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# Cryoglobulins as indicators of upregulated immune response in schizophrenia

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**Abstract**

**Objective:** In the present work the concentration of abnormal immune complexes, cryoglobulins (Cgs), in the blood of schizophrenic patients was determined, and immunochemical composition of these complexes was studied.

**Patients and methods:** Eighty multiple-episode schizophrenia-affected subjects (55 medicated, 25 drug-free) and 40 healthy controls were involved in the study. Cgs were isolated by exposure of blood serum samples to precipitation at low temperature followed by extensive washings of Cg-enriched pellets. The immunochemical composition of Cgs was analyzed using different electrophoretic and immunoblotting systems.

**Results:** Significantly increased blood serum levels of type III Cgs were detected in all schizophrenia-affected subjects, as compared to controls. We also revealed the presence of C1q and C3 complement proteins and their activation products in Cgs isolated from the blood of schizophrenic patients.

**Conclusions:** The results of the present study suggest that Cgs are involved in schizophrenia-associated upregulated immune response by binding the complement proteins, activating the complement cascade and triggering aberrant apoptosis.


*Keywords:* Cryoglobulins; Complement proteins; Immunoglobulins; Schizophrenic patients

## Introduction

There is a considerable evidence to suggest a role for upregulated immune response in the pathogenesis of schizophrenia, since alterations in both the innate and adaptive immunity, including autoimmune and inflammatory component, were described in this pathology [1–6]. However, the molecular pathomechanisms responsible for schizophrenia-associated immune abnormalities still remain obscure.

Cryoglobulins (Cgs) are abnormal immune complexes, where both antigen and antibody are presented by immunoglobulins (Igs), and which reversibly precipitate at low temperatures. Cryoglobulinemia has been associated with a number of diseased conditions and considered as a nonspecific marker of the stimulation of the immune system, inflammation and autoimmune sensitization [7–12]. In certain diseased conditions the ability of Cgs to bind complement components and activate the complement system, the major mediator of the immune response, was observed [13,14]. On the other hand, several studies including our own results suggest about the involvement of the complement dysfunction in pathomechanisms of schizophrenia [15–18]. These findings generate interest in studying Cgs in schizophrenia. The present study, for the first time, demonstrates the elevated levels of Cgs in the blood of schizophrenic patients and provides detailed information on the immunochemical composition of these complexes.

## Patients and methods

### *Study population*

In this study multiple-episode schizophrenic patients were involved. Fifty-five patients (females 24, mean age±SD 42.6±9.1 years, age at the first-onset of illness mean±SD 21±7.5 years, duration of illness mean±SD 15.2±6.7 years) were

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treated with typical antipsychotic medications and 25 (females 10, mean age±SD 40±9 years, age at the first-onset of illness mean±SD 26±7, duration of illness mean±SD 15±8) were drug-free over a period of 10 weeks before blood sampling. All the affected subjects were recruited at the Nubarashen Republic Psychiatric Hospital of the Ministry of Health of Armenia and diagnosed by two independent experienced psychiatrists as paranoid schizophrenics according to ICD-10 criteria (F20.0). Among schizophrenia-affected subjects, forty-one had a positive family history of the disease (30 medicated and 11 drug-free). Forty-nine schizophrenia-affected subjects (37 medicated and 12 drug-free) and twenty-two healthy subjects were nicotine-dependent (smokers).

As a control group, 40 physically and mentally healthy volunteers (females 18; mean age±SD 41±9 years) without family history of schizophrenia or other psychiatric disorders were entered in this study. The healthy subjects were free of any medication for at least 1 month prior to blood sampling.

Subjects with concurrent diseases or conditions interfering with the aim of the study, such as acute or chronic infections, autoimmune diseases, malignancies, any surgical interventions within the previous 12 months, and those on immunosuppressive drugs, were excluded. Acute or chronic infections (e.g., ongoing viral or bacterial infection, active tuberculosis) were ruled out by physical inspection, body temperature measurement, and a blood screening for inflammation markers (C-reactive protein, erythrocyte sedimentation rate, and differential blood count). Absence of autoimmune diseases, malignancies, surgical interventions and immunosuppressive drug usage was checked based on relevant medical history and medical examination forms. All subjects were in a good physical condition in terms of blood and urine tests, hemoglobin, hematocrit, serum electrolytes, complete blood count, blood sedimentation rate, blood urea, standard set of venereal disease tests, electrocardiogram and electroencephalogram. They have no sign of malnutrition, non-alcoholic and non-addicted (with no current or past diagnosis of substance abuse or dependence).

Schizophrenia-affected subjects were interviewed by psychiatrists and informed that all measurements would be performed for research purposes only with no immediate therapeutic value. All subjects gave their informed consents to provide 10 mL of venous blood. The study has been approved by the Ethics Committee of the Institute of Molecular Biology NAS RA.

### Methods

All chemicals, not otherwise specified, were from Sigma-Aldrich Co. (USA).

### Blood sampling and isolation of Cgs

Cgs precipitation and purification procedures were based upon earlier developed approaches and recommendations [14,19,20]. Using pre-warmed equipment, 10 mL of blood was collected by venipuncture at 9:00–10:00 a.m. from each study subject and maintained at 370 °C until coagulation was complete. Serum was isolated by centrifugation at 3000×*g* for 30 min at 37 °C and stored with an antiseptic (sodium azide, 0.01%) at 4 °C for 7 days to precipitate low-concentration and late-precipitating Cgs. Cgs were isolated by centrifugation at 3000×*g* for 15 min at 4 °C. The supernatant was discarded, and the pellet was washed 3 times with a minimal volume of ice-cold 0.9% NaCl in 0.01 M Na-phosphate buffer pH 7.4. To check the reversible nature of the cryoprecipitate and to avoid residual contamination with erythrocytes, fibrin, etc., washed Cgs were resuspended in 50–100 μL of the same buffer and incubated at 37 °C for 1 h. The suspension was centrifuged warm at 10,000×*g* for 15 min, and any remaining insoluble material was discarded. Aliquots (20 μL) were stored at −30 °C until further use. Before use the aliquots were thawed, incubated at 37 °C and (if necessary) diluted with mentioned above buffer.

Total concentration of protein in Cgs was determined according to the method of Lowry et al. [21] using bovine serum albumin as a standard.

### Immunological analysis of Cgs

For characterization of Ig composition of Cgs, we used immunoblotting procedure described by Musset et al. [19] with slight modification. Cgs samples (10 μL; 0.25–0.5 mg/mL) were subjected to electrophoresis at 37 °C on 15×10-cm glass plates with 5 mm of 1.5% agarose gel (Bio-Rad Laboratories, Inc., USA) using Wide Mini-Sub Cell GT system electrophoresis equipment (Bio-Rad Laboratories, Inc., USA). Electrophoresis was performed for 1.5 h at a constant voltage (120 V) in a barbital buffer (pH 8.6, 64 mM). The gel was rinsed with distilled water and, without delay, covered with a sheet of nitrocellulose (Amersham Biosciences, USA) of the same dimensions as gel, followed by a sheet of filter paper (Whatman no. 1, S.T.P., France) and two sheets of blotting paper. The sandwich was then placed between two sheets of glass under pressures of 1 kg for 5 min and then 5 kg for 15 min. The blot was oven dried and immersed in a 5% solution of powdered skimmed milk for 1 h at 45 °C followed by incubation for 45 min at room temperature with alkaline phosphatase-conjugated goat anti-human monoclonal antibodies specific to IgG γ-chain (dilution 1:30,000), IgM μ-chain (dilution 1:10,000), IgA α-chain (dilution 1:15,000), Ig λ-chain (dilution 1:10,000) or Ig κ-chain (dilution 1:10,000). Dilution was performed by adding the mixture containing 5% skimmed milk, 0.01% Tween 20 and 0.15 M NaCl. At the end of incubation blots were washed 4 times with 100 mL of 0.01% Tween 20 in 0.15 M NaCl. Enzyme activity was revealed using 10 mL solution of 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) prepared just before use according to manufacturer's recommendations. After 10 min the reaction was stopped by washing with distilled water. Polyclonal IgG (1.7 mg/mL), IgM (0.25 mg/mL) and IgA (0.3 mg/mL) (all from pooled normal human plasma), monoclonal IgG (3.4 mg/mL), IgM and IgA (0.3 mg/mL) (all from human myeloma) were used as standards and subjected to electrophoresis (5 μL of each) together with the samples. The standards were purified according to previously described procedures [22] and were judged by sodium dodecyl