immune complexes are positive; however, there may be many more patients in whom cryoglobulins are present, but no measurements are made. Moreover, sampling, separation, and storage of serum may cause detected levels to be lower than actual levels.

Types of cryoglobulins

Brouet et al[5] studied 86 patients with cryoglobulinemia and identified three groups of cryoglobulins. Type I is a single homogeneous monoclonal immunoglobulin with only one class or subclass of heavy and/or light chains; corresponding diseases involve IgM, IgG, IgA, or Bence Jones protein. When the monoclonal protein is isolated by cold precipitation other immunoglobulins are not found. Brouet et al[4,5] reported that in type I cryoglobulinemia, serum levels are usually high (1 to 30 mg/mL) and the immunoglobulin readily precipitates in the cold; of 11 IgG cryoglobulins studied, 6 (55%) were IgG1, 4 IgG2 (36%), and 1 IgG3. This is an unusually high frequency of IgG2 compared to Virella's report[7] in which it was only 10%, most likely related to the fact that different diseases were studied. Type II comprises mixed cryoglobulins with a monoclonal component which acts as an antibody against polyclonal IgG (i.e., rheumatoid factor activity). Most are IgM–IgG, though IgG–IgG and IgA–IgG can also occur; and serum levels of type II cryoglobulins are usually high. Type III includes mixed polyclonal cryoglobulins which are consistently heterogeneous; they are composed of one or more classes of polyclonal immunoglobulins, and sometimes nonimmunoglobulin molecules such as $beta_1$, C3, or lipoproteins. Most are also immunoglobulin–anti-immunoglobulin immunoglobulin–anti-immunoglobulin immunoglobulin–anti-immunoglobulin im-

common type of cryoglobulins, serum levels of type III cryoglobulins are usually very low (0.1 to 1 mg/dL). Brouet et al[4] initially reported that 25% of their patients had type I, approximately 26% type II, and 50% type III; in a follow-up study of 231 patients, the corresponding percentages were 26, 21, and 53%.[5]

Chemical and physical properties

Solubility effects

Two studies have demonstrated the importance of concentration and temperature on precipitation.[4,8] Increasing the concentration of a purified cryoglobulin results in an increase of the temperature at which precipitation occurs. Cryoprecipitation occurs more readily and at higher temperatures in more concentrated solutions, whereas lower temperatures are required to initiate cryoprecipitation in more dilute protein solutions. There may be a progressive increase in the precipitated fraction as the temperature is lowered. Meltzer et al[8,9] showed that the concentration necessary for cryoprecipitation varied with different proteins, ranging from 0.5 to 4 mg/mL of pure cryoglobulins; mixed IgG–IgM cryoglobulins were precipitated most easily at levels as low as 0.5 mg/mL. Middaugh et al[10] studied monoclonal cryoimmunoglobulins in 5 patients and one dog and showed the loss of low-temperature insolubility at low protein concentration. The amount of soluble protein was found to depend on total cryoglobulin concentration over a wide range of concentrations, suggesting the presence of cold-induced aggregates in equilibrium with the solid phase. Although each cryoprotein was soluble at 38° C, insolubility became dectectable below 20° C at a protein concentration of 2.5 mg/mL. As shown by Brouet et al,[4,5] IgM predominates in disease, generally undergoing cryoprecipitation at lower concentrations than IgG. Differences in the concentration and temperature dependence of cryoprecipitation, as well as both qualitative and quantitative differences in the effect of solutes, suggest that the physicochemical basis for cold-dependent insolubility of proteins differs. Scoville et al[11] studied the IgG2 crystalline monoclonal cryoglobulin WEB and found that below a critical concentration of 0.6 mg/mL, cryoprecipitation did not occur. Aggregation exhibited a concentration-dependent lag time, suggesting that nucleation is

important to precipitation. Finally, Vialtel et al[12] investigated the kinetics of polymerization of human monoclonal cryoimmunoglobulins at low temperatures[12] and found that around a critical concentration of 2–3 mg/mL, a characteristic concentration-dependent lag phase was observed following thermal equilibration, with the only stable intermediate being the dimer. The initial rate of self-assembly was proportional to the product of the monomer concentration and that of the dimer promoter. Depolymerization was three times faster than polymerization and proportional to the concentration of polymers, suggesting that polymerization of monoclonal cryoimmunoglobulins is controlled by nucleation and that dimerization is the rate-limiting step. The interaction site between monomers also appeared to be in the Fab region. Polymerization of monomers was induced only by autologous dimers, showing that hypervariable regions play a specific role.

Primary structure: amino acid sequence

The aforementioned differences in solubility between cryo- and noncryoimmunoglobulins indicate that there are differences in the amino acid sequences of these proteins. Because monoclonal cryoimmunoglobulins are available in pure form, they are studied most frequently. Brouet et al[4,5] showed that the amino acid composition of light and heavy chains of an IgG1 kappa cryoglobulin were similar to that of a noncryo-IgG1 molecule. Middaugh et al[10] studied the Fab and Fc fragments of 5 human and one canine monoclonal cryoimmunoglobulins, but (with one exception) were unable to detect any differences between cryoglobulin and noncryoglobulin proteins. Litman et al[13] found no differences in the amino acid composition of the heavy and light chains of an IgG1 kappa monoclonal cryoimmunoglobulin, compared to noncryoglobulin reference proteins. Erickson et al[14] examined the amino acid sequences of the V_H domains from 2 human monoclonal cryoimmunoglobulins (McE and Hil) and noncryoglobulins using metric analysis and found that neither cryoglobulin sequence contained an unusual insertion or deletion of residues; however, each contained two unprecedented residues in the outer beta-sheet structure of the V_H domain. Gerber-Jenson et al[15] studied the amino acid sequence of the heavy-chain variable region of McE, a monoclonal IgM cryoimmunoglobulin which has been shown to involve solubility characteristics rather than antigen-antibody complex formation in cold precipitation. Previous structural characterization of this protein by Middaugh et al[10] revealed atypical conformation, gel filtration, and amino acid composition associated with the Fab fragment (Fabμ). Primary structural analysis indicated that the N-terminal regions of the light chain and Fc pentamer (Fcμ_5) are similar to the corresponding regions of immunoglobulins which lack cryoglobulin behavior. Analysis of the intact Fabμ fragment yielded a single N-terminal sequence which was identical to that of the isolated light chain. Lopez de Castro and Chiu and their colleagues[16,17] have determined the amino acid sequence of the variable region of the light (λ) and heavy chain (V_H) for the human myeloma cryoimmunoglobulin IgG Hill. They found slight differences in the amino acid sequence that could account for differences in the nature and conformation of this protein compared to its noncryoglobulin counterparts.

Electrostatic interactions

The influence of changes in pH, ionic strength, and solute interactions on solubility suggests that electrostatic and hydrophobic forces are important to cryoprecipitability. Brouet et al[4,5] showed that acid and alkaline pH, high ionic strength, and low concentrations of urea (0.5 M), guanidine hydrochloride (0.2 M), and sodium dodecylsulfate (0.1%) all resulted in a decreased cold precipitation. Meltzer and Franklin[8] have reported similar findings.

Cryoimmunoglobulins represent the outer edge of the solubility distribution of total serum immunoglobulins. Solubility is abnormal when high concentrations of a particularly insoluble immunoglobulin are produced during the course of the disease. Because of their homogeneous nature, monoclonal cryoglobulins (type I) have been analyzed to evaluate the phenomenon of cryoprecipitation. Middaugh and Litman[18] studied the effect of the solute on the cold-induced insolubility of monoclonal cryoimmunoglobulins and found that neutral salts inhibited (i.e., they had a low relative electric field at the ion surface) cryoprecipitation to a degree paralleling the Hofmeister (lyophobic) series of ions ($SCN^- > ClO_4^- > I^- > Br^- > Cl^-$ for anions and $Ca^{++} > Mg^{++} > NH_4^+ > K^+$, Na^+ for cations) while high concentrations of nonchaotropic neutral salts (NaCl, $CaCl_2$) appeared to have little effect on precipi-

tation. (Chaotropic salts enhance the solubility of hydrophobic compounds in water.) Hydrophobically substituted solutes (i.e., ones exhibiting reduced solute-solvent and increased solute-solute interactions) resulted in an apparent enhancement of cryoprecipitation that increased along with the length of the hydrocarbon chain. Middaugh and Litman also noted that, in general, agents which enhance the solubility of polar compounds in water favored cold-induced precipitation. While interactions between charges were not directly responsible for cryoprecipitation, electrostatic interactions were believed to play a key role in its occurrence in most cases. The apparent enhancement of cryoprecipitation seen with the hydrophobically substituted solutes suggested that intermolecular hydrophobic interactions were not immediately responsible for cryoprecipitation in any of the monoclonal cryoimmunoglobulins examined, while the inhibition of cryoprecipitation by sugars and halothane suggested that hydrogen bonding may play a role in stabilization of the cryoprecipitate. Agents that inhibit cryoprecipitation were found to lower the temperature at which precipitation begins, while cryoprecipitation was shifted to a higher temperature in the presence of enhancing solutes. Such agents decreased both the temperature at which conformational changes are initiated and their magnitude. Hydrophobic chromatography, a technique based on the increased hydrophobic interaction between a protein and an immobilized ligand at high salt concentrations, was used by Litman et al[13] to evaluate the macromolecular conformation. These findings suggested that a variety of subtle alterations in macromolecular structure may account for the anomalous temperature insolubility characteristic of monoclonal cryoimmunoglobulins. Middaugh et al[10] have suggested that cryoglobulin behavior may reflect heterogeneous solubility of immunoglobulins, and that cryoglobulins may represent the intrinsically less soluble members of the population. If such is indeed the case, then a comprehensive survey of cryoglobulins should detect proteins exhibiting only marginal cryoglobulin behavior. Middaugh et al[19] studied the thermodynamic basis behind the abnormal solubility of cryoimmunoglobulins and suggested that (*a*) no significant conformational changes occur between 0 and 35°C and (*b*) hydrophobic effects are unimportant. The lack of such effects with both normal and cryoimmunoglobulins suggests that interactions are dominated by polar groups, e.g., charge-charge and dipole-dipole interactions and hydrogen bonds). In Middaugh's study,[10] human proteins reacted with the kappa light chain but not with the lambda chain. The maximum pH compatible with cryoprecipitation was 5.5 to 9.5. Meltzer et al[8,9] found that cryoprecipitability was greatest at a pH of 5.5 to 7.0 and decreased salt concentration. Spectroscopic analysis indicated that the loss of cryoprecipitability at an acidic pH is accompanied by changes in protein structure, whereas at a basic pH the effect appears to occur prior to any detectable conformational changes. Contrary to their previous studies,[18] the non-chaotropic neutral salt NaCl inhibited cryoprecipitation of four proteins; however, proteins were inhibited by chaotropic salts (NaSCN, $MgCl_2$) as shown previously. These studies and others[19] suggest that intermolecular hydrophobic interactions are not directly responsible for cryoprecipitation, and that electrostatic (ionic) interactions are a major force in the cold-induced insolubility of some proteins. Litman et al[13] demonstrated inhibition of cryoprecipitation of the IgG1 kappa monoclonal cryoimmunoglobulin by the neutral salt NaCl, supporting Middaugh's 1978 study[10], but not their 1977 findings.[18] This also suggests that electrostatic interactions are involved in cryoprecipitation. Weber and Clem[20] investigated the molecular mechanism of cryoprecipitation and cold agglutination of an IgM lambda Waldenström cryoglobulin (designated MAT) isolated from the plasma of a patient with lymphoproliferative disease. Either elevated temperatures (above 32° C) or exposure to a relatively low concentration of a dissociating solvent (1M guanidine-HCl) proved to be sufficient to prevent intermolecular association. Mixtures of the Fab fragments and the Fc pentamer demonstrated the temperature-dependent association of these two soluble fragments.

Secondary and tertiary structure

Studies of monoclonal cryoimmunoglobulins indicate that any differences between amino acids are slight; however, they may still be sufficient to cause changes in the secondary or tertiary structure of these proteins (referring to the ordered conformation caused by interactions of neighboring amino acid residues and to the arrangement of polypeptide chains resulting from interactions between distant amino acid residues, respectively). Brouet et al[5] conducted differential spec-

troscopic analysis of cryoimmunoglobulins and showed that tryptophan exposure occurred at lower temperatures. Middaugh and Litman[21] compared the acrylamide inhibition of the intrinsic tryptophan fluorescence of cold-soluble monoclonal and monoclonal cryoglobulin IgM proteins, which is capable of interacting differently with buried and exposed indole side chains. They observed that the tryptophan residues of cryoglobulins were exposed to acrylamide more thoroughly than those of noncryoglobulin IgM, suggesting dynamic structural alterations of the cryoglobulins. Brouet et al[4,5] demonstrated in circular dichroism studies that the secondary ordered beta structure was not affected by lowering the temperature and that only the near-ultraviolet portion of the spectrum was modified. Meltzer et al[8,9] studied cryoimmunoglobulins in 29 patients but were unable to isolate a single structural feature that could be associated with cryoimmunoglobulins in comparison to their cold-soluble counterparts, although about half of the proteins studied had an atypical tertiary structure. The lack of cryoprecipitability of cryoglobulin subunits or bivalent fragments and the failure of the fragments to inhibit or enhance cryoprecipitability of the parent molecules argue against classical antibody-antigen complex formation. The properties of an isolated kappa monoclonal IgM cryoglobulin (McE) and five noncryoglobulin cold-soluble proteins, as well as their constituent monomeric subunits and Fcμ_5 and Fab fragments, were compared under both native and partially denaturing conditions by Middaugh et al.[22] The results suggested that the cryoglobulin anomaly is more likely a result of differences in tertiary rather than secondary structure.

Carbohydrate- or lipid-associated properties

Several investigators have noted carbohydrate abnormalities of cryoimmunoglobulins in comparison to noncryoglobulins—specifically, differences in sialic acid content—suggesting that the differences are related to carbohydrate rather than protein content. Brouet et al[4,5] showed that the carbohydrate composition of several cryoglobulins was not markedly abnormal, but that sialic acid was absent. Zinneman et al[23] studied a mixed IgG-IgM cryoglobulin and found that neither component had the qualities of a cryoglobulin by itself. The carbohydrate composition of the 19S component was normal, but the 7S component lacked sialic acid and contained only 20% of normal hexose and hexosamine, whereas fucose was present in normal quantities. The 19S component yielded only a minimal cryoprecipitate with pure normal IgG; but when it was added to normal pure IgG from which sialic acid had been removed by incubation with neuraminidase, a dense cryoprecipitate resulted on chilling. On analysis of cryoglobulin from a patient with multiple myeloma, sialic acid, hexose, hexosamine, and fucose were absent. These studies suggested that the IgM component is an IgG-specific antibody which has become an antigen with the loss of sialic acid. Removal of sialic acid from the globulin molecule may alter its solubility and also reduces its negative charge. Athineos et al[24] studied the antigenicity of native and desialized orosomucoid in rabbits. Orosomucoid contains large amounts of galactose, mannose, glucosamine, and sialic acid, which is limited to terminal nonreducing positions and thus might be expected to be an important determinant of immunochemical specificity. While sialic acid is normally antigenic, desialization of orosomucoid with neuraminidase significantly enhanced antigenicity. Litman et al[13] conducted a carbohydrate analysis on a monoclonal cryoimmunoglobulin and showed that fucose, hexosamine, and sialic acid were reduced by 50%, 50%, and 80%, respectively. Decreased sialic acid has been suggested as a basis for the cold-dependent insolubility of other monoclonal IgG cryoglobulins[25]; however, removal of sialic acid from noncryoglobulin reference proteins failed to induce cryoglobulin behavior. In Weber and Clem's study of MAT,[20] determinants found on human and canine erythrocytes could be distinguished; however, treatment with neuraminidase rendered them unreactive. Papain also diminished the reactivity of canine red cells, but had little if any effect on human erythrocytes. This reactivity proved to be anti-glycolipid-dependent. Treatment of MAT with mild alkali followed by neuraminidase resulted in release of more than 85% of sialic acid and concomitant loss of cryoprecipitation, so that sialic acid was believed to be essential for expression of both cryoprecipitation and cold agglutination. These findings appear to conflict with those of Zinneman et al,[23] who noted that reduced sialic acid content contributed to cryoprecpitation; however, it would appear that some sialic acid is required, or else an optimum amount must be present following

conformational changes in order to provide the charge effects necessary for cryoprecipitation. Lewis et al[25] conducted a detailed analysis of cryoglobulin associated with lipids, in a patient presumed to have lymphocytic lymphoma. They found that 95% of protein-stainable cryoglobulin had the mobility of gamma-globulin and only 3% migrated as beta-globulin; in addition 90% of the lipid-stainable material migrated as beta-lipoprotein. Addition of whole serum or low-density beta-lipoprotein to the macroglobulin fraction at temperatures of 32° C or lower resulted in precipitation. Unlike other studies, low-density lipoprotein, not gamma-globulins, was necessary for the macroglobulin to be cold-precipitable. These authors hypothesized that the relatively infrequent occurrence of cryoglobulinemia in patients with macroglobulinemia may be due in part to the low levels of serum cholesterol and beta-lipoproteins frequently observed in patients with hypergammaglobulinemia or macroglobulinemia. Kodama[26] observed serum IgG antilipoprotein-autoantibody activity in refrigerated samples (4° C) from a patient who had plane xanthoma but without multiple myeloma. Following acid dissociation of the complex, the polyclonal IgG fraction was shown to precipitate with lipoproteins; thus the cryoglobulins were demonstrated to be immune complexes of polyclonal IgG-anti-lipoprotein autoantibody and both alpha- and beta-lipoproteins.

Biological properties

One of the simplest ways of detecting antigen-antibody reactions in vitro is by precipitation. The amount of the precipitate is often increased by cooling due to decreased solubility. Thus the presence of cryoprecipitates containing immunoglobulins in serum raises the possibility that they are immune complexes. Neither Middaugh et al[10] nor Litman et al[13] found any evidence of formation of cold-dependent antigen–antibody complexes in their studies of monoclonal cryoimmunoglobulins. However, Brouet et al[4,5] showed that several monoclonal IgMs acted directly on blood group antigens. Cellular studies in patients with type II IgG-IgG or IgA-IgG cryoglobulins and multiple myeloma showed that malignant cells synthesized a monoclonal IgG or IgA which was able to bind polyclonal aggregated Ig. On the other hand, they could not detect monoclonal immunoglobulin by electrophoresis or immunoelectrophoresis of purified type II cryoglobulin. Meltzer and Franklin[8] analyzed mixed cryoglobulins and demonstrated that both fractions were necessary for cryoprecipitability and rheumatoid factor activity. Reported combinations of mixed cryoglobulins, in decreasing order of occurrence, include IgM-IgG, IgM-IgG-IgA, IgA-IgG, and IgG-IgG; IgG was always the antigen, and both monoclonal and polyclonal components have been detected.[4,9]

While antigen-antibody complexes have been found in cryoprecipitates in various diseases, the presence of immune reactants has raised the question of their relationship in the pathogenesis of disease. Davis et al[27] showed that cryoproteins of patients with autoimmune diseases such as systemic lupus erythematosus (SLE) contain antigen-antibody complexes and are rich in antibodies to nuclear ribonucleoprotein as well as both single- and double-stranded DNA; both DNA and anti-DNA were enriched relative to serum levels in the cryoprecipitates. Cryoproteins have been detected in patients with viral hepatitis and infectious mononucleosis. Hepatitis B antigen and antibody have been found in patients with extrahepatic manifestations of acute viral hepatitis, such as arthritis and nephritis. Levo et al[28] have demonstrated hepatitis B antigen and antibody in cryoprecipitates from patients with essential mixed cryoglobulinemia and showed that there is an association between cryoprotein and decreased serum complement levels, suggesting that complement activation occurs in patients with cryoproteinemia. Complement proteins have been found in cryoprecipitates as well. Wilson et al[29] studied cryoprecipitates in 12 patients with essential monoclonal and mixed cryoglobulinemia, as well as cryoproteinemia associated with rheumatoid arthritis and Sjögren's syndrome, and assessed their ability to activate complement by either classical or alternative pathways. C1q, C4, C3, and factor B were found in most cryoprecipitates, and the terminal complement proteins C5, C6, and C9 were also detected in most patients. In 3 patients whose cryoprecipitates contained IgG and IgA, but not IgM, no complement proteins were detected. The serum from most patients contained a significant titer of anti-gamma globulin. In about 75% of seropositive rheumatoid arthritis patients, serum and cryoprecipitates were positive for rheumatoid arthritis precipitin. These antibodies were enriched 32- to 80-fold in the cryoprecipitates, compared to the serum. Serum of 7 patients was positive

for antinuclear antibody. Antibodies to single- or double-stranded DNA, nuclear ribonucleoprotein, and Sm antigen were not detected in any cryoprecipitates. Seven patients had below-normal CH_{50} levels, 6 had decreased levels of C1q and C4, and 2 had abnormally low levels of factor B. In all but one case, the cryoprecipitates activated the classical complement pathway, while two-thirds of the cryoprecipitates significantly activated the alternative pathway.

The detection of a wide variety of immune reactants and complement in cryoproteins provides additional evidence that cryoprecipitation from serum may be a manifestation of pre-existing immune complexes. Demonstration of the immune-complex nature of most mixed cryoglobulins suggests that such complexes may be responsible for acute vasculitis in vivo. Mixed cryoglobulins share anti-IgG antibodies (i.e. rheumatoid factor activity, which commonly resides in the IgG component).[30] The accumulating evidence that immune complexes of various kinds are present in cryoprecipitates suggests that immune reactants are present in both tissue and serum. Hardin[31] studied the relationship of type III cryoglobulins in immune complexes determined by ^{125}I-C1q-binding in serum of patients with rheumatic or infectious diseases. Experimentally produced cold-soluble immune complexes behaved like cryoimmunoglobulins in the presence of increased concentrations of certain normal serum proteins that were reversibly cryoprecipitable by themselves, and whose polypeptide constituents were similar to the nonimmunoglobulin components of cryoglobulins isolated from patients with rheumatic or infectious disease. These findings imply that type III cryoglobulins depend on nonimmunoglobulin components for their cold-insolubility and that cryoprecipitability is not a property of immune complexes themselves, but rather of a group of large serum proteins. One such substance is fibronectin, though it is not the only one related to cryoglobulin formation. In inflammatory disease, concentrations of some of these proteins, responding as acute-phase reactants, may increase to the point where intermolecular attractive forces become prominent, particularly in the cold. Lowering the temperature favors insolubility of macromolecules, especially at higher solute concentrations. Cold-augmented molecular aggregation between these nonimmunoglobulin proteins and immune complexes could decrease their collective solubility, resulting in the cryoprecipitation of otherwise cold-soluble immune complexes. Fibronectin has been implicated in the cryoprecipitation of fibrin–fibrinogen complexes and has been suggested as playing a role in cryoglobulinemia, having been found in the cryoprecipitates of patients with collagen and autoimmune disease.[32] Levo[33] searched for fibronectin in 32 cryoprecipitates from patients with acute hepatitis, chronic active hepatitis, essential mixed cryoglobulinemia, or inflammatory bowel disease. Most cryoproteins of patients with essential mixed cryoglobulinemia and inflammatory bowel disease were of the mixed IgG-IgM type, and all contained fibronectin. In contrast, most cryoproteins of patients with hepatitis were composed of polyclonal IgM and were devoid of fibronectin. While it is possible that fibronectin characterizes and facilitates the formation of mixed cryoglobulins, it does not seem to be necessary for cryoprecipitation. Ginder et al[34] studied the relationship of pancytopenia to mixed cryoglobulinemia in vivo and showed that cryoglobulin (or a material attached to it) was capable of suppressing precursor cells. Depletion of cryoglobulin by means of plasmapheresis resulted in reversal of inhibitory activity. Cryoglobulin of 3 patients with collagen vascular disease who had normal peripheral blood counts, as well as 2 others with mixed cryoglobulinemia, failed to demonstrate anti-precursor cell activity. Precursor cell antibody activity due to anti-DNA antibody was also demonstrated in a patient with active SLE and pancytopenia.

Relationship of disease to cryoglobulinemia

Since Wintrobe and Buell's report of the association of cryoimmunoglobulins and multiple myeloma in 1933,[2] they have been reported in other diseases as well. In their study of 178 patients with cryoimmunoglobulins (49 type I, 49 type II, and 80 type III), Brouet et al[35] attempted to establish a possible correlation between the immunochemical characteristics of the cryoglobulins and the patient's symptoms. They found that 80% of patients had some symptoms which could be related to the cryoglobulins, though pathological importance was sometimes difficult to assess. The incidence of neurological symptoms was relatively high (17%); however, many patients had vasculitis on muscle biopsy, and the

relationship between vasculitis and cryoglobulin was not established. The same was true of hemorrhagic symptoms in patients with myeloma or macroglobulinemia. Cutaneous symptoms varied according to the type of cryoglobulin. Immune complex vasculitis was found in patients with mixed cryoglobulinemia and occasionally in patients with monoclonal cryoglobulins. In contrast, patients with monoclonal cryoglobulins had severe symptoms (skin necrosis, acryocyanosis, vasomotor symptoms) which may have been related to vascular deposition of cryoglobulins. Patients with mixed cryoglobulins and a monoclonal component may have both types of injury. Brouet et al also reported that the incidence of nephritis was similar in patients with various types of cryoglobulins. Recurrent glomerular injury was observed in patients with monoclonal cryoglobulins, or mixed with a monoclonal component, whereas renal disease was slowly progressive in patients with mixed polyclonal cryoglobulins. Hematological (lymphoid) malignancy was seen in 75 patients, including multiple myeloma, Waldenström macroglobulinemia, chronic lymphocytic leukemia, and malignant lymphoma. Autoimmune diseases were the most frequent associated conditions (69/178), reflecting the fact that these cases were collected in a department devoted to hematology and clinical immunology; included in this group were SLE, periarteritis nodosa, Sjögren's syndrome, rheumatoid arthritis, autoimmune hemolytic anemia, and thrombocytopenic purpura. Cold-induced symptoms were often present several years before the diagnosis of multiple myeloma and increased in severity with time. Presenting symptoms related to cryoglobulinemia were found in 80% of patients and were present one to 10 years before the diagnosis was made. Glomerular lesions were far in excess of those observed in macroglobulinemia without cryoglobulin and were considered to have major prognostic significance. Mixed cryoglobulin was a frequent finding in diseases known (or presumed) to have an autoimmune mechanism. Symptomatic cryoglobulin preceded the onset of overt autoimmune diseases for many years in 10 patients. Glomerulitis occurred in 6 out of 17 patients with Sjögren's syndrome, emphasizing the pathogenetic role of cryoglobulin, since glomerular injury is exceptional in this disease when no cryoglobulins are present. In 20 patients with a tentative diagnosis of essential cryoglobulinemia at the onset of disease, autoimmune diseases or hematologic malignancy developed two to 20 years later, suggesting that these patients may have undergone chronic antigenic stimulation and that circulating antigens bound to antibodies and rheumatoid factors were present in the cryoglobulins. In 17 patients with essential mixed cryoglobulinemia, there was evidence of chronic hepatitis. Five other patients with mixed cryoglobulins and hepatitis also had malignant lymphoproliferative or autoimmune disease. Brouet et al suggested that in some cases the underlying illness may facilitate the development of chronic hepatitis. Nightingale et al[36] studied the possible inheritance of cryoglobulinemia in a family in which 10 members of three generations had IgM-IgG cryoglobulinemia and suggested that the pedigree was characteristic of autosomal dominance. No underlying disease that could account for the cryoglobulinemia was identified in any patient, nor could it be linked to HLA-A and HLA-B locus haplotypes, blood group antigens, or immunoglobulin Gm allotypes. This study demonstrated that mixed cryoglobulinemia can be inherited and have varying clinical manifestations.

Liver disease and cryoglobulinemia

Jori and Buonanno[37] studied 11 patients with essential cryoglobulinemia who were found to have either persistent or aggressive chronic hepatitis on hepatic biopsy, but no other underlying disease. Cryoglobulin concentrates greater than 1 g/100 mL were found in 7 patients and values of less than that in 4; cryoglobulins were mixed. In comparison, 3 of 5 patients with cryoglobulinemia and cirrhosis were shown to have pure IgG cryoglobulins. Serum IgM was significantly higher in hepatitis than in cirrhosis. Several patients had no clear-cut evidence of liver disease, as expressed by either subjective complaints, hepatosplenomegaly, or functional abnormalities. Thus it appears that liver involvement may be a constant feature of the cryoglobulin syndrome. Initial transition to cirrhosis was suggested by the severity of the histological changes observed in 2 patients. Since the level and composition of serum cryoglobulins did not differ significantly between patients with cirrhosis and those with chronic hepatitis, the pathogenic mechanism may be similar in both diseases in patients with cryoglobulinemia.

Hepatitis B (HbsAg) surface antigen has been detected in the serum of patients with a variety of diseases, and immune complexes of this antigen and antibody have been implicated in damage to various organs. Various investigators have reported a high incidence of cryoproteins in immune complex syndromes, both in man and experimental animals, and have demonstrated that these represent complexes of antigen and antibody. Such cryoproteins were shown to possess biological properties usually attributable to immune complexes, and to have both clinical and immunopathological significance. McIntosh et al[38,39] studied serum from patients with acute HbsAg-positive hepatitis, chronic hepatitis B, and a variety of HbsAg-negative liver diseases as well as normal subjects, seeking to determine the presence and nature of cryoproteins. Their incidence was highest in acute HbsAg-positive hepatitis, including all 14 patients evaluated by macrotechnique and 20 out of 30 patients evaluated by micro-technique (67%). It was also high in chronic HbsAg-positive hepatitis and carriers of chronic HbsAg. Cryoproteins were detected far less frequently in HbsAg-negative patients and were not found at all in normal subjects. The cryoprecipitates from the hepatitis patients were composed of mixed immunoglobulins (predominantly IgM) and also contained traces of C3 and rheumatoid factor with concentration of HgsAg and antibody. Patients with chronic hepatitis, but no vasculitis, had both detectable antigen and antibody, while those with associated vasculitis or nephritis had large amounts of antigen but little antibody. These observations support the concept that pathogenic mechanisms analogous to those in acute and chronic serum sickness in experimental animals play a role in hepatitis B-associated diseases. While cryoproteins are not specific for HgsAg-positive liver disease, their presence may indicate circulating immune complexes and their nature may reflect the clinical syndrome involved. These studies suggest that investigation of cryoproteins in hepatitis may have both clinical and immunopathogenic value.

Ozawa et al[40] described a patient who initially manifested features of acute immune complex disease, nephritis, and liver disease. At autopsy, histological studies of the liver and kidneys were made in the hope of establishing a definitive immune complex pathogenesis for renal disease. Granular deposition of IgG, C3, C4, and HbsAg along the glomerular basement membrane was noted, and HbsAg was seen in liver sections as well. Light microscopy of the kidney revealed increased cellularity and an increase in the mesangial matrix of the glomeruli, as well as tubular degeneration and scattered necrosis. Elution studies demonstrated that the glomerular-bound immunoglobulin contained an antibody to HbsAg. Since the ratio of the antibody titer per mg of IgG in the eluate to the supernatant serum after cryoprecipitation was approximately 37:1, the authors suggested that the presence of immunoglobulin in the glomeruli was due to selective deposition rather than nonspecific trapping of antibody from the circulation.

Levo et al[28] reviewed a large series of patients with essential mixed cryoglobulinemia and were struck by the high frequency of clinical and laboratory evidence of liver involvement. Thirty such patients with purpura ($n = 28$), arthralgia ($n = 24$), weakness, and frequently kidney involvement ($n = 15$) but no well-defined connective tissue disease, lymphoproliferative neoplasia, or chronic infection were studied. Only 2 patients had obvious liver disease at the time of diagnosis. Half of the serum samples from patients with essential mixed cryoglobulinemia contained anti-Hbs and 3 out of 25 were positive for HbsAg. None of the controls had HbsAg. The frequency of positive results was increased when the cryoglobulins were examined; 74% were positive for either HbsAg or its antibody. Electron microscopy of some cryoprecipitates indicated the presence of virus particles. These data suggest that an infectious agent (most commonly hepatitis B virus) is frequently involved in the pathogenesis of essential mixed cryoglobulinemia. These authors suggested that viral infection could initiate chronic immune disease, and that the virus can persist despite a good immune response. Many of the pathological manifestations attributed to hepatitis B virus seem to be mediated by the host's immune response rather than by the direct effects of the virus itself. There is also evidence of involvement of HbsAg-Ab complexes in polyarteritis nodosa, as well as serum hepatitis. Jori et al[41] studied the frequency of cryoglobulinemia in chronic liver disease and histologically confirmed the presence of chronic inflammatory liver disease in 150 patients. Cryoglobulins were detected in 44 patients (29%). The incidence of cryoglobulins was higher among patients with

chronic aggressive hepatitis (43.5%), compared to 38% in chronic persistent hepatitis, 36.6% in cirrhosis with active hepatitis, and 9.4% in inactive cirrhosis. Most patients with cryoglobulinemia were women. Thus a recognizable cause of liver damage was lacking more often in cryoglobulin-positive patients (75%) than in negative ones (47%). This study suggests that cryoglobulinemia can be detected in about one third of patients with chronic inflammatory liver disease which is not associated with other conditions that may lead to altered immunoglobulin synthesis. Both HbsAg positivity and evidence of alcoholic liver disease were obtained more often in cryoglobulin-negative than in positive cases. Thus neither infection nor alcohol abuse seems to play a significant role in causing cryoglobulinemia associated with chronic liver disease. While the clinical association of cryoglobulinemia with chronic active liver disease has practical importance, the relationship between cryoglobulinemia and liver disease remains unclear.

Essential cryoglobulinemia

In the absence of demonstrable disease, the diagnosis of essential mixed cryoglobulinemia is made when cryoglobulins are present. In 1 such case, Kumar et al[42] demonstrated antibodies to not only the surface antigen but also the core antigen of hepatitis B virus in the cryoprecipitate. Considering the findings of other authors,[28,39] in which three fourths of cases were thought to be "essential," one may wonder whether cryoglobulinemia is ever really essential, or simply a reflection of the inadequacy of present diagnostic techniques.

Invernizzi et al[43] recently reported on 166 patients seen between 1966 and 1981. Seventy-nine patients were diagnosed as having secondary or essential cryoglobulinemia. All but 2 patients were symptomatic, most commonly exhibiting purpura and arthralgia. Thirty-five patients were followed up for eight to 17 years. Membranous proliferative glomerulonephritis developed in 13 (37%), more than half of whom died of renal insufficiency. Cirrhosis was diagnosed in 4 (11%) after four to nine years, and 4 others (11%) later demonstrated lymphoproliferative disease. Of the remaining 14 (40%), 2 were still asymptomatic at the time of publication and 12 had both purpura and arthralgia.

Renal disease and cryoglobulinemia

The work of Meltzer and Franklin[8,9] strongly suggests that under certain circumstances, immune complexes are cryoglobulins which can be deposited in the glomeruli, thereby inducing glomerulonephritis. Druet et al[44] surveyed the frequency of cryoglobulinemia in 76 patients with various forms of glomerulopathy, as well as the composition of the cryoglobulins, comparison with the intraglomerular deposits revealed by immunohistochemical methods, the level of serum complement, and the presence of rheumatoid factor. The occurrence of cryoglobulin in glomerulopathy was significantly higher than in controls with arteriosclerosis obliterans of the lower extremities, and it was statistically significant in active proliferative endocapillary glomerulonephritis, membranoproliferative glomerulonephritis, and lupus glomerulonephritis. Fifty-one cryoglobulins (67%) contained IgG and IgM, and 6 of them also contained C3. IgG, IgA, and IgM were found in 16 cases (21%), together with C3 in one case. IgG was the only immunoglobulin present in eight cases (10.5%) and was associated with C3 in three. One cryoglobulin contained IgG and IgA. Rheumatoid factor was rarely found. In most of the patients with acute endocapillary proliferative glomerulonephritis, C3 was the only protein found in the glomerular tuft. In membranoproliferative glomerulonephritis, there was good correlation between the immunoglobulins in the cryoprecipitate and those in the tuft. In the patients with SLE, glomerular disease was demonstrated by immunochemistry.

Bengtsson et al[45] isolated a monoclonal cryoglobulin from a man with a history of skin lesions on exposure to cold who was later found to have renal impairment and nephrotic syndrome with glomerular involvement. Unlike Druet et al[44] who reported that renal disease was associated with polyclonal immunoglobulins, only monoclonal IgG was detected. Ponticelli et al[46] also reported a case of monoclonal IgG cryoglobulinemia associated with renal failure. While glomerular proliferative lesions are rarely associated with monoclonal cryoglobulins, the presence of mesangial proliferation, vasculitis, low concentrations of serum C3, and rheumatoid activity in patients with cryoglobulin is consistent with an immune complex disease. These authors suggested that the mechanism may be the same as in

mixed cryoglobulinemia, *i.e.*, the IgG is a mixture of antigens and antibodies. Germain et al[47] described a case of type II cryoglobulinemia associated with membranoproliferative glomerulonephritis and vasculitis. The patient's response to cyclophosphamide and prednisone both clinically and serologically correlated with improvement in the glomerular and interstitial lesions on renal biopsy, although skin and renal vasculitis persisted over a two-year period. These observations suggest that different immunopathological mechanisms are involved in vascular and glomerular injury. Infiltration of macrophages was demonstrated in the glomerular capillary lumen, while polymorphonuclear cells predominated in the vascular lesion. Cordonnier et al[48] compared renal biopsy specimens and in vitro cryoprecipitates in a patient with mixed IgG-IgM cryoglobulinemia associated with glomerulonephritis. The cryoprecipitates and walls of the renal capillary loops were made up of cylindrical or ring-shaped bodies 20 to 25 nm in diameter. Since these bodies were similar in both the glomeruli and cryoprecipitates, they were believed to consist of IgG and IgM. The evidence suggests that this was probably an immune complex disease.

McIntosh et al[38] studied serial serum samples taken over a six-year period (1967–1974) from patients with immunologic and nonimmunologic renal disease and acute infection as well as normal controls. The presence of cryoproteins correlated with serial analyses of renal function, as well as morphological, immunohistological, clinical, and serologic observations. A large number of the cryoproteins found in renal disease were thought to be mediated by immune complexes. Cryoprecipitates were not detected in the other patients. The presence of fibrinogen in a serum cryoprecipitate was always associated with rapidly progressive disease and a poor prognosis, while persistence of cryoproteinemia was associated with progression to end-stage renal disease. Detection of serum cryoglobulins was found to be better than serum complement as an index of clinical and morphological activity of immune complex renal disease. These findings suggest that cryoglobulins are not merely an incidental finding and have both diagnostic and prognostic significance.

Zimmerman et al[49] studied a patient with nephrotic syndrome, recurrent purpura, chronic active hepatitis, and ascites who had mixed IgM-IgG cryoglobulins. Their data strongly suggested that the cryoglobulin precipitate acts as an immune complex and very likely plays a role in the pathogenesis of glomerular changes. IgG, IgM, C3, and C4 were all present in the capillary thrombi as well as along peripheral capillary loops in the renal glomeruli. The liver contained advanced active cirrhosis with features suggesting transition from chronic active hepatitis. It is not known whether hepatic disease precedes cryoglobulin formation or vice versa. Unlike the kidneys, the liver seems to be less susceptible to experimental immune complex injury. Possibly primary hepatic disease results in immunoglobulin abnormalities which in turn lead to cryoprecipitation. While various drugs have been used to treat mixed cryoglobulinemia, we know of no therapy that offers prolonged benefit in slowing its progression. We therefore suggest that management be directed toward lowering the amount of circulating cryoglobulin in an effort to alter the course of the renal injury. Monga et al[50] evaluated patients with mixed IgG-IgM cryoglobulinemia, glomerulonephritis, and membranoproliferative disease, with emphasis on the histological, immunofluorescence, histochemical, and electron microscopic findings in the intraluminal thrombi. Ultrastructural analysis demonstrated that the hyaline bodies actually corresponded to clusters of macrophages. The authors argued that the cells were monocytes and that, based on size considerations alone, the IgG-IgM complexes would tend to form an aggregate in the subendothelial space, where they would conceivably be phagocytized.

Andrejak et al[51] found cryoprecipitates in 16 out of 51 kidney transplant recipients, suggesting the presence of circulating immune complexes. A transient cryoprecipitate was found in 10 patients (20%), 9 of whom had intercurrent infection. Mixed cryoglobulinemia was not considered an absolute contraindication to transplantation even when it was thought to be the cause of renal disease. Cordonnier et al[48] have demonstrated renal involvement in more than 20% of patients with cryoglobulinemia. Zimmerman et al[49] and Monga et al[50] have implicated the precipitation of IgM-IgG immune complexes in the kidneys.

McPhaul[52] tested the serum of 206 consecutive patients with renal disease, 98 normal subjects, and 16 patients with SLE but no apparent renal disease for cryoglobulin precipitates. Cryoglobulins occurred relatively infrequently (11%) in mild cases of glomerulonephritis not associated

with systemic disease, but more so (45%) in renal disease with SLE, SLE without clinical renal disease (43%), or renal disease treated by dialysis and/or transplantation (30%). They also found that the cryoprecipitates contain antigen-antibody complexes. In a follow-up study,[53] McPhaul and Montgomery studied cryoprecipitates from 17 patients undergoing renal biopsy and 2 patients without renal disease. Their data likewise show that cryoprecipitates may contain enhanced concentrations of circulating autoantibodies and/or tissue antigens, possibly as immune complexes. Carloss and Tavassoli[54] reported a case of acute renal failure due to precipitation of cryoglobulins in the renal vascular system (specifically type I monoclonal IgM) while the patient was in a cool operating room (20° C), emphasizing the possibility of such complications in cryoglobulinemia. Thus not only cold weather but also cold indoor environments should be avoided by patients with cold sensitivity or Raynaud's phenomenon.

Musculoskeletal disease (rheumatoid arthritis) and cryoglobulinemia

Cryoglobulin and immune complexes are found in the synovial fluid in rheumatoid arthritis and may therefore play a role in the pathogenesis of inflammation. Serum of such patients occasionally contains elevated levels of immune complexes. Weisman and Zvaifler[55] assessed the incidence and biological significance of cryoglobulins in rheumatoid arthritis, paying particular attention to optimal conditions for their detection and characterization, since in previous studies, cryoglobulins were not always seen in rheumatoid arthritis despite the high incidence of vascular and joint involvement and the presence of immune complexes. When serum samples were kept at 4° C, precipitation was minimal at 24 hours and maximal at 72 hours. Twelve out of 38 patients with rheumatoid arthritis had significant amounts of cryoglobulins (≥ 0.15 mg/mL), and 3 of these 12 patients had vasculitis (including neuropathy). This group of 12 patients was compared with 5 other patients who had vasculitis and cryoglobulinemia, 8 patients with vasculitis alone, 9 patients with rheumatoid arthritis but no vasculitis, and all 35 patients with rheumatoid arthritis but no vasculitis. The mean C3 concentration in the patients with vasculitis was significantly lower than that for the patients with rheumatoid arthritis but no vasculitis. Levels of total protein and immunoglobulin (IgG and IgM) were significantly higher in the cryoglobulins from patients with vasculitis than those from rheumatoid patients without vasculitis. There was a statistically significant relationship between IgM content and rheumatoid factor titers of vasculitis cryoglobulins. Serial studies of vasculitis patients treated with cyclophosphamide disclosed a correlation between clinical evidence of vasculitis and the presence of cryoglobulins. The presence of antigen (IgG) and antibody components (largely IgM rheumatoid factor) indicated that widespread vascular complications of rheumatoid arthritis are mediated in part by circulating immune complexes.

There is a marked association of extra-articular manifestations (vascular, visceral, and granulomatous lesions) in relation to rheumatoid arthritis. Circulating immune complexes, IgM rheumatoid factor, and complement have been implicated in the pathogenesis of some of these manifestations.[55] Erhardt et al[56] studied two measurements of circulating immune complexes in patients with severe rheumatoid arthritis—cryoglobulinemia and ^{125}I-C1q-binding activity—and also correlated them with the clinical features. Serum cryoglobulin protein concentrations ranged from 0 to 10 μg/mL in normal subjects and exceeded 10 μg/mL in 20 out of 28 patients with extra-articular disease. Serum immunoglobulin levels were higher in patients with extra-articular disease than in those with uncomplicated rheumatoid arthritis. C1q binding activity was increased in about half of these patients, particularly those with extra-articular disease. There was a positive correlation between C1q binding activity and cryoglobulin; 20 patients with extra-articular disease (71%) had cryoglobulinemia and 16 (58%) had increased C1q binding activity. The presence of cryoglobulinemia was a better index of extra-articular disease than C1q binding activity. There was a significant correlation between serum C1q binding activity and the erythrocyte sedimentation rate (ESR), but not between cryoglobulinemia and the ESR. No correlation was found between cryoglobulinemia or C1q binding activity and the articular (Ritchie) index or joint score. These results support the concept that cryoglobulins do not merely reflect levels of circulating immunoglobulins but also concentrate immune complexes.

In their study of cryoglobulinemia in connective tissue disease, Invernizzi et al[43] showed that 4 out of 15 patients with Sjögren's syndrome, 9

out of 34 with SLE, and 6 out of 48 with progressive systemic sclerosis had cryoglobulinemia. None of the 55 patients with uncomplicated rheumatoid arthritis or the 7 with Behçet's syndrome had significant cryoglobulinemia. Steere et al[57] reported serum cryoimmunoglobulins in patients with attacks of Lyme arthritis and in some patients with a characteristic skin lesion (erythema chronicum migrans) that sometimes precedes arthritis. Arthritis developed in 7 patients who had cryoimmunoglobulins concurrent with the skin lesion but not in 4 patients without cryoimmunoglobulins. These findings support the hypothesis that skin and joint lesions have a common origin and suggest that circulating immune complexes may play a role in the development of Lyme arthritis. In such cases, cryoglobulins may be associated with neurological abnormalities (cranial nerve palsy, sensory radiculopathy, or aseptic meningitis) or with abnormal myocardial conduction. Inman[58] has recently reviewed the rheumatic manifestations of hepatitis B virus infection and suggested that the virus has a direct cytopathic effect. Electron micrographs of synovial tissue revealed masses of particles measuring 20 to 25 nm in the endothelial cytoplasm.

While most reports of joint involvement and cryoglobulinemia are concerned with mixed immunoglobulins of an immune complex nature, Langlands et al[59] have reported finding crystallizable IgG-lambda cryoprotein in the synovial fluid of a patient with peripheral erosive arthritis and tenosynovitis. The same crystallizable paraprotein was found in serum incubated at 4° C but could be inhibited in vitro by D-penicillamine. Crystals were found in the synovial tissue and Bowman's corneal membrane.

Neurological disease and cryoglobulinemia

Essential or primary mixed cryoglobulinemia usually presents as polyarthralgia and purpura complicated by liver and kidney disease, with neurological manifestations only rarely being described. Peripheral neuropathy occurs in 7% to 15% of patients with cryoglobulinemia. Most patients have symmetrical subacute distal sensorimotor polyneuropathy; however, some have acute mononeuropathy. Chad et al[60] described a case of vasculitic neuropathy and essential cryoglobulinemia in which the peripheral neuropathy was completely relieved by lowering the level of cryoglobulins by plasmapheresis.

Several possible mechanisms have been proposed to explain the pathogenesis of cryoglobulinemic neuropathy, especially immunologically mediated demyelination, microcirculatory occlusion, and vasculitis involving the vasa nervorum. Several authors have shown that demyelination may occur and may be immune-mediated. Deposition of immunoglobulin on myelin sheaths has also been demonstrated. Monoclonal antibody has been shown to be directed against the myelin of the peripheral nerves. Anti-peripheral nerve myelin antibodies have been found in patients with polyneuropathy and paraproteinemia and also in persons with inflammatory polyneuritis. Electron microscopy has shown cryoglobulins within the walls and lumina of the vasa nervosum and in the endoneural space, serving as evidence of disturbance of the local microcirculation. Several authors have described vasculitis in the vasa nervorum of patients with mixed cryoglobulins. There is evidence that vasculitis can affect other tissues, with mixed cryoglobulins acting as immune complexes that activate the complement system. Pines et al[61] described a woman with mixed cryoglobulinemia where the most prominent features were involvement of the central nervous system, acute psychosis, abnormal electroencephalographic changes. They suggested that mixed cryoglobulinemia be included in the differential diagnosis of connective tissue disease involving the central nervous system.

Treatment of cryoglobulinemia

Treatment of cryoglobulinemia is generally directed toward minimizing the signs and symptoms of the primary disease. In patients with Raynaud's phenomenon, treatment is aimed at minimizing cold exposure. Periactin is useful when urticaria occurs, as this is caused by histamine release mediated by cryoprecipitate formation. Treatment of leg ulcers is aimed at preventing secondary bacterial infection, while dependent purpura can be minimized by avoiding prolonged standing. Patients with joint lesions can be treated with aspirin or other anti-inflammatory agents. Methods aimed at reducing the production or circulating concentration of cryoglobulins include drug therapy and plasma treatment or replacement. Gorevic et al[62] followed up 40 patients with mixed cryoglobulinemia for up to 21 years (1960–1978). Many medications were used, including nonsteroidal anti-inflammatory drugs, antihistamines, cryoproheptadine, dex-

tran (for vasculitic ulcers), corticosteroids, penicillamine, and immunosuppressive therapy. In uncontrolled studies, it was observed that severely ill patients did relatively better on steroids and/or immunosuppressive therapy, but that remission (if achieved) was short and side effects often necessitated discontinuation of the drug. Geltner et al[63] studied the effect of combined steroid and immunosuppresive therapy and plasmapheresis. All 5 patients with mixed cryoglobulinemia who had renal, neurological, and vascular involvement showed improvement both clinically and on laboratory testing. The mean cryocrit decreased from 12% to 2%. All 4 patients with renal disease exhibited improvement, and 2 patients with vasculitic ulcers on the lower extremities had complete healing. Complement levels showed no significant change, and no improvement was seen in the neuropathic motor defect. The mean period of treatment until improvement was three months. Combined treatment produced rapid remission of the major clinical manifestations which lasted after therapy was discontinued.

The value of plasmapheresis alone or combined with drug therapy in immune complex diseases such as mixed cryoglobulinemia is a subject of intense investigation. Ginder et al[34] demonstrated the value of plasmapheresis in the treatment of mixed cryoglobulinemia, and stressed the importance of immunosuppressive drug therapy to avoid a relapse of humoral immune response. Abnormal immune complex handling has been attributed to overload of the reticuloendothelial system or intrinsic dysfunction. Lockwood et al[64] have shown that plasmapheresis enhances clearance of immune complexes by the spleen in patients with nephritis and/or vasculitis, resulting in unexpectedly marked and often prolonged improvement even after plasmapheresis is stopped. Berkman and Orlin[65] studied the use of plasma exchange in 5 patients with cryoglobulinemia (3 mixed, 1 monoclonal IgM, and 1 monoclonal IgG). In each case, symptoms improved markedly and cryoglobulin levels decreased. Circulating levels of mixed and monoclonal IgM cryoglobulins were reduced more easily than those of IgG cryoproteins, which was believed to be related to their removal from the intravascular pool with little or no equilibration with the extravascular pool. Procedures were carried out at room temperature, with reinfusion through a blood warmer. Only in one case did plasma form an unmistakable cryoprecipitate on exposure to room air for 15 minutes. Meltzer et al[8,9] demonstrated a direct relationship between cryoprotein concentration and the temperature at which precipitation occurs, which increases along with protein concentration. Therefore, it would appear desirable to ensure that the environment is controlled during the procedure.

L'Abbate et al[66] carried out selective removal of plasma cryoglobulins in 2 patients with mixed cryoglobulinemia associated with severe membranoproliferative glomerulonephritis and angiitis. Plasma was separated with a continuous flow centrifuge, collected in chilled plastic bags, and centrifuged at 0° C for 15 minutes at 3,000×g. The cryoprecipitate was discarded and the supernatant reinfused after rewarming. The cryocrit decreased by 74%, cryo-IgM by 50%, cryo-IgG by 50%, and cryo-rheumatoid activity by 67%. Serum levels of C3 and C1q fell by 20% and 7%, respectively, while fibrinogen decreased by 30%. Both patients showed marked clinical improvement. Unlike plasma exchange, depletion was more specific and there was no need for foreign plasma products. McLeod and Sassetti[67] employed a variant of this techique in the treatment of cryoglobulinemia. In the first exchange, plasma was replaced with 5% albumin; in subsequent exchanges, fluid was replaced by autologous plasma from which the cryoglobulin had been removed. The plasma which contained cryoglobulin was refrigerated more than 48 hours to promote cryoprecipitation, after which it was centrifuged and the supernatant plasma separated and used as the exchange fluid in the next treatment. Three patients were treated in this way, and all responded with reduced cryoglobulin level and an improved clinical picture.

Malchesky et al[68] reported on the on-line technique of membrane plasma separation and cryofiltration, i.e., removal of macromolecules by cooling the plasma to a selected temperature no lower than its freezing point and then filtering it through a porous membrane. Heparinized plasma was separated from whole blood and cooled to near-freezing, thereby promoting the formation of cryogel or cryoprecipitates which were in turn removed by filtration through a second membrane. Unlike the other techniques, precipitation of macromolecules was not required. In this technique, the material separated from the plasma and retained by the filter is referred to as cryogel; this is quite different from

cryoglobulin or cryoprotein as defined previously. Cryogel is formed from plasma following rapid cooling (generally less than 20 minutes) and filtration and may contain other solutes in addition to cryoprotein. Cryofiltration allows removal of many pathological macromolecules and the return of albumin, so that there is no need for replacement fluid when the procedure is performed no more than twice a week. In studies of rheumatoid arthritis, the cryogel concentrated immune complexes relative to albumin.[69] While this has been the major application of cryofiltration to date, other diseases have also been treated in such a fashion, including cryoglobulinemia, rheumatoid vasculitis, Sjögren's syndrome, nephrotic syndrome, sclerosing cholangitis, cold-induced hemolytic anemia, chronic alcoholic liver disease, SLE, and peripheral polyneuropathy. Abe et al[70] employed cryofiltration to treat 1 patient with Sjögren's syndrome and 3 with essential cryoglobulinemia and noted significant clinical improvement as well as reduced cryoglobulin levels in all cases.

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