Cryoglobulinemia in Echinococcosis (37150)

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Mixed type cryoglobulins have been recently associated with a number of inflammatory, bacterial, viral and protozoan infections (1). Although they differ in the various conditions, they usually consist of a "rheumatoid-like" factor, IgG and occasionally complement fractions, most probably circulating *in vivo* as soluble immune complexes (2–4).

Cryoglobulins have never been previously associated with helminthic infections. In this study it was shown that mixed IgM, IgA, IgG cryoglobulinemia is a frequent finding in patients with hydatid cyst disease.

Materials and Methods. Blood samples from 37 patients with echinococcosis were obtained from the "Policlinico Umberto I," Rome. Diagnosis was established on the basis of surgical findings. The cyst localization was hepatic in 62%, pulmonary in 22% and multiple in 16% of the patients studied. Blood was allowed to clot at room temperature and the sera were left at 4° for 1 to 2 wk and then centrifuged at 2000 rpm in a cold centrifuge. The precipitate was resuspended and washed 4 times in cold isotonic phosphate buffered saline (pH 7.2), incubated at 37° for 1 hr and centrifuged at 2500 rpm for 30 min at room temperature and the supernatant, containing the cryoglobulins, was preserved for subsequent use.

The nitrogen content of the samples was determined by a modified Ninhydrin method as described by Schiffman, Kabat and Thompson (5).

Immunodiffusion was performed using a 0.7% agarose in Veronal–glycine buffered saline (pH 7.6). Wells were cut 4.5 mm with centers 7.5 mm apart.

Immunoelectrophoresis of cryoglobulins was performed by the micromethod of Scheidegger (6) using 1% agarose and 0.1 *M* Veronal buffer (pH 8.6).

Rabbit anti-whole human serum was prepared according to Hirschfeld (7). Commercially available specific antisera against human IgG, IgM, IgA, IgE and α-2-macroglobulins were used. Antisera against β_{1C}, β_{1E}, k and λ chains were kindly supplied by Dr. C. L. Christian. Antiserum against sheep hydatid fluid was prepared in rabbit according to Kagan, Norman and Allain (8). The antiserum was adsorbed with normal human serum before use.

The IgG, IgM and IgA concentrations of cryoglobulins were measured in plates for radial immunodiffusion containing the corresponding specific antibodies.

Complement levels in the sera were estimated by the method of Kent, Burkantz and Rein (9). For testing anticomplementary activity of cryoglobulins 20 μl of the sample and 0.18 ml of Isosaver buffer (9) were added to 0.20 ml of a 1 to 10 dilution of fresh normal human serum and incubated for 30 min at 37° followed by 1 hr at 4°. This mixture (0.20 ml) was subsequently incubated for 30 min at 37° with 0.60 ml of sensitized sheep blood cells and with 0.70 ml of Isosaver, then centrifuged at low speed and the OD_{541} of the supernatant was read in a Bausch & Lomb colorimeter. The anticomplementary activity was expressed as complement (CH_{50} units) fixed by the sample compared to the control.

Rheumatoid factor (RF) activity was estimated by sensitizing O Rh+ (D+) human blood cells with an anti-D antiserum (kindly supplied by Dr. C. L. Christian); cells were washed and resuspended as a 0.5% suspension. Serial dilutions of sera and cryoglobulins were performed in duplicate

in a Takatsy microtitrator (Cooke Engineering Co., Alexandria, VA) and agglutination patterns were checked after 12 hr at room temperature and at 4°, respectively. Suitable controls were run at the same time.

Mercaptoethanol treatment of cryoglobulins was performed by incubation of equal volumes of 0.2 *M* 2-mercaptoethanol and sample for 45 min at 37° followed by dialysis against Isotris buffer (10) (pH 7.4) for 24 hr.

Results. As shown in Table I cryoproteins are a common finding in patients with active echinococcosis, both in the preoperatory (91%) and in the immediate postoperatory conditions (79%). They were also present in 2 patients with infected cysts, but in none of 5 patients with calcified cysts. They usually require a long time of storage at 4° to precipitate (from 1 to 2 wk).

Cryoproteins isolated from 23 samples of sera of 14 patients with active echinococcosis and from 2 with infected cysts were subjected to further analysis.

Table II shows the composition of these 16 cryoglobulins, their amount in the serum, the RF activity of both the cryoglobulins and the original sera and the level of complement in the sera. As shown in Table II all cryoglobulins are present at rather low concentrations in the serum. Analysis by immunodiffusion against specific antisera and by radial immunodiffusion of cryoglobulins isolated from 14 patients with active cysts showed that 9 of them reacted with anti-IgG, anti-IgM and anti-IgA (IgM was usually the main component); 2 of them reacted with anti-IgM and anti-IgG; 2 others with anti-IgA and anti-IgG and in one of these (C.C.) the IgA concentration was similar to the IgG; finally one cryoglobulin reacted only with anti-IgG. Both cryoglobulins from patients with infected cysts reacted only with anti-IgG. None of the cryoglobulins reacted with an anti-IgE, anti-β_{1C}, anti-β_{1C}, anti-α-2-macroglobulins and with an antiserum against sheep hydatid fluid. They also did not show any antibody activity when tested on immunodiffusion against sheep hydatid fluid at a concentration of 10 mg/ml, although the original sera in the same conditions gave at least one line of precipitation.

TABLE I. Cryoprecipitation in 37 Patients with Echinococcosis.

Stage of disease	Cryoprecipitation (positive sera/total no. of cases)
Active echinococcosis[a]	
preop	10/11
postop	15/19
Infected cysts[b]	2/2
Calcified cysts	0/5

[a] Stage of the disease in which one or more cysts are present containing living protoscolices.

[b] Stage of the disease in which secondary infection of the cyst with pyogenic bacteria has occurred.

Immunoelectrophoretic analysis of the cryoglobulins never revealed the presence of albumin or other contaminants.

Total hemolytic complement has been measured in 11 sera (Table II) and found to be within normal range (160–210 CH_{50} units) or slightly increased in 9 and reduced in 2 (G. L. and P. S.). It did not bear any significant relationship to the amount of cryoglobulins present in the sera. However, all cryoglobulins showed strong anticomplementary activity.

Sera and cryoglobulins have been also tested for RF activity; RF was absent in all sera except one at low titer (1:80). On the contrary it was present in 15 out of 16 cryoglobulins tested and the degree of positivity increased if incubation was carried out at 4°. The absence of reactivity with unsensitized cells excluded the presence of cold agglutinins. Mercaptoethanol treatment of the samples destroyed RF activity when only IgM and IgG were present; it lowered but it did not destroy the activity in the other cases indicating that the reactivity was at least in part due to IgA or IgG rheumatoid factor.

Discussion. Cryoglobulinemia of mixed type is commonly found in patients with a number of chronic inflammatory diseases (1). Compared with other previously described conditions, the cryoglobulins in echinococcosis show some peculiarities in their composition.

TABLE II. Composition of Cryoglobulins, Rheumatoid Factor (RF) Activity and Complement Levels in 18 Sera of 16 Patients with Echinococcosis.

Patients with active disease	Cyst localization	Amount of cryoprecipitate (μg N/ml serum)	Immunoglobulins present in the cryoprecipitate (μg/ml serum)[a]			RF activity in the serum	RF activity in the cryoprecipitate (minimum active concn μg N/ml)	CH_{50} units in the serum
			IgG	IgM	IgA			
M.T.[b]	Pulmonary	12.4	14	31	+	—	55.6	nd[c]
Dg.P.[b]	Pulmonary	6.2	10	18	+	—	11.7	219
A.C.	Pulmonary	8.2	11	26	+	—	—	nd
C.B.	Hepatic	3.6	+	+	+	—	15.0	292
M.D.	Pulmonary	14.1	20	31	+	—	45.2	nd
L.D.	Hepatic	10.9	+	19	+	—	21.8	222
143	Hepatic	5.0	+	+	+	—	10.2	nd
P.S.	Hepatic	2.9	+	+	+	—	12.5	135
R.N.	Pulmonary	nd[c]	+	+	+	1:80	(1:8)[d]	193
M.A.	Hepatic	3.5	+	+	—	—	4.4	nd
G.L.								
(24h preop)	Hepatic	1.3	+	+	—	—	—	145
(24h postop)		10.1	+	11	—	—	47.0	145
(3wk postop)		0.3	+	—	—	—	2.8	210
C.C.	Hepatic	14.7	12	—	11	—	34.6	196
G.I.	Hepatic	1.6	+	—	+	—	9.6	220
D.P.	Hepatic	2.3	+	—	—	—	2.0	200
Patients with infected cysts								
R.B.	Hepatic	3.0	+	—	—	—	21.7	nd
R.A.	Pulmonary	2.9	+	—	—	—	5.9	nd

[a] Presence of immunoglobulins was tested by both immunodiffusion and radial immunodiffusion. Values (μg/ml serum) are given in those cases only, where the concentration of the specific immunoglobulin in the preparation of cryoprecipitate was in the range of reliability of the latter method.

[b] On these patients serial samples have been tested. The results obtained were similar to those reported in the Table.

[c] Not done.

[d] RF activity is expressed as a titer since nitrogen content of the corresponding cryoprecipitate is not available. The cryoprecipitate was dissolved in 0.1 buffered saline volume of the original serum.

Indeed, in the majority of the cases they contain all the major classes of immunoglobulins, IgG, IgM and IgA, even though IgM is the main component and IgA is present in small amounts in all cases with the exception of one, where it has a concentration similar to the IgG. Mixed IgA–IgG cryoglobulinemia has been seldom described in the literature (11–13) and its significance is unknown, but it is intriguing that in 2 previously reported cases it was associated with a chronic infection, a toxoplasmosis and a syphilis (11). Moreover, prolonged immunization of rabbits with bacterial antigens (Yersinia, Brucella, Vibrio) has been shown to induce IgA–IgG cryoglobulinemia (13). On the other hand, cryoglobulins in echinococcosis show most of the properties common to other mixed cryoglobulins, *i.e.*, the long time of storage to precipitate, the RF activity and the anticomplementarity. In spite of the very strong anticomplementarity, it was impossible to demonstrate complement components in the cryoglobulins, such as β_{1C} and β_{1E}. This failure may be due to the low sensitivity of the method of detection and the low concentration of the cryoglobulins. The same considerations may hold true for the failure to show any hydatid cyst antigen or antibody activity in the cryoglobulins. Moreover, other antigen–antibody systems cannot be excluded in the case of infected cysts. To clarify this point more cryoprecipitate should be available for immunizing experimental animals. The presence of RF and its antigen indicate that at least part of the cryoglobulins may circulate as soluble immune complexes, and since mercaptoethanol treatment did not always destroy the RF activity, the existence in the same serum of various antigen–antibody systems, such as IgM–IgG, IgA–IgG or IgG–IgG can be postulated.

It is possible that cryoglobulins are a consequence of the prolonged immunization of the host by parasytic antigens, a mechanism common to other chronic infections (1). The absence of cryoprecipitates in the sera of the 5 patients with calcified cysts supports this hypothesis. Because of the particular nature of this disease in which a source of antigens, the cyst(s), is in close contact with the host, it is likely that under certain circumstances there is a continuous or massive release of cyst fluid in the bloodstream leading to the formation of large amounts of immune complexes. It is well known that a spontaneous or traumatic rupture of the cyst may lead to serious anaphylactic reactions. Although IgE was not detectable by immunodiffusion in the cryoglobulins, the level in the serum, which is not yet available in this preliminary study, would be of interest since it plays a prominent role in hypersensitivity reactions associated with helminthic infections.

In our series we have found a reduced serum complement only in 2 out of 11 sera. Preliminary studies on a few patients in which serial samples were collected before and after the removal of the cyst showed in some cases a sensitive increase of the serum complement level after surgery, even though it was previously within the normal range, indicating that the presence of the cyst led to a chronic consumption of complement.

In the course of this study we have observed a cutaneous rash in 2 patients and, occasionally, mild anemia and albuminuria. In no way, however, can such manifestations be related, at present, to an immune mechanism, and further studies are necessary to clarify the clinical significance of the cryoglobulins and of other eventually circulating antigen–antibody complexes in echinococcosis.

Summary. Cryoglobulinemia is shown to be a frequent finding in active echinococcosis. Cryoglobulins are of mixed type containing in most cases IgM, IgG and IgA. They show RF and anticomplementary activity. Complement components (β_{1C} and β_{1E}) and parasitic antigens or antibodies were not detectable. Serum complement levels in these patients were usually within normal range. The origin and possible clinical significance of cryoglobulins are discussed.

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