Temperature Curves of Different Types of Cryoglobulins

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Cryoprecipitation after repeated cooling was studied in patients with cryoglobulinemia of different origin, in whom the presence of cryoglobulins with specific physicochemical properties depended on the disease. Analysis of cyclic characteristics demonstrates the qualitative composition of precipitate in this condition.

Key Words: *cryoglobulins; cryoglobulinemia; cryoprecipitation; temperature hysteresis*

Cryoglobulinemia (CGM), the main or concomitant factor in many autoimmune, lymphoproliferative, and infectious diseases associated with a much more severe course and poor outcome of the diseases. In Sjogren's syndrome the development of CGM correlates with systemic manifestations, including lung and liver involvement, Raynaud's phenomenon, and lymphadenopathy. CGM is believed to reflect abnormalities in lymphocyte proliferation. In rheumatic diseases CGM correlates with systemic manifestations and is regarded as a marker of inflammatory processes leading to damage to the vascular wall. Hepatic and sometimes renal involvement, particularly in hepatitis B and C, is due to CGM [1-4].

CGM involves an essential increase in the blood concentrations of cryoglobulins (CG) characterized by abnormal solubility at temperatures below 37°C. The mechanisms of CGM are unknown, because CG are a heterogeneous group of immunoglobulins, represented in various diseases by monoclonal (type 1), mixed mono- and polyclonal (type 2), and polyclonal (type 3) immunoglobulins.

Study of physicochemical properties of various types of CG and their changes under different temperature will help to solve many problems of CGM.

We investigated cryoprecipitation of CG of different types exposed to repeated heating-cooling cycles.

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MATERIALS AND METHODS

Cryoglobulins isolated from the sera of patients aged 17-60 with CGM were examined. There were 2 patients with multiple myeloma, 2 with systemic lupus erythematosus accompanied by Raynaud's syndrome and 1 without it, 2 with nephritis caused by hemorrhagic vasculitis with skin involvement, and 3 with chronic glomerulonephritis. All the patients were examined during disease exacerbation. Donor sera (n=10) served as the control.

Sera were isolated by incubation of blood in a TC-8OM-2 thermostat at 37°C for 2 h and subsequent centrifugation (3000g, 10 min). These conditions minimize the loss of CG during clot retraction and prevent binding to the complement components [10]. Cryoprecipitation of CG upon cooling from 37°C to 4°C was evaluated spectrophotometrically on a Spektromom-208 by optical density (OD) at 500 nm. In order to rule out overrated results because of precipitation of immune complexes containing normal immunoglobulins on the cold, these complexes were removed from the serum [2]. To this end, 14% polyethyleneglycol (PEG-6000, Difko) in veronal-medinal buffer (pH 8.6), was added to the serum to a final concentration of 7% at 37°C. After incubation (37°C, 2 h) the sample was placed into warm centrifuge tubes, centrifuged at 2500g, and the supernatant was separated from the precipitate. The supernatant was again incubated at 37°C for 2 h in order to dissolve the cryoprecipitate, which could form during centrifugation. CG solutions were examined during cyclic cooling to

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4°C in a refrigerator and subsequent heating to 37°C. Cyclic heating and cooling of CG solution was repeated until changes in OD became significant. In parallel, the temperature of CG solution was measured (0.1°C). A 7% PEG solution in buffer served as the control. From these data, temperature curves of cryoprecipitation OD were plotted for various types of CG. The results were processed using nonparametrical Wilcoxon's paired test.

RESULTS

Temperature curves of cryoprecipitation of the analyzed CG solutions were different (Fig. 1). Analysis of CG solutions in patients with multiple myeloma, which were characterized by the presence of type 1 monoclonal CG, showed virtually coinciding loop-like curves (Fig. 1, *a*). In each thermal cycle, cooling to 4°C and heating to 37°C resulted in a 3-fold decrease in OD indicating intense formation of cryoprecipitate during temperature drop. Repeated formation of cryoprecipitate by type 1 CG during repeated cooling has been described [8]. Our data therefore confirm the current opinion on thermal stability of monoclonal CG.

However, in our experiments the curve reflecting a relationship between OD and temperature and the amount of cryoprecipitate formed during heating and cooling were different (Fig. 1, *a*). Therefore, the OD-temperature relationship is characterized by hysteresis. We can speak about temperature delay in the restructuring of CG macromolecule. CG, whose molecular weight is high, possess numerous inter- and intramolecular bonds in the cryoprecipitate and require time for relaxation and energy for adapting to new thermal conditions. Though loop-like curves of cyclic cryoprecipitation for type 1 CG virtually coincided in shape during cooling-heating cycles, the OD of CG solution at 4°C increased with the number of cycles (Fig. 1, *a*). The latter may be due to the fact that after each stage of cryoprecipitation on the cold, a small part of CG remained unsolved during heating.

Thermal curves of cryoprecipitation of CG solutions from patients with mixed CGM differ from the above curves (Fig. 1, *b*). A clear-cut hysteresis curve reflecting the OD-temperature relationship during the first cooling-heating cycle and decreased cryoprecipitation and the area of hysteresis loop during subsequent cycles are characteristic of these CG. In a patient with systemic lupus erythematosus with Raynaud's syndrome, heating-cooling cryoprecipitation curves virtually did nor differ starting from the fourth cycle. Thus, repeated heating-cooling cycles reduced the capacity of mixed CG to resuspend and form a new cryoprecipitate. The observed decrease in OD and hence, in the amount of cryoprecipitate at 4°C from cycle to cycle is not unexpected, because we know that mixed CGM are characterized by the presence of mainly types 2 and 3 CG in the blood (Fig. 1, *b*). These latter CG are thermolabile and lose their cryoproperties during cooling [8]. Similar curves were plotted for a patient with nephritis caused by hemorrhagic vasculitis [11,12].

Temperature curves of CG from patients with chronic glomerulonephritis without systemic symptoms are different (Fig. 1, *c*). First cooling of CG solution was accompanied by an increase in OD, which indicated cryoprecipitation, but subsequent heating did not lead to a decrease in OD, characteristic of dissolution of cryoprecipitate, but to its increase. Further heating-cooling cycles negligibly increased OD at 37°C and at 4°C. Similar curves reflecting the formation of cryoprecipitate were plotted for all patients with chronic glomerulonephritis and for a patient with systemic lupus erythematosus without Raynaud's syndrome. A stronger temperature dependence of OD during heating and subsequent thermal cycles can be due to the pre-

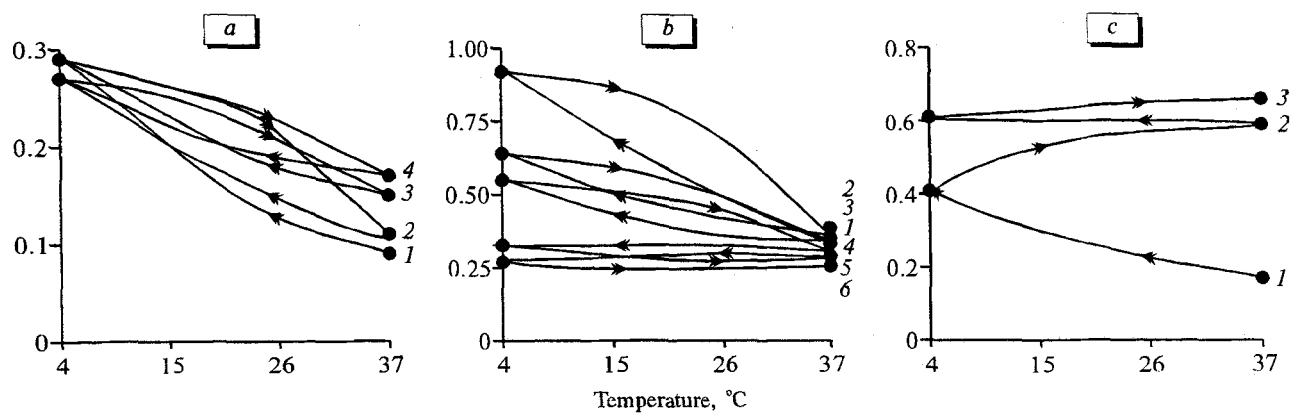

Fig. 1. Thermal curves of cryoglobulin cryoprecipitation in patients with multiple myeloma (*a*), mixed cryoglobulinemia (*b*), and chronic glomerulonephritis (*c*). Ordinates: optical density, arb. units. Curves represent the time course of formation and destruction of cryoprecipitates during cooling-heating cycles. Numbers of curves correspond to numbers of cycles.

sence of primarily type 3 CG and a considerable amount of pyroglobulins in the patient's serum, which contribute to the formation of insoluble cryoprecipitate at temperatures above 37°C. Hence, CG with specific physicochemical properties and different ability to form cryoprecipitate in repeated cooling-heating cycles are found in the sera of patients with CGM of various origin. Analysis of the cyclic pattern of cryoprecipitation of CG isolated from patient's serum demonstrates the qualitative composition of cryoprecipitate in this or that disease and is therefore useful for practical laboratories.

REFERENCES

1. A. A. Baranauskaite, E. L. Nasonov, and S. K. Solov'ev, *Ter. Arkh.*, No. 2, 23-27 (1991).
2. N. A. Konstantinova and A. Yu. Kirsanov, *Lab. Delo*, No. 11, 62-65 (1989).
3. E. Ya. Margulis, V. G. Savchenko, and V. A. Vasil'ev, *Ter. Arkh.*, No. 7, 102-107 (1985).
4. T. Berney and T. Shibata, *J. Immunol.*, **147,** No. 15, 3331-3335 (1991).
5. P. Cacoub and F. L. Fabriani, *Am. J. Med.*, **96,** No. 2, 124-132 (1994).
6. T. Deuel and P. Hoffsten, *Ibid.*, **68,** No. 5, 633-796 (1980).
7. A. Kolovidouris and P. Jonson, *Ann. Rheum. Dis.*, **37,** 444-448 (1978).
8. G. W. Litman and C. Schefferli, *Mol. Immunol.*, **10,** No. 8, 707-718 (1981).
9. L. Musset and M. L. Diemert, *Clin. Chem.*, **38,** No. 6, 798-802 (1992).
10. F. Sansonno and L. Gesnaldo, *Hepatology*, **25,** No. 5, 1237-1244 (1997).
11. M. Tren-Delenburg and J. A. Schefferli, *Ann. Rheum. Dis.*, **57,** No. 1, 3-5 (1998).
12. M. Weisman and N. Zvaifler, *J. Clin. Invest.*, **56,** 725-739 (1975).