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Circulating immune complexes as possible inflammatory markers in posttraumatic stress disorder

L.P. Hovhannisyan

Institute of Molecular Biology of Armenian National Academy of Sciences 7 Hasratyan st., 0014, Yerevan

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Posttraumatic stress disorder is a chronic anxiety disorder (PTSD) [5] that can develop in a person after exposure to a terrifying event (or after witnessing or learning about such an event) or ordeal in which grave physical harm occurred or was threatened. The disorder is characterized by symptoms of avoidance/numbing, hyperarousal and reexperiencing [5, 20]. Traumatic event can exert an excessive demand on regulatory functions, particularly if the stressor is excessive or prolonged, resulting in risk for excessive inflammation [8]. Some studies suggest that the immune system regulation might be disturbed in individuals with PTSD and demonstrate an excess of inflammatory actions of the immune system, which may contribute to health declines in these individuals [1,2,6,9,10,12,13,15,16,20,21,23]. It is currently unknown to what extent depressive symptoms might confound the association between a clinical diagnosis of PTSD and inflammation, and due to insufficiency of relevant data the molecular picture of the immune system abnormalities in PTSD is yet unclear.

Circulating immune complexes (CIC) are major mediators of inflammation, modulators of both cellular and humoral immune response. Formation of CIC is a normal reaction of the organism to the presence of foreign or autoantigen. In healthy organism CIC are easily eliminated from circulation by phagocytosis (Fig. 1). In pathologic conditions inappropriate clearance or deposition of CIC may occur and affect the development of the immune response at multiple levels. CIC may deposit in endothelial or vascular structures provoking prolonged inflammatory response through permanent activation of the complement cascade. Thus, the negative effects of increased CIC concentration may badly affect disease progression and outcome [14, 17, 22].

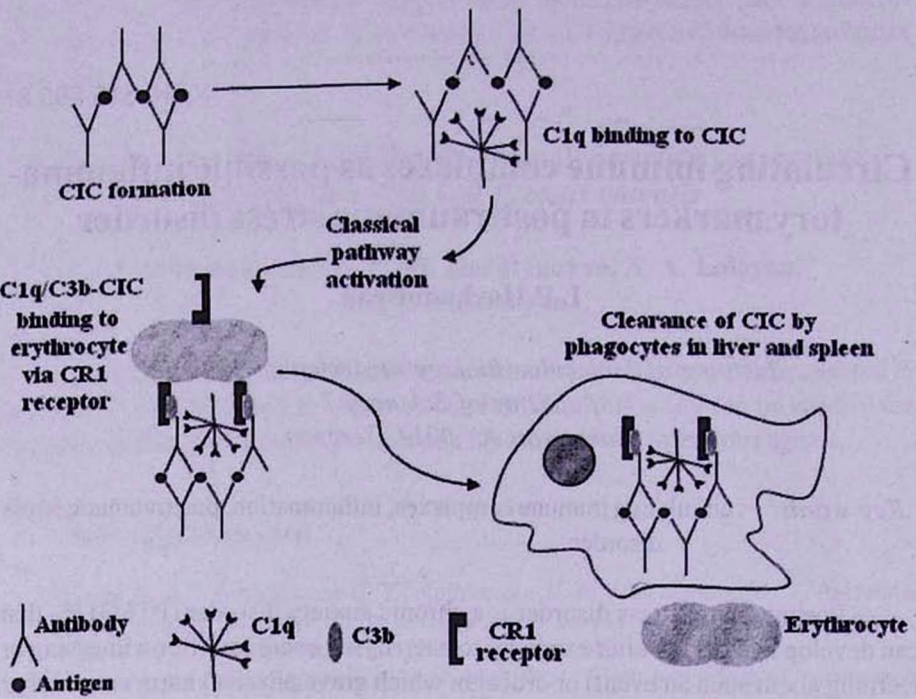

Figure 1. Formation and clearance of CIC [14].

C1q – complement component, initiating point of the complement classical pathway; CR1 – complement receptor 1; C3b - activation cleavage product of the complement C3 component.

In the total CIC population, CIC attached to the complement activation products, C1q component and cleavage products of C3 component (C3b, iC3b, C3dg) are usually observed [18]. The present study was focused on determination of the levels of total CIC, CIC containing C1-q, and CIC containing C3 cleavage products in patients with PTSD in comparison to healthy subjects.

Material and Methods

Patients were hospitalized at the Stress Center of the Ministry of Labor and Social Affairs of Armenia and diagnosed according to the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (DSM-IV (SCID-I)) [7] and the Clinician Administered PTSD Scale (CAPS) [3]. The control group involved 40 age- and sex-matched healthy volunteers leading an active and independent life without any history of physical or sexual abuse or other major trauma, defined as being free of current or past psychiatric disorders as determined by structured interview (SCID-I).

After obtaining permission from the Ethical Committee of the Institute of

Molecular Biology NAS RA and informed consents from 50 PTSD patients, blood samples were taken by venipuncture at 9:00-10:00 a.m. After 1 hour of coagulation at 4°C the serum was separated by centrifugation.

CIC were isolated from fresh sera of affected and healthy subjects using earlier described procedure. First an equal volume of 7% polyethylene glycol (PEG, 6 kDa) was added to the equal volume of serum up to 3.5% final concentration. Then the mixture was incubated at 4°C for 18-20 h followed by centrifugation at 3000 g for 15 min. The pellet containing total CIC was dissolved in 0.1 N NaOH. The concentration of CIC was determined by measuring absorbency at 280 nm and expressed in the units of optical density (A280) [4].

Concentrations of C3d (peptide fragment of C3b, iC3b and C3dg) and C1q containing CIC (C3d-CIC and C1q-CIC, respectively) in the sera were measured by Enzyme Linked Immuno-Sorbent Assay (ELISA) using commercial kit (Human, Germany). In brief, 96-well plates coated with C1q or C3d were filled with 100 µl of serum diluted (1:101 for C1q and 1:11 for C3d) with phosphate buffer (0.01M, pH 7.0) and incubated for 1 h. The wells were washed 3 times with 300 µl of Tris-HCl buffer (0.01 M, pH 6.9), after which 15 µl of anti-human-immunoglobulin G conjugated to peroxidase was added and incubated for 30 min. The wells were again washed 3 times with 300 µl of the same buffer. Then100 µl of 3,3′,5,5′-tetramethylbenzidin solution (1.2 mM in 3 mM hydrogen peroxide, pH 3.7) was added per well and incubated for 10 min. All procedures were performed at room temperature. The reaction was ended by adding 15 µl of sulphuric acid (0.5 M) per well and the absorbance at 450 nm wave length was read. For calibration curve different dilutions of standard serum were used. Standard sera for calibration and positive control were treated in the same way as experimental serum patterns. Concentrations of C1q-CIC and C3d-CIC were expressed in mg/ml of serum.

Statistical analysis was performed by Student's two tailed t-test using "Graphpad Prism" (GraphPad Software Inc., USA) software. $P<0.05$ was considered as significant.

Results and Discussion

According to the results obtained, mean values of the total serum CIC, C3d-CIC and C1q-CIC were 1.5, 2.1 and 1.58 times significantly higher ($p<0.0055$, $t=2.88$; $p<0.0004$, $t=3.784$; $p<0.02$, $t=2.311$, respectively) than in case of healthy subjects. The results obtained are presented in Table 1 and Fig. 1, 2.

Table 1

Serum levels of the total CIC, C3d-CIC and C1q-CIC in PTSD patients and healthy subjects

Parameter	Healthy subjects			PTSD patients		
	Total [CIC] A280	[C3d-CIC] μg/ml	[C1q-CIC] μg/ml	Total [CIC] A280	[C3d-CIC] μg/ml	[C1q-CIC] μg/ml
M	0.12	20.38	42.69	0.18	42.36	67.50
SE	0.004	3.126	3.847	0.027	3.303	6.291
SD	0.027	12.510	15.390	0.130	21.910	41.730
Min	0.060	8.000	24.000	0.017	11.000	27.000
Max	0.180	57.000	72.000	0.120	108.000	204.000

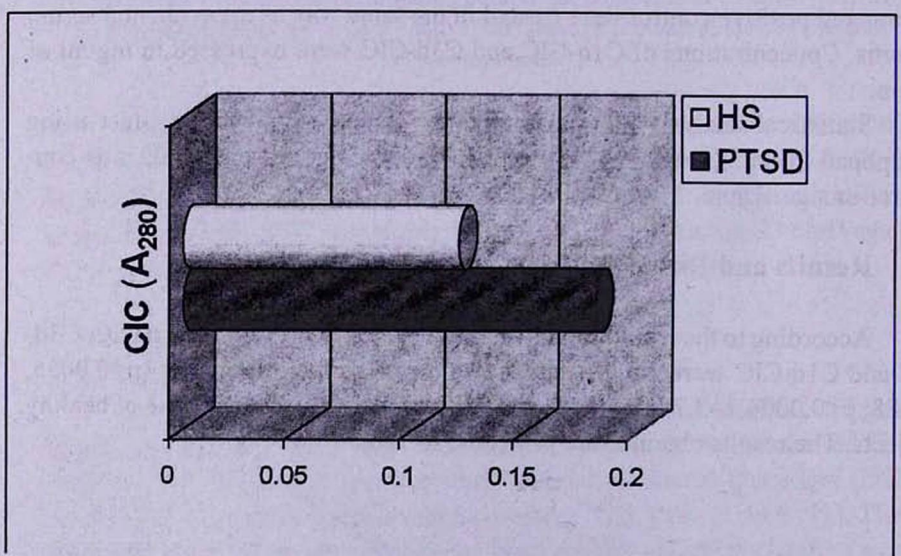

Fig. 1. Mean values of total CIC in the serum of PTSD patients and healthy subjects (HS) ($p<0.0055$)

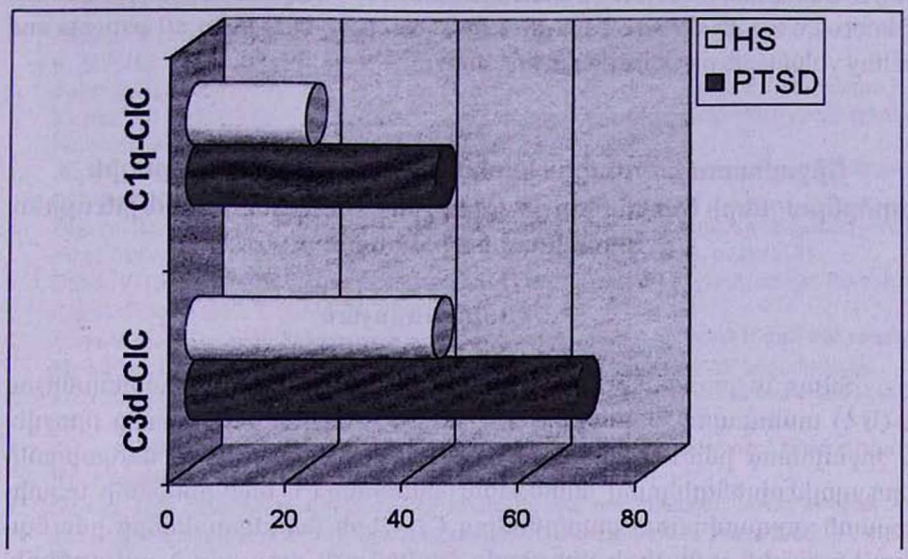

Fig. 2. Mean values of C3d-CIC and C1q-CIC in the blood serum of PTSD patients and healthy subjects ($p<0.0004$ and $p<0.02$, respectively)

The data obtained indicate that pathogenesis of PTSD is associated with high blood levels of both total CIC, C1q-CIC and C3d-CIC. The increased levels of total CIC in circulation may reflect either decline in their clearance or the presence of the excess of foreign/ autoantigens. The presence of C1q-CIC and C3d-CIC in the total CIC population strongly suggest about the activated state of the complement cascade in PTSD that is consistent with our previous data demonstrating complement classical pathway activation in PTSD [11]. C1q is the initial point of the complement classical pathway and C3 is a triggering point for C3-convertase, which launched the terminal complement pathway, leading to formation of the membrane attack complexes [19].

The results presented in this study provide additional evidence in support of the previous observation demonstrating the increased inflammatory response in PTSD patients [10, 11]. The central problem here is to reveal triggers and particular pathogenetic impact of inflammation in PTSD. Further studies with the involvement of more patients and with determination of the functional state of other inflammatory markers and regulators of the immune response are requested to clarify this problem.

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Շրջանառու իմունային համալիրները որպես բորբոքային գործընթացների հնարավոր ցուցանիշներ հետվնասվածքային սթրես համախտանիշի ժամանակ

Լ.Պ. Հովհաննիսյան

Տվյալ աշխատանքում հետվնասվածքային սթրես համախտանիշով (ՀՎՍՀ) տառապող հիվանդների և առողջ անձանց արյան մեջ որոշվել են շրջանառու իմունային համալիրների (ՇԻՀ) ընդհանուր մակարդակները պոլիէթիլենգլիկոլով նստեցման եղանակով և կոմպլեմենտի ակտիվացման արգասիքներ պարունակող ՇԻՀ-երի մակարդակները իմունաֆերմենտային անալիզի միջոցով: Համաձայն ստացված տվյալների, ՀՎՍՀ-ով տառապող հիվանդների մոտ նկատվում է ինչպես ընդհանուր ՇԻՀ-երի, այնպես էլ կոմպլեմենտի ակտիվացման արգասիքներ պարունակող ՇԻՀ-երի մակարդակների հավաստի աճ՝ համեմատած առողջ անձանց հետ: Այս արդյունքները հանդիսանում են ՀՎՍՀ-ի պաթոգենեզում բորբոքային գործընթացների ներգրավվածության ևս մեկ ապացույց:

Циркулирующие иммунные комплексы как возможные воспалительные маркеры при посттравматическом стрессовом расстройстве

Л. П. Оганесян

В настоящей работе с помощью ПЭГ-преципитации были определены общий уровень циркулирующих иммунных комплексов (ЦИК) и с помощью ИФА – уровни иммунных комплексов, содержащих продукты активации комплемента в крови, у больных посттравматическим стрессовым расстройством (ПТСР) и здоровых лиц.

Согласно полученным данным, как общий уровень ЦИК, так и уровни ЦИК, связанных с продуктами активации комплемента, у больных ПТСР по сравнению со здоровыми лицами были значительно повышены. Эти результаты предоставляют дополнительные доказательства вовлечения воспалительных процессов в патогенез ПТСР.

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