Biologic and Clinical Significance of Cryoglobulins

A Report of 86 Cases

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Eighty-six patients with cryoglobulinemia repeatedly underwent complete immunochemical and clinical evaluation during the course of their disease. Immunochemical analysis of the purified cryoglobulins allowed us to classify them into three groups. Type I cryoglobulins are made of isolated monoclonal immunoglobulin: IgM (11 cases), IgG (7 cases), IgA (2 cases) or Bence Jones protein (1 case). Type II cryoglobulins are mixed cryoglobulins with a monoclonal component possessing antibody activity towards polyclonal IgG. These cryoglobulins were mainly IgM-IgG (19 cases), sometimes IgG-IgG (2 cases) or IgA-IgG (1 case). Type III cryoglobulins (43 cases) are mixed polyclonal cryoglobulins, i.e., composed of one or more classes of polyclonal immunoglobulins and sometimes nonimmunoglobulin molecules such as beta1C or lipoprotein. Most of these type III cryoglobulins are also immunoglobulin-anti-immunoglobulin immune complexes. This classification enabled us to establish correlations between the biologic findings and the clinical features as well as the underlying diseases.

Cutaneous and vasomotor symptoms were most severe in patients with type I and II cryoglobulins. The usual clinical picture in patients with type II or III cryoglobulins consisted of chronic vascular purpura and mild Raynaud's phenomenon. Renal and neurologic involvement were more frequent in patients with type II and III cryoglobulins, and were of major prognostic significance. In our series, immunoproliferative and autoimmune disorders were the most frequent diseases associated with cryoglobulinemia. The former were associated with type I or II cryoglobulins and the latter mainly with type III cryoglobulins. Of note is that idiopathic cryoglobulinemia accounted for nearly 30 per cent of the cases despite repeated careful clinical evaluation and a mean follow up of 9 years.

In 10 per cent of the cases, acute and severe symptoms necessitated emergency treatment with plasmapheresis and chemotherapy which allowed a satisfactory initial remission in all but one patient. Conversely, no treatment was definitively effective in patients with chronic symptoms such as vascular purpura.
Most proteins involved in cryoprecipitation are immunoglobulins. Cryoglobulins with a single monoclonal immunoglobulin were recognized first, the most frequent being immunoglobulin G (IgG) or M (IgM). Following LoSpalluto's report [1], cryoglobulins composed of two or more immunoglobulins belonging to different classes were described and called mixed cryoglobulins, most of which are in fact immune complexes [2-4]. IgM-IgG cryoglobulins are the most frequent type of mixed cryoglobulins, and the IgM antibody can be either monoclonal or polyclonal [4,5], whereas the antigen is polyclonal IgG. Monoclonal cryoglobulins with or without antibody activity are found mostly in immunoproliferative diseases. Mixed cryoglobulins are frequent in autoimmune disorders. A number of microbial, viral or parasitic infections are often associated with asymptomatic and transient cryoglobulins.

We describe 86 patients with cryoglobulinemia who were studied and followed in a department of hematology and immunopathology. Our chief aim was to establish in individual patients possible correlations between the immunochemical characteristics of the cryoglobulin and the clinical symptoms or associated diseases.

IMMUNOCHEMICAL STUDIES

Isolation and Purification of the Cryoglobulin. Blood was obtained from the fasting patient in a room kept at a temperature of 37°C, and the blood, collected without anticoagulant, was allowed to clot at 37°C. The serum, after centrifugation at 37°C, was transferred into narrow tubes which allowed easy detection of minute amounts of cryoprecipitate. The serum samples were checked for cryoglobulin until the 8th day. The cryoprecipitate was purified by centrifugations and washings at 4°C, the two first being followed by redissolution of the cryoprecipitate at 37°C. The cryoglobulin level was evaluated by measuring ultraviolet absorption at 280 µm of an acid dissolved aliquot of the cryoprecipitate. When the amount of cryoglobulin was appreciable, the cryoglobulin level was evaluated by centrifugation of the serum in a hematocrit tube at +4°C or by comparing the serum protein level before and after cryoprecipitation. The initial temperature of cryoprecipitation was defined as the higher temperature at which the serum became opalescent. This parameter showed great variability (+10°C to +36°C) from patient to patient.

Immunoechemical Analysis. The purified cryoprecipitate was characterized by electrophoresis (at a concentration of 8 to 15 mg/ml) and by immunoelectrophoretic study of the purified cryoglobulin against antisem to whole human serum and monospecific antisem to gamma, alpha and mu chains (Figure 1). The monoclonal or polyclonal nature of the immunoglobulin(s) found in the cryoprecipitate was mainly assessed by typing of light chains. In mixed cryoglobulins, the depolymerization by thiol reagents often enabled the immunoelectrophoretic determination of the light chain type(s) of the IgM component. However, when the amount of cryoglobulin was low, purification of its immunoglobulin components by preparative ultracentrifugation in a sucrose density gradient at acid pH was necessary prior to light chain typing by double diffusion analysis in gel (Figure 2).

Following this analytical study, we classified the cryoglobulins into three categories: (1) monoclonal cryoglobulins made of immunoglobulins with only one class or subclass of heavy and/or light chain; (2) mixed cryoglobulins with a monoclonal component made of immunoglobulins belonging to two different classes, one of which is monoclonal (we have separated this category of cryoglobulins because of the outstanding significance of the presence of a monoclonal immunoglobulin within a mixed cryoglobulin); and (3) polyclonal mixed cryoglobulins which are made of heterogeneous immunoglobulin molecules belonging usually to two or more different classes and sometimes of additional serum proteins. This classification is arbitrary, but it has been found useful for the study of clinicobiologic correlations in patients.

Monoclonal Cryoglobulins (Type I Cryoglobulins). As shown in Table I, 25 per cent of the 86 cryoglobulins were purely monoclonal. The IgM cryoglobulins

![Figure 1. Immunoelectrophoretic pattern of various types of cryoglobulins. 1, monoclonal IgG. 2, monoclonal IgM. 3, mixed IgM-IgG cryoglobulin with monoclonal IgM. 4, mixed polyclonal IgM-IgA-IgG cryoglobulin. A = polyvalent horse antiserum to normal human serum previously absorbed by IgG globulins. B = rabbit antiserum monospecific for gamma heavy chains.](image)
are the most frequent within this group. We have found two IgA kappa and one Bence Jones lambda cryoglobulins. The serum level of these monoclonal cryoglobulins is usually high (1 to 30 mg/ml), and the immunoglobulin readily precipitates in the cold. Usually, a flocculent precipitate is observed; in other instances, such as our two IgA cryoglobulins, a gelatinous precipitate occurred. A single cryoglobulin (IgG2 kappa) gave a crystalline precipitate. Six IgG cryoglobulins belonged to the IgG1 subgroup, four to the IgG2 and one to the IgG3. Thus the frequency of IgG2 class is unusually high as in another report [6]. The relative frequency of kappa or lambda light chain types of IgG or IgM cryoglobulins was similar to that observed among noncold precipitable monoclonal immunoglobulin.

The cryoprecipitability of the monoclonal immunoglobulin was affected by various physicochemical parameters. Increasing the concentration of the purified cryoglobulin resulted in an increase of the temperature at which precipitation occurred. Acid and alkaline pH, high ionic strength and low concentrations of urea (0.5 M), guanidine hydrochloride (0.2 M) or sodium dodecyl sulphate (0.1 per cent) all resulted in a decrease in the amount of cold precipitate. These results and those from previous reports [7–9] strongly suggest that weak electrostatic and hydrophobic forces occur during cold-induced denaturation.

Our results pointed to the major importance of the over-all structure of the immunoglobulin molecule for cold precipitation to occur. Fab, F(ab')2 and Fc fragments as well as gamma chains from a IgG1 kappa cryoglobulin did not precipitate in the cold. However Saha et al. [10] observed a slight cryoprecipitation of isolated gamma chains and of the F(ab')2 fragment from an IgG cryoglobulin. Dimerization of Fab

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**Figure 2. Identification of the heavy and light chains of the two components of a mixed IgM lambda-IgG cryoglobulin.**

Upper, 19S and 7S fractions separated by preparative ultracentrifugation in a sucrose gradient. Lower, immunodiffusion studies of the 19S and 7S fractions with antisera against mu chains (A); gamma chains (B); kappa chains (C); lambda chains (D).

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**Table 1** Immunochcmical Classification and Serum Levels of the 86 Cryoglobulins

<table>
<thead>
<tr>
<th>Immunochcmical Type of the Cryoglobulin</th>
<th>Frequency</th>
<th>Cryoglobulin Level</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;1 mg/ml</td>
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<tr>
<td>Type I, monoclonal cryoglobulin</td>
<td>24.5%</td>
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<tr>
<td>IgM</td>
<td>13%</td>
<td>10%</td>
</tr>
<tr>
<td>IgG</td>
<td>8%</td>
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<tr>
<td>IgA</td>
<td>2.5%</td>
<td></td>
</tr>
<tr>
<td>Bence Jones</td>
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<td>IgM-IgG</td>
<td>22%</td>
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</tr>
<tr>
<td>IgG-IgG</td>
<td>2.5%</td>
<td></td>
</tr>
<tr>
<td>IgA-IgG</td>
<td>1%</td>
<td></td>
</tr>
<tr>
<td>Type II, mixed cryoglobulin with a monoclonal component</td>
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<td></td>
</tr>
<tr>
<td>IgM-IgG</td>
<td>43%</td>
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<tr>
<td>IgM-IgG-IgA</td>
<td>7%</td>
<td></td>
</tr>
<tr>
<td>Type III, mixed polyclonal cryoglobulin</td>
<td>50%</td>
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or F(ab′)2 fragments of two IgG cryoproteins was shown to occur in ultracentrifugal studies [10,11]. Reduced and alkylated IgM subunits and mu heavy chains obtained from eight of our cryomacroglobulins were not able to precipitate in the cold. In a single report the reduction to monomeric subunits did not abolish cold precipitability [9]. Hybrid IgM molecules made of subunits of cryo- and noncryomacroglobulins still retained some degree of cryoprecipitability. The Bence Jones lambda cryoprotein was a mixture of a noncovalent dimer (80 per cent) and of a native monomer (20 per cent). The isolated covalent dimer precipitated at +4°C above a molar concentration of 4.10^{-5} M. The isolated monomer or the reduced alkylated covalent dimer was unable to cryoprecipitate at 4.10^{-4} M although ultracentrifugal analysis at 37°C showed that it was in the form of a noncovalent dimer, demonstrating the importance of the interchain disulfide bridge in the cryophenomenon. Amino acid analysis revealed a high content of tyrosine residues (10 per monomer). Difference spectrum studies showed that tryptophan exposure occurred when the temperature decreased. Circular dichroism analysis showed that the secondary ordered beta structure was unaffected by temperature decrease and that only the near ultraviolet spectrum was modified.

In accordance with previous data [12], the amino acid composition of light and heavy chains of an IgG1 kappa cryoprotein was similar to that of a noncryo-IgG1 molecule. Carbohydrate analysis revealed a striking lack of sialic acid. However the carbohydrate composition of several other cryoglobulins showed no remarkable abnormality. Cryoglobulins cannot be presently considered as abnormal molecules with a unique size, shape or charge characteristics. Moreover it is noteworthy that one monoclonal IgG1 kappa cryoglobulin of our series exhibited an antistreptolysin antibody activity [13]. X-ray studies of crystalline immunoglobulin may provide a suitable model for a better understanding of the cryoprecipitation phenomenon [14].

Mixed Cryoglobulins with Monoclonal Component (Type II Cryoglobulins). These cryoglobulins are made of two immunoglobulin components, one of which is monoclonal. This variety accounts for 26 per cent of our cryoglobulins and IgM-IgG cryoglobulins are by far the most frequent within this group (Table I). The serum level of these cryoglobulins is usually high. However the serum level of the monoclonal component is sometimes too low to give a characteristic spike, or the interaction between the two components of the cryoglobulin can mask the electrophoretic homogeneity of the monoclonal protein.

IgM represents the monoclonal component of the mixed IgM-IgG cryoglobulins. Isolation of the monoclonal IgM was performed by gel filtration in pH 4.5, 0.2 M acetate buffer. The two separate components isolated from the cryoprecipitate were each soluble in the cold. The cryoprecipitability was restored by the addition of purified IgG or of IgG Fc fragments to the monoclonal IgM but not by the addition of unrelated IgM to the monoclonal IgG of the cryoprecipitate. F(ab′)2 fragments of the IgG were able to combine with IgG. Metzger and Stone provided most revealing data upon the thermodynamic properties of the reaction between such monoclonal IgM and IgG [2,3,15]. The IgM has an homogeneous binding constant (6.9 10^4 M^{-1}). Each of the 10 Fab fragments of IgM combines with a single antigenic determinant of human IgG although the pentamer molecule binds only five IgG molecules. The solubility characteristics of the small IgM-IgG complexes are determined by the salt concentration, the pH or temperature of the environment, but these parameters do not modify the equilibrium constant between the monoclonal IgM and IgG.

In our series, the monoclonal IgM had kappa light chains except in two cases in which lambda chains were found. This is of interest since almost all reported monoclonal IgM with anti-IgG activity had kappa light chains. This situation is most probably relevant to the antibody combining site specificity. In two cases, a mixed IgM kappa-IgG cryoglobulin showed an anti-I antibody-like activity. Further studies of these peculiar cryoglobulins are obviously needed.

In two cases IgG-IgG cryoglobulins were found. Ouchterlony double diffusion experiments demonstrated that these two purified cryoprecipitates each contained two IgG subclasses (respectively, IgG3-IgG1 and IgG3-IgG2) and both types of light chains. In one of these cases, no monoclonal spike was apparent on electrophoresis of the serum or even of the isolated cryoglobulin. The immuno-electrophoretic study disclosed the presence of both kappa and lambda molecules in the IgG bow. However myeloma plasma cells of this patient synthesized in vitro only IgG3 molecules with lambda light chains. IgG3 is the prevalent subclass among the monoclonal IgG antibodies with an anti-IgG activity [16]. Complex formation between Fab or F(ab′)2 fragments of monoclonal IgG rheumatoid factors and normal IgG or their Fc fragments was first shown by Grey et al. [16] in ultracentrifugal experiments. The binding constant is weak (3 10^4 M^{-1}) [17]. The detection of these mixed IgG-IgG cryoglobulins is sometimes very difficult since the monoclonal immunoglobulin may account for almost all the protein in the cryoprecipitate and F I latex test is seldom positive. Since we have not performed systematically such ultracentrifugal experiments, we may have underestimated the real frequency of these mixed IgG cryoglobulins in our series.

The monoclonal component of the IgA-IgG cryo-
globulin was an IgA1 kappa protein which gave a highly viscous cryogel with polyclonal IgG at 35°C. Such monoclonal IgA with anti-lgG antibody activity have seldom been reported [18,19], and the thermodynamic parameters of the reaction have not been thoroughly worked out.

A peculiar pattern of specificity for these IgM, IgG or IgA monoclonal rheumatoid factors has not yet emerged. The monoclonal IgM of our mixed IgM-lgG cryoglobulins often reacted only with some subclasses of aggregated monoclonal IgG. Such restricted specificity has been described in several laboratories [16,20,21]. A monoclonal IgM was shown to cryoprecipitate with IgG molecules which were devoid of sialic acid [22]. More than two-thirds of monoclonal rheumatoid factors react only with primate IgG, and the remainder react both with human and rabbit IgG [23,24]. Cross idiotopic specificities between various monoclonal IgM rheumatoid factors were demonstrated in two reports [25,26]. Similar amino acid sequences in the hypervariable regions of the heavy or light chains may account for some of these cross reactions [26].

Mixed Polyclonal Cryoglobulins (Type III Cryoglobulins). This group accounts for half of our cryoglobulins. IgM-lgG cryoglobulins were the most frequent. The level of these cryoglobulins was usually very low (0.1 to 1 mg/ml) (Table 1). No monoclonal immunoglobulin could be detected by electrophoretic or immunoelectrophoretic studies of the purified cryoglobulin. In only two-thirds of these cases was the amount of cryoglobulin sufficient to allow further studies. Both kappa and lambda chains were found in the various immunoglobulin components of the cryoprecipitate isolated by preparative ultracentrifugation in acidic medium. Rheumatoid activity, as shown by positive F II latex test, was usually demonstrated either at 37°C with the isolated cryoglobulin or at +4°C with its purified IgM component. Waaler Rose sheep red blood cell reaction was occasionally positive in low titer. Chavin and Franklin [27] demonstrated the anti-lgG antibody activity of the polyclonal IgM in such a mixed cryoglobulin. Although IgA-containing cryoglobulins are not unusual in Wager et al.’s experience [28], we have in several cases detected only minute amounts of IgA by double diffusion analysis of the dissolved precipitate at high concentration. However, in four mixed polyclonal IgM-lgG-lgA cryoglobulins, each of the three major classes of immunoglobulin was present in appreciable amounts (Figure 1), and the F II latex test was positive. Two polyclonal IgM cryoprecipitates were described in patients with trypanosomiasis [5]. Various components of the complement system may be found in polyclonal IgG or IgM-lgG cryoprecipitates [29–31]. In some such cases, C1q presumably plays a major role in the cryoprecipitability since IgG and C1q isolated from the IgM-lgG-C1q cryoglobulins studied by Stastny and Ziff [29] precipitated in the cold when mixed in the absence of IgM. Cryoglobulins made of beta lipoprotein and monoclonal IgM were described [32]. The true immunologic nature of this interaction was not demonstrated. The presence of small amounts of deoxyribonucleic acid (DNA) in some cryoprecipitates has been reported [33], but the significance of this finding is still uncertain.

Apart from rheumatoid factor activity, antibody activity at high titers was very seldom reported in isolated polyclonal cryoglobulins. Antinuclear activity disappeared after cryoprecipitation in the serum of patients with infectious mononucleosis [34]. We found such a decrease in antinuclear factor activity in only 1 of 10 cryoprecipitating sera with very high levels of antinuclear factors. However nuclear antigens were not found in this cryoprecipitate. In other studies, antibacterial, antiviral, anticardiolipid, antinuclear or red cell antibody activities found in the serum were not significantly decreased in the supernatant after precipitation in the cold, and the corresponding antibody activities were usually not concentrated in the cryoprecipitate [29,35–37].

One can visualize the significance of these polyclonal cryoglobulins in at least two ways: (1) the cryoglobulin may be made of different classes of antibodies directed against a same unidentified antigen; and (2) alternatively the cryoglobulin could be due to the interaction of polyclonal rheumatoid factors with IgG molecules bound to an unknown antigen. The second hypothesis seems more appealing; it could account for most of the findings in human subjects and is in accordance with recent experimental data [38].

CLINICAL STUDY

Full clinical investigation included, if not contraindicated, extensive hematologic study with lymphangiogram and bone marrow biopsy; hepatic, renal and neurologic evaluation; and search for clinical or biologic autoimmune disorders. Cutaneous, muscular and renal biopsies were performed if clinically indicated. These studies were repeated in many patients during the follow up.

Presenting Symptoms. In two thirds of our patients, skin lesions or vasomotor attacks were the major presenting symptoms. Cold sensitivity was apparent in less than half of these patients. In rare instances, the initial symptoms consisted of renal failure, mucosal bleeding, visual disturbance or abdominal pains. In four cases, the diagnosis of cryoglobulinemia was suggested by unusual routine laboratory findings such as an increased sedimentation rate at 37°C contrasting with a normal rate at room temperature, rouleaux formation and false results in blood counts, especially in electronic procedures. In 25 per cent of the cases,
the cryoglobulin was detected through a systematic survey for cryoproteins. More than half of those with high level type I cryoglobulins and only 15 per cent of those with mixed cryoglobulins were asymptomatic.

**Cutaneous and Vasomotor Symptoms.** Vascular purpura was the most frequent symptom, usually beginning in the lower limbs. In 25 per cent of the cases, the purpura gradually extended to the thighs and the lower part of the abdomen, or to the buttocks. In a few cases, the arms were involved. The face and the trunk were always spared. Palatal or gastric purpura was found in two cases. Petechia and/or papula were the most common lesions (Figure 3, left). Necrotic purpura was found in four patients. Bullous or vesicular lesions were infrequent. Ecchymoses, erythematous spots and dermal nodules were associated with the purpura rashes in 20 patients, and in 10 patients the clinical picture was that of the so-called Gougerot trisymptom. Skin biopsy was performed in 30 patients. The usual picture was an acute angitis characterized by fibrinoid necrosis of the vascular walls and perivascular infiltration of pyknotic polymorphonuclear leukocytes. The vessels of the upper dermis were most often affected. In addition, intravascular hyalin deposits corresponding most likely to the cryoprotein were found in four cases.

Cold enhanced the purpura rashes in only 30 per cent of the cases. On the other hand, crops of purpura were often triggered by standing or sustained efforts. In eight cases, the purpura was eventually triggered by drugs (penicillin, acetyl salicylic acid, vaccins) or upper respiratory tract infections. Most patients had successive purpuric rashes, usually once or twice a month, lasting for 1 or 2 weeks and leading, after some years, to brownish pigmentation of the skin. The rashes were often preceded by itching or a burning sensation localized to the purpuric areas. Painful swelling of the ankles was frequently observed. In two patients, a single rash occurred although the cryoglobulin did not disappear. In two other patients with infrequent purpura for many years, sudden and repeated rashes were followed by the onset of a polyneuropathy or a glomerulonephritis.

Skin necrosis and/or supramalleolar ulcers were found in 5 per cent of our cases. Necrosis affected the tip of the nose, the ears, fingers, toes or legs. In all instances the limited area of skin necrosis appeared after exposure to severe cold.

Urticaria and livedo were constantly induced by the cold and usually associated with vascular purpura. In one patient with IgM kappa cryoglobulin the skin lesions were most unusual. Orange skin infiltration was present on the hands, arms, face, scalp and nose. Involvement of the nails was also observed (Figure 3, right). Ultrastructural studies showed widespread proteinaceous deposits inside and outside the vessels of the dermis which stained by anti-mu and anti-kappa antiseraums on immunofluorescence.

**Mild or severe Raynaud's phenomenon** was the presenting symptom in 15 per cent of our cases and was most often associated with vascular purpura. In 25 per cent of the patients these symptoms were very severe. The syncopal attacks often worsened with time, and necrosis of the fingertips was observed in three cases. Cyanosis or erythrocyanosis induced by cold was found in 9 per cent of our cases. In some patients acrocyanosis was associated with circumscribed painless white or purple areas dissem-

![Figure 3. Left, typical cutaneous lesions in a patient with type III cryoglobulin: extensive vascular purpura with livedo and leg ulcer. Right, orange plaques on hand and forearm (which were shown to be caused by vascular and extravascular deposition of IgM kappa cryoglobulin) and nail lesions in a patient with IgM cryoglobulin.](image)
inated all over the body occurring dramatically, for instance, during sea baths.

As shown in Table II, vascular purpura or mild vasomotor symptoms were often found in patients with mixed cryoglobulins (type II or III). On the other hand, skin necrosis, necrotic purpura and severe Raynaud’s phenomenon were hallmarks of patients with type I or II cryoglobulins. In patients with mixed cryoglobulin, cutaneous or vasomotor symptoms usually ran a chronic course with no dramatic changes in the clinical symptoms over the years. Conversely, in those with monoclonal cryoglobulins, mild cutaneous symptoms may precede for years major vascular or visceral injuries.

These findings are in keeping with previous reports, although correlations between clinical and immunochemical data have rarely been worked out [39–44]. Meltzer et al. [4] first stressed the importance of purpura as well as arthralgias in the clinical pattern of mixed cryoglobulins. Immunofluorescent studies in patients with acute vasculitis showed the deposition of IgG, IgM and beta 2C in the vessel walls [42,45]. A monoclonal or mixed cryoglobulin is found in about 20 per cent of the patients with cold urticaria [46]. In a case reported by Costanzi and Coltman [47], the monoclonal IgG cryoglobulin presumably interacted with C1 esterase. Urticaria may be transferred passively to normal recipients with isolated cryoglobulin [47]. Distal necrosis, extensive gangrene and recurrent necrosis of the fingers, leading to successive amputations, have been observed in patients with type I cryoglobulins [48–51]. Widespread intravascular deposition of proteinaceous material has been noted in these patients [52–54].

Renal Symptoms. Glomerular injury was found in 21 per cent of our patients, disregarding those with systemic lupus erythematosus. Anuria and glomerulonephritis were the presenting symptoms in only two patients. In eight patients, renal damage was found during the first evaluation. In the other eight patients, clinical or laboratory evidence of glomerulonephritis developed 6 months to 10 years after the initial evaluation. Subacute or chronic glomerulonephritis was the usual clinical pattern (14 patients).

A renal biopsy specimen was obtained from 17 patients. Diffuse glomerulonephritis with cell proliferation and endomembranous deposits was found in eight patients, with scattered polymorphonuclear neutrophils and/or acute vasculitis of small vessels in some. Endomembranous deposits were usually moderate and discrete (sometimes with wire loop aspects), whereas in three patients the deposits almost occluded the capillary lumen (Figure 4). In two instances, ultrastructural studies showed intermembranousendothelial localization of the deposits. In one case, crystalline inclusions were found in endothelial cells. Four other patients (including the two with sudden anuria) showed large endomembranous deposits without any cell proliferation. Focal glomerulitis with endomembranous or subepithelial deposits was found in four patients. In the last case, the picture was that of a membranous glomerulonephritis. In 14 cases, immunofluorescent studies of the biopsy specimens were performed and showed positive staining with anti-Ig and anti-beta 2C antisera with granular deposition along the basement membrane. The immunoglobulin class(es) present in these glomerular deposits were identical to those found in the cryoglobulin.

In five patients, renal failure was the major cause of the death. Four patients, still living, have had chronic renal insufficiency for 2 to 12 years.

Meltzer et al. [4] stressed the frequency of glomerulonephritis in patients with mixed cryoglobulin and showed the occurrence of voluminous endothelial deposits and cell proliferation. Subsequent reports confirmed these findings [55–60]. Isolated deposits nearly occluding the capillary lumen were reported in a few patients with monoclonal cryoglobulins [52,60,61]. The present data show that the incidence of renal injury is highest in those with type II cryoglobulins (Table II), but no clear-cut correlation emerged from the comparison between the immunochimical type of the cryoglobulin and the histologic pattern. Diffuse glomerulonephritis with endomembranous deposits was observed in patients with all three types whereas isolated massive deposits were found only in those with type I or II, i.e., with monoclonal components. The biopsy specimen disclosed no histologic abnormalities in three additional patients with type III mixed polyclonal cryoglobulins and normal urinary findings. Conversely, voluminous endomembranous deposits were found in two patients.

### TABLE II Incidence of Symptoms Observed in 86 Patients

<table>
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<tr>
<th>Symptoms</th>
<th>General Incidence (%)</th>
<th>Incidence (%) According to Cryoglobulin Type</th>
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<tr>
<td></td>
<td>II</td>
<td>III</td>
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<tr>
<td>Cutaneous symptoms</td>
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<tr>
<td>Vascular purpura</td>
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<td>15</td>
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<td>Distal necrosis</td>
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<td>Urticaria</td>
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<td>Livedo</td>
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<tr>
<td>Leg ulcers</td>
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<td>Raynaud’s phenomenon</td>
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**November 1974** The American Journal of Medicine Volume 57 781
with severe cold-induced symptoms and high levels of type I or II cryoglobulin despite normal laboratory tests.

Neurologic Symptoms. Neurologic involvement was found in 17 per cent of our patients and was more frequent in those with type III cryoglobulinemia than in those with type I or II cryoglobulinemia (Table II). In nine patients, paresthesia and numbness were the main features. The motor defect appeared some months or years later, and progressed in a distal and symmetrical fashion. It was ascertained in each case by electromyogram and neuromuscular biopsy. The latter was of no help in elucidating the mechanism of the neurologic injury. The sensory motor neuropathy usually had a protracted course, and the neurologic defect stabilized 2 to 4 years after its onset. However, in two patients, the neurologic symptoms disappeared after several years of follow-up. In four other patients, the neurologic symptoms appeared abruptly and progressed in an asymmetric course, and in three of these, muscle biopsy showed a picture of periarteritis nodosa. Central neurologic involvement was found in only two patients: one with cryomacroglobulinemia had a transient hemiplegia and the other with type III cryoglobulinemia had a temporary blindness.

Logothetis et al. [98] reviewed the neurologic involvement in patients with cryoglobulinemia. Although they reported an incidence of only 7 per cent, the over-all clinical presentation agreed with our findings and those of others [4,62]. Central neurologic involvement was seldom reported [4,48,63]. Cerebral hyaline thrombi and purpura were found in a patient with myeloma [63]. The distinctive features of cryoglobulinemic neuropathy are difficult to delineate. Whether the cryoglobulin of the underlying illness is responsible for the neurologic symptoms was debatable in six of our patients who were affected with periarteritis nodosa, multiple myeloma or Waldenström's macroglobulinemia.

Miscellaneous. Painful abdominal crisis was observed in three patients, one without satisfactory explanation, one with hematemesis due to gastric purpura and one with intestinal infarction due to mesenteric arterial occlusion by hyaline thrombi.

Hemorrhage were observed only in six patients with multiple myeloma or macroglobulinemia. This is in keeping with other reports [52,53,64], and it is difficult to decide if the cryophenomenon plays a crucial role in the bleeding tendency. However in two patients, the Ivy test was markedly abnormal at 20°C and returned to normal values at 37°C. In one of these, the level of factor V was less than 5 per cent at 20°C and was normal at 37°C.

Granular sludge in conjunctival and retinal vessels was very frequent, mainly in those with monoclonal cryoglobulins. Retinal vein thrombosis was found in the first reported case of cryoglobulinemia [64].

Comments: Two main mechanisms may be responsible for the clinical findings we describe in patients with cryoglobulinemia.

Intravascular deposits of cryoglobulin were first suggested on clinical (cold-induced symptoms of vascular insufficiency) as well as histologic (occlusion of various sized vessels) grounds. It was assumed that cryoprecipitation occurred in vivo or that local hyperviscosity enhanced sludging in small vessels and/or major vascular insufficiency. However similar intravascular deposits may be demonstrated in visceral vessels which are protected from thermic variations. Attention should thus be paid to abnormalities in the local microcirculation which are likely to occur in the kidney since protein concentration and hematocrit are increased at the end of the capillary loops, lead-
The demonstration of the immune complex nature of most mixed cryoglobulins led to postulate that such complexes may be responsible of in vivo acute vasculitis. This hypothesis is heightened by several facts: (1) histologic similarities between the cutaneous or renal lesions found in patients with mixed cryoglobulinemia and in those with acute serum sickness; (2) immunofluorescence demonstration of specific deposition in the vascular walls of the immunoglobulin found in the cryoprecipitate; (3) detection of circulating immune complexes at 37°C by ultracentrifugal studies of some sera with mixed cryoglobulins [4,5,6,57,65,66]; and (4) decrease of the serum complement level at the onset of visceral injuries in mixed cryoglobulinemia. However, we found low total complement level in monoclonal as well as in mixed cryoglobulins and were unable to establish correlations between complement levels and renal lesions. In vitro experiments showed anticomplementary activity of isolated cryoglobulins [67,68]. Rother et al. [69] demonstrated a two stage complement activation: complement was bound to the cryoglobulin at +4°C but the reaction went to completion only at +37°C.

It is clear from Table II that the symptoms related to the mechanical vascular insufficiency are mainly found in patients with type I or II cryoglobulins, the levels of which are usually high. On the other hand, acute cutaneous vasculitis and glomerulonephritis are most frequent in patients with type II and III mixed cryoglobulins. Patients with type I cryoglobulins may exhibit features of both varieties of injuries, i.e., vasculitis in the skin and glomerular obstruction without cell proliferation in the kidney. However, exceptions to these schematic rules were found: some patients with minute amounts of type III cryoglobulins had prominent symptoms of vascular insufficiency; two patients with monoclonal IgG cryoglobulins exhibited an immune complex type of nephritis with granular deposition of beta 2 C and IgG endomembranous thrombi, although the possibility of mixed IgG-IgG cryoglobulin had been ruled out. The pathogenetic importance of cryoglobulins is sometimes difficult to assess since some signs and symptoms, usually considered as directly related to the cryoglobulin, may be due to the associated diseases or may be found in patients with noncryoprecipitating monoclonal immunoglobulin or rheumatoid factors.

ASSOCIATED DISEASES (TABLE III)

Hematologic Diseases. Multiple myeloma: Six per cent of the myeloma proteins studied in our laboratory were cryoglobulins. Eight patients in this series had multiple myeloma. Four had symptoms directly related to the cryoglobulin. Extreme cold sensitivity with severe vasomotor symptoms and/or cutaneous necrotic lesions were constant findings in these patients. One patient with mixed IgG-IgG cryoglobulin also exhibited extensive vascular purpura with acute vasculitis. Polyneuropathy was a dramatic event in another patient in whom transient clinical improvement was obtained twice with extensive plasmapheresis and chemotherapy. No correlation could be established between the severity of the cold-induced symptoms and the level of the cryoglobulin. Our patients with the most dramatic symptoms had cryoglobulins which precipitated above 33°C. It is noteworthy that cold-induced symptoms were often present several years before the diagnosis of multiple myeloma was made and increased in severity with time. This finding outlines the need for repeated evaluation of these patients. Such symptoms should allow a relatively early diagnosis of myeloma, and treatment at this stage may prevent the occurrence of life-threatening symptoms, such as extensive gangrene, which were reported in early cases [48,50,51].

Waldenstrom’s macroglobulinemia: Nearly 10 per cent of monoclonal IgM were cryoglobulins in our experience. Fifteen patients in this series had Waldenstrom’s macroglobulinemia. Seven had a type I cryoglobulin and eight a type II cryoglobulin with a monoclonal IgM. The strikingly high incidence of anti-IgG antibody activity of monoclonal IgM in serum as well
as on the surface of leukemic lymphocytes [70] suggests that autoantibody-synthesizing clones are more likely to undergo neoplastic transformation.

Presenting symptoms related to the cryoglobulinemia were found in 80 per cent of these patients and were present from 1 to 10 years before evaluation leading to diagnosis. Raynaud’s phenomenon was present in 10 patients, vascular purpura in 7 (6 of whom had mixed cryoglobulins), glomerular injury in 5 and neurologic symptoms in 3. Kidney biopsies showed massive intracapillary deposits in three patients (two with IgM and one with IgM-IgG cryoglobulin) and proliferative nephritis in two patients with mixed cryoglobulinemia.

The presence of a cryoglobulin may modify the usual symptoms of Waldenstrom’s macroglobulinemia [53,65]. Vascular purpura or Raynaud’s phenomenon are not seen in patients without cryoglobulinemia. Glomerular lesions are far in excess of those observed in macroglobulinemia without cryoglobulinemia. An extreme decrease in IgG levels has been reported in patients with mixed IgM-IgG cryoglobulinemia [57,66] and was present in three of our patients. Waldmann et al. [66] demonstrated in such a patient a decreased synthesis of IgG but also an increased catabolism of those subclasses of IgG which combined with the monoclonal IgM.

Other hematologic diseases: A diagnosis of chronic lymphocytic leukemia was made in three patients, and that of well differentiated lymphocytic lymphoma in six. In two patients, the lymphoma was disclosed several years after the diagnosis of cryoglobulinemia and negative hematologic evaluation. All these patients had a type II or III mixed cryoglobulin, usually at a low level in small amounts. A systematic survey should be made to determine the Registry of cryoglobulinemia in these diseases is lower than 2 per cent. Major symptoms could be assigned to the cryoglobulin in six of 14 patients, and two died of renal failure. Of note is that, in addition to the lymphoproliferative disease, 10 patients had autoimmune hemolytic anemia and two had Sjogren’s syndrome.

Cryoglobulinemia was also found in two patients with Hodgkin cell sarcoma and in individual patients with Hodgkin’s disease, myelofibrosis, reticulonodular sclerosis or polycythemia vera.

Autoimmune Diseases. Mixed cryoglobulin is a frequent finding in diseases with a proved or presumed autoimmune mechanism, and it belonged to type III in 0 of 3 cases. A symptomatic cryoglobulinemia may occur for many years before the onset of overt autoimmune diseases in five patients.

Systemic lupus erythematosus: Four patients with a proven systemic lupus erythematosus had a transient type III cryoglobulin and two of them had glomerular nephritis. In one patient, a cryoglobulin was found 15 years after the onset of the disease and some months before a relapse.

Various type III cryoglobulins (IgM-IgG, IgM-IgG-C1q, IgG-C1q) are found in active systemic lupus erythematosus [29,30,56,71] and are most frequent when a glomerulitis and/or a low level of complement are present [29]. The titer of antinuclear antibodies was not higher in the cryoprecipitate than in whole serum [29]. Koffler et al. [72] showed that rheumatoid factor activity was present in the renal capillary deposits of a patient with systemic lupus erythematosus and mixed cryoglobulin.

Sjogren’s syndrome: In eight patients, Sjogren’s syndrome was associated with a type II or type III cryoglobulin. In accordance with previous findings [73,74], all these patients exhibited, besides the sicca syndrome, extrasalivary symptoms such as vascular purpura, Raynaud’s phenomenon, polynuropathy and necrotizing angiitis. The occurrence of glomerulitis in three of our patients is striking since glomerular injury is exceptional in Sjogren’s syndrome without cryoglobulinemia [73] and emphasizes the pathogenetic role of the cryoglobulin. The course of the disease was complicated in three patients by the occurrence of a lymphoma.

Other autoimmune disorders: Four patients had definite rheumatoid arthritis. No evidence of malignant arthritis was documented in these patients despite a long-standing history. One patient with probable rheumatoid arthritis had a crystallizable IgG2 cryoglobulin with necrotic purpura. We did not study the synovial fluid in which different varieties of cryoproteins have been described [75]; their significance is still uncertain.

We found a cryoglobulin in two patients with idiopathic thrombocytopenic purpura and in seven patients with autoimmune hemolytic anemia. Four of the latter were affected with cold agglutinin disease and the IgM kappa cryoglobulin exhibited strong anti-I activity.

Four patients with type III cryoglobulinemia had the clinical and histologic features of classic periarteritis nodosa. In two, the vascular disease was associated with Sjogren’s syndrome. The occurrence of cryoglobulins in periarteritis nodosa has seldom been reported [76-78]. In view of the possible role of circulating immune complexes involving Australia antigen in the pathogenesis of vascular injuries [79], it is of interest that one of our patients had acute hepatitis 6 years before the onset of periarteritis nodosa. Australia antigen was present at high concentration in this serum.

Essential Cryoglobulinemia. The cryoglobulinemia appeared idiopathic in 29 patients despite careful evaluation during follow-up. Mixed polyclonal cryoglobulins were the most frequent. Five of these pa-
patients had a monoclonal IgG. The first symptoms had appeared from 3 to 20 years before evaluation. Two patients had severe proliferative glomerulonephritis, two had necrotic purpura and one died from mesenteric arterial obstruction. Although no patient had evidence of myeloma or of other malignancy, chemotherapy was prompted by the severity of the symptoms. Only two patients had apparently essential type II cryoglobulinemia, one with IgM kappa-IgG cryoglobulin, the other with a high level of IgA kappa-IgG cryoglobulin. Because of extreme cold sensitivity with permanent erythrocytosis, the latter patient needed repeated plasmapheresis and chemotherapy. Dramatic improvement ensued, and the patient is still free of symptoms 5 years later.

Among the 22 patients with essential type III cryoglobulin, a female prevalence was observed. Patients were 35 to 70 years old at the onset of symptoms. The mean duration of the disease averages 9 years at the present time. Vascular purpura and Raynaud's phenomenon were the main symptoms. Arthralgias were found in half of these patients. Mild leukopenia was a common finding. Moderate polyclonal hypergammaglobulinemia was frequent (mean 1.7 g/100 ml). Transient positive Coombs' test without hemolytic anemia or a significant titer of speckled antinuclear antibodies was found in nine patients. The course was benign in 11 patients. In four others the myalgias, paresthesias or vasomotor symptoms were most disabling. In the last seven patients, renal or neurologic injuries appeared some months or years after the onset of the cutaneous symptoms. In some of these patients, fever and weight loss were suggestive of diffuse vasculitis.

It is worth emphasizing that the diagnosis of essential cryoglobulinemia should be considered only after extensive evaluation and follow-up. In 10 of our patients the initial tentative diagnosis of essential cryoglobulinemia had to be dismissed 2 to 10 years later when autoimmune diseases or hematologic malignancies became apparent. Comments: The incidence of the various associated diseases in our patients differs from that of other large series [4,7,28,80] and reflects the fact that our own cases were collected in a department devoted to hematology and clinical immunology. In our series, it appears clearly from Table III that patients with immunoproliferative diseases mainly have type I or II cryoglobulins, whereas in most patients with autoimmune disorders, the cryoglobulin belongs to type II or type III. It is not astonishing that both groups of associated diseases are found in patients with type II cryoglobulinemia. The autoantibody nature of the monoclonal IgM in this variety of cryoglobulins and its high incidence among Waldenström's macroglobulins points to the possible relationship between autoimmunity and malignancy. Indeed in our patients, 10 (12 per cent) had both lymphoproliferative and autoimmune diseases. Since essential cryoglobulinemia accounts for 33 per cent of the cases included in this study, it is noteworthy that we have found no cryoglobulin in 100 normal control subjects under 60 years old and 4 per cent in older healthy subjects. Using different isolation procedures, Cream [81] found a much higher incidence of minute amounts of cryoglobulin in normal control subjects.

Cryoglobulinemia in infectious diseases is not included in this series. Mixed cryoglobulins are encountered in the acute phase of viral illnesses such as infectious mononucleosis or cytomegalovirus infection [34,82-84], in subacute bacterial endocarditis [39,85], leprosy [86,87], primary and secondary syphilis [88] and in the course of parasitic infections such as trypanosomiasis [5,39]. A high frequency of mixed cryoglobulins has been found in acute poststreptococcal nephritis or in various types of subacute nephritis [31,89]. Chronic hepatic diseases may be associated with cryoglobulinemia [4,90-92]. A 4 per cent incidence of mixed cryoglobulin was found in a systematic survey of 100 patients with alcoholic cirrhosis [93]. A direct role of the cryoglobulin in the genesis of the various visceral injuries found in patients with infectious, renal or hepatic diseases has not been established. These cryoglobulins belong usually to type III, sometimes to type II and are often transient; their level is usually very low. In contrast to our findings in hematologic and autoimmune disorders, symptoms directly related to the presence of a cryoglobulin are rarely reported in these diseases. However the occurrence of cryoglobulins in infectious diseases may have an important pathogenic significance since cryoglobulins were found in the course of active experimental immunization with protein or nonprotein antigens [94,95], recalling current etiologic concepts of autoimmunity. In this respect the finding of IgM cryoglobulins in 25 per cent of NZB mice aged 4 months or older is of interest [96].

TREATMENT

The treatment of the underlying diseases and of the symptoms linked to the cryoglobulin should be evaluated separately. There is a sharp contrast between the dramatic effectiveness of symptomatic treatment in many patients with severe manifestations and the little benefit obtained in most patients with chronic symptoms such as vascular purpura. In the former patients, plasmapheresis is the main emergency treatment [97]. When indicated, plasmapheresis should be performed with withdrawal of 400 to 600 ml of plasma each day until sustained clinical improvement is attained, which is usually after 2 to 4 weeks. Plasmapheresis should then be performed
usually twice a week until a stable reduction of the cryoglobulin level is maintained. When the initial temperature of precipitation is high, plasmapheresis should be carried out at 37°C, and the separation of plasma from blood cells should be performed with care in order to avoid hemolysis. Such long-term plasmapheresis therapy was applied to eight patients in this series. They had type I or II cryoglobulinemia with high levels. The clinical diagnosis was Waldenstrom's macroglobulinemia in five patients, idiopathic monoclonal gammopathy in two and multiple myeloma in one. The indication for plasmapheresis rested upon the presence of one or more of the following severe symptoms: vascular insufficiency (Raynaud's phenomenon or distal necrosis), renal and/or neurologic involvement. A satisfactory clinical improvement was obtained in all but one patient and was only transient in two. In five patients the initial improvement was followed by a satisfactory remission under chemotherapy, and they are doing well without plasmapheresis after 2 to 5 years of follow up. Other emergency therapeutic procedures, such as supportive therapy of renal failure, may be rewarding. In two patients three episodes of anuria were successfully treated by hemodialysis, whereas in two others acute renal failure led to death despite therapeutic efforts.

In cryoglobulinemia without severe symptoms and in the absence of an underlying disease requiring active treatment, the decision as to the need of treatment and its choice are difficult. Beneficial results can be obtained by minimizing cold exposure. Avoidance of prolonged standing is most important in patients with purpura. Hydroxychloroquine has occasionally been of some benefit in our experience. When manifestations with a relatively poor prognosis, such as renal failure or neurologic involvement, are present, or when the patient experiences disabling paresthesias or myalgias, more active therapy is legitimate. Prednisone and/or so-called immunosuppressive agents have met with limited success [45,97] but have been effective in a few of our patients. Improvement appeared related to the anti-inflammatory effects of these drugs rather than to any reduction in the concentration of the cryoglobulin. It is noteworthy that cytotoxic drugs may modify the antigen:antibody ratio and therefore precipitate immune complex vasculitis. In our limited experience, penicillamine, which has been used for treatment of IgM monoclonal or mixed cryoglobulinemia, has been ineffective.

The choice and effectiveness of cytotoxic agents in cryoglobulinemia associated with multiple myeloma, Waldenstrom's macroglobulinemia or malignant lymphoma are the same as in random patients with these diagnoses. In patients with monoclonal cryoglobulinemia and without overt myeloma or macroglobulinemia, the hazard of severe manifestations related to the cryoglobulinemia justifies active treatment. In this whole group of patients, the reduction of the level of cryoglobulin usually leads to diminution in the symptoms related to the cryoglobulinemia. In patients with autoimmune disease, the presence of a cryoglobulin does not usually modify the therapeutic attitude. Controlled therapeutic trials, which have not been performed in the present series, are certainly needed in order to evaluate the effectiveness of various treatments in mixed polyclonal cryoglobulinemia.

ACKNOWLEDGMENT

We are grateful to Dr. L. Morel-Maroger for interpreting the kidney biopsies, to Miss Y. Signoret for excellent technical assistance and to Mrs. Miglierina for her help in preparing this manuscript.

REFERENCES


14. Terry WD, Matthews BW, Davies DR: Crystallographic stud-
significance of cryoglobulins—broquet et al.

15. Stone MJ: Studies on monoclonal antibodies. I. The specificity and binding properties of a Waldenstrom macro-


20. Capra JD, Kehoe JM, Winchester RJ, Kunkel HG: Structural function relationships among anti-gamma-globulins anti-


24. Stone MJ, Metzger H: The specificity of a monoclonal macroglobulin (γ M) antibody reactivity with primate γ G im-


26. Kunkel HG, Agnello V, Joslin FG, Winchester RJ, Capra JD: Cross-idiotypic specificity among monoclonal IgG pro-


29. Stastny P, Ziff CL: Insoluble complexes and comple-


44. Barr DP, Engle RL, Russ EM: Cryoglobulinemia: a case re-


48. Barr DP, Engle RL, Russ EM: Cryoglobulinemia: a case re-


SIGNIFICANCE OF CRYOGLOBULINS—BROUET ET AL


93. Schlegel N, Rueff B: Personal communication.


