Classic and Alternative Complement Cascades in Post-Traumatic Stress Disorder

L. P. Oganesyan, G. M. Mkrtchyan, S. H. Sukiasyan*, and A. S. Boyajyan

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Hemolytic activity of classic and alternative complement cascades and blood concentrations of TNF-α, IL-1β, and IL-6 were measured in patients with post-traumatic stress disorder. The results attest to hyperactivity of the classic complement cascade associated with elevated content of proinflammatory cytokines and hypoactivation of the alternative complement cascade in patients with post-traumatic stress disorder in comparison with healthy individuals.

Key Words: *post-traumatic stress disorder; pro-inflammatory cytokines; complement system*

Post-traumatic stress disorder (PTSD) is a complex, severe, and chronic mental disease belonging to the group of anxious-depressive disorders, that can develop as a prolonged response to a terrifying or catastrophic stressful event. Traumatic event inducing PTSD leave a indelible trace in patient's psychics and than permanently and insistently recurs in his imagination. The patients with PTSD are characterized by a clinically significant grave emotional state, reduced adaptive capacity of the organism, disturbances in social, professional and other important spheres [3,6]. The incidence of PTSD varies from 1 to 14% depending on population. Examination of high-risk individuals revealed apparent increase in the incidence of PTSD (up to 58%). According to statistical data, 70% of the world population is permanently exposed to factors promoting PTSD development, and 20% of them become ill. Moreover, the number of PTSD patients increases by 3.6% per year and according to some predictions, 8-10% of human population will be affected after 10 years [3,6].

The molecular bases of etiology and pathogenesis of PTSD are poorly studied; however, some reports suggest that dysfunction of the immune system can contribute in the disease development [1,12].

Pathological mechanisms underlying the development of immune disorders in PTSD and causal relationship between these disturbances and other pathophysiological processes characterizing PTSD remain unclear. On one hand, disorders in the hypothalamic–pituitary–adrenal and sympathetic–adrenal systems typical of PTSD [4,15] can modulate the immune response; on the other hand, the immune system regulating the above systems can trigger or exacerbate pathological processes through the feedback mechanism [9,11].

The complement system is the major effector of the immune system, it acts at the interface of the innate and adaptive immunity and interacts with both cellular and humoral systems of immune recognition. Structural and functional defects in this mediator lead to the development of inflammatory and autoimmune disorders and aberrant apoptosis contributing to a number of diseases, including mental ones [2,8].

Here we evaluate functional activity of classic and alternative complement cascades and the relationship between disturbances in these pathways in PTSD.

MATERIALS AND METHODS

Patients with PTSD in chronic stage (n=31; 27 male and 4 female; mean age 42.0±4.6 years) were exa-

Institute of Molecular Biology, National Academy of Sciences of Armenia.; *Armenia Stress-Center, Erevan, Armenia. ***Address for correspondence:*** aboyajyan@sci.am. A. S. Boyajyan

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mined. PTSD was diagnosed using criteria [6] by psychiatrists in Armenia Stress Center. In all cases, the traumatic event provoking the disease took place 13 years ago.

Healthy controls (n=31; 27 male and 4 female; mean age 39.0±43.1 years) were volunteers without family history of PTSD and other mental disorders. Healthy volunteers were never exposed to factors inducing PTSD and had no mental disieases.

Blood samples were collected from the ulnar vein at 9:00-10:00 at fast (during hospitalization immediately after diagnosis specification, but before the start of treatment). None of the patients had tumor or autoimmune diseases, acute infections, and surgical interventions; none of the patients received immunodepressants at least 12 months before blood sampling and other drugs at least 30 days before blood collection. All subjects gave consent for participation in the study. This study was approved by Ethical Committee of the Institute of Molecular Biology.

Immediately after blood collection, the samples were placed on ice; after clotting, they were centrifuged at 3000g for 10 min and the serum was collected. Serum samples were stored at -30°C and defrosted immediately before use.

Serum hemolytic activity was determined for classic and alternative complement pathways (CH50 and AH50, respectively) and for C3 component C3 (C3H50) [4], a convergent site for all three pathways of complement activation and the initial step of the alternative pathway.

For AH50 measurements, rabbit erythrocytes were used as the target cells, sheep erythrocytes sensitized with rabbit antibodies to sheep erythrocytes were used for CH50 и C3H50. The amount of serum inducing 50% hemolysis in the reaction mixture was taken as 1 unit of hemolytic activity.

Serum levels of TNF-α, IL-1β, and IL-6 were determined by ELISA using Stat Fax 3200 apparatus (Awareness Technology Inc.) and appropriate commercial kits (R&D Systems Europe Ltd.).

Statistical data processing was carried out using Student's t test and analysis of correlation based upon Pearson's correlation coefficient (r). The differences were significant at $p<0.05$.

RESULTS

Mean serum content of CH50 in patients with PTSD was by 2.1 times higher than in healthy people ($p<0.0002$, Table 1). However, mean serum levels of AH50 and C3H50 in patient with PTSD were by 1.7 ($p<0.0001$) and 1.5 ($p<0.0306$) times lower than the corresponding values in healthy subjects.

A significant correlation was found between the contents of AH50 and C3H50 (r=0.57; $p<0.027$) in patients with PTSD. No significant correlations between other studied parameters in patients and healthy individuals were found.

Serum levels of proinflammatory cytokines TNF-α, IL-1β, and IL-6 in patients with PTSD significantly surpassed the normal: by 1.2 ($p<0.048$), 1.6 ($p<0.032$), and 1.5 ($p<0.038$) times, respectively. A positive correlation was observed between the levels of these cytokines and CH50 (r=0.44, p=0.007; r=0.35, p=0.048 and r=0.48, p=0.03 for TNF-α, IL-1β and IL-6, respectively).

Thus, pathogenesis of PTSD is characterized by dysfunction of the complement system manifesting in hyperactivity of the classic cascade and reduced activity of the alternative cascade. Due to the capacity of complement to self-potentiation and production of various inflammatory mediators, there are special mechanisms preventing continuous complement activation. Despite considerable contents of water soluble and membrane-bound complement regulators in cells, they are insufficient in some pathological conditions. Uncontrolled hyperactivation of the classic cascade invariantly leads to the development of inflammatory reactions and aberrant apoptosis. The observed hyperactivity of the classic complement cascade probably leads to exhaustion of C3 complement component with subsequent inhibition of the alternative complement cascade triggered by this component.

Recent studies showed that immune system disturbances, *e.g.* at the level of the development of inflammatory reactions in CNS and at the systemic level, can contribute to PTSD pathogenesis [5,7,10,13,14]. Previous studies revealed increased levels of proinflammatory cytokines in patients with PTSD [7,10,13], which agrees with our findings. We previously revealed high levels of circulating immune complexes in PTSD

TABLE 1. Serum Levels of AH50, CH50, and C3H50 in Patients with PTSD and Healthy Individuals (M±m)

Group	AH50	CH50	C3H50
PTSD patients	52.30±3.37	375.00±29.52	37.570±4.198
Healthy individuals	87.62±2.13	176.00±24.56	55.92±10.11
p	<0.0001	<0.0002	<0.03

patients [5], which are markers of autoimmune and inflammatory reactions and known activators of classic complement cascade [2]. Our study showed, that PTSD patients also have disturbances in the complement system, an important component of the inflammatory response. The destructive changes in CNS in PTSD induce activation of the classic complement cascade and trigger inflammatory reactions, including formation of proteolytic complement activation products (opsonins, anaphylatoxins, and cytolytic membrane-attacking complexes) and expression of proinflammatory cytokines. These processes lead to structural and functional damage to the blood-brain barrier and to induction of autoimmune and inflammatory reactions at the systemic level. This assumption requires further experimental studies.

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