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ABERRANT IMMUNE COMPLEXES AND CYTOKINE PRODUCTION IN SCHIZOPHRENIA

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In the study the influence of cryoglobulins isolated from the blood of patients with schizophrenia on the production of a number of cytokines by mononuclear cells was shown.

Cryoglobulins – cytokines – mononuclear cells – schizophrenia

Աշխատանքում ցույց է տրված շիզոֆրենիայով հիվանդների արյունից անջատած կրիոգլոբուլինների ազդեցությունը մոնոնուկլեար բջիջների կողմից մի շարք ցիտոկինների արտադրության վրա:

Կրիոգլոբուլիններ – ցիտոկիններ – մոնոնուկլեար բջիջներ – շիզոֆրենիա

В работе показано влияние криоглобулинов, выделенных из крови больных шизофренией на продукцию ряда цитокинов мононуклеарными клетками.

Криоглобулины – цитокины – мононуклеарные клетки – шизофрения

A considerable evidence suggests a role for upregulated immune response in the pathogenesis of schizophrenia (SCZ), since alterations in both the innate and adaptive immunity including autoimmune and inflammatory components were described in this pathology at both central and peripheral levels [1]. The results of our previous study revealed the detectable blood levels of type III cryoglobulins (Cgs) in SCZ and found the presence of complement activation split products in these complexes [2]. Therefore, we propose that in SCZ Cgs may be implicated in disease-associated inflammatory reactions. The aim of this study was to investigate the potential ability of Cgs isolated from the blood of SCZ patients stimulate production of a number of cytokines, namely interleukin(IL)-1β, IL-6, IL-10, IL-8, tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein-1 (MCP-1), by PBMCs. The choice of these mediators and modulators of the immune response was based upon the earlier reported data including also our own results indicating their altered levels in the blood of SCZ-affected subjects [1].

Materials and methods. Fifty five patients with chronic paranoid SCZ (ICD-10 code: F20.0) treated with typical neuroleptics (males/females: 34/21, mean age ± SD: 45.8 ± 8.4 years, mean age at the first-onset of illness ± SD: 17.4 ± 8.2 years, mean duration of illness ± SD: 28.4 ± 7.6 years) and 12 physically and mentally healthy subjects without family, past or present history of SCZ or other psychiatric disorders (males/females: 6/6, mean age ± SD: 23.2 ± 1.2 years) were involved in this study. Cgs were purified from the blood of affected subjects according to earlier described procedure [2] and kept at -30^{o}C until further use. Concentration of Cgs was determined by measuring total protein according to the method of Lowry et al. [3] using bovine serum albumin as a standard. PBMCs were obtained from heparinized blood of healthy subjects

using standard Ficoll-Paque (Amersham Pharmacia Biotech) density gradient centrifugation. Thereafter PBMCs were diluted to $3x10^5$ cells/ml by Roswell Park Memorial Institute (RPMI)-1640 medium (Life Technologies, UK) supplemented with 1% glutamine, 1% penicillin–streptomycin, 1% HEPES and 1% fetal bovine serum and incubated in the humidified incubator at 37^0C with 5% CO_2. IL-10, IL-1β, IL-6, IL-8, TNF-α, and MCP-1 levels in culture medium were determined by enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Gen-Probe Diaclone, France) according to manufacturer's instructions. Concentration of cytokines was expressed as pg/ml. Data analysis was performed by "GraphPad Prism-3.0" software (GraphPad Software Inc, USA). Ordinal descriptive statistics, Student's two-tailed t-test were used for data analysis. Values of $p<0.05$ were considered as significant. The data for each given parameter was obtained in 10 independent experiments (with 3 repeated measurements in each) and is expressed as mean ± standard deviation (mean±SD).

Results and Discussion. To determine whether Cgs from SCZ patients induce IL-10, IL-1β, IL-6, IL-8, TNF-α, and MCP-1 production by PBMCs, the later were incubated with various concentrations of Cgs (range: 0.2-1.0 mg/ml) for 24 hours. After incubation the levels of cytokines were measured in culture medium. In order to assess the potential influence of gender difference on measured parameters, we compared the effects of Cgs from male patients with those from female patients on PBMCs from male and female healthy subjects, respectively. We detected that Cgs (starting from concentration 0.2 mg/ml) induced production of pro-inflammatory and chemotactic cytokines by PBMCs. Thus, after incubation of PBMCs with Cgs in concentration of 1 mg/ml the mean levels of IL-1β, IL-6, TNF-α, IL-8, and MCP-1 in the culture medium were 2.1, 12, 2.4, 5.5 and 3.7 times significantly higher ($p<0.05$) than basal levels of corresponding cytokines estimated before the incubation. While this effects were much more pronounced when Cgs and PBMCs from female subjects were used, the detected differences were statistically insignificant ($p>0.05$) indicating that the gender difference does not affect the production of these cytokines by PBMCs induced by Cgs. In case of IL-10 no influence of Cgs on production of this anti-inflammatory cytokine by PBMCs was observed.

The results obtained in the present study suggest that in vitro Type III Cgs isolated from the blood of SCZ patients may induce the expression of pro-inflammatory and chemotactic cytokines IL-1β, IL-6, TNF-α and IL-8, MCP-1, respectively, by PBMCs. No influence of Cgs on anti-inflammatory cytokine IL-10 production was by PBMCs was observed. As it was already mentioned in the introduction earlier studies demonstrated increased levels of IL-1β, IL-6, TNF-α, IL-8, MCP-1 in the blood of SCZ-affected subjects [1] providing evidence on the involvement of systemic inflammatory reactions in pathogenesis of SCZ. In addition, it was shown that monocytes of SCZ patients stimulated by lipopolisaccharide released significantly higher amounts of IL-1β and TNF-α than those of healthy subjects and that leukocyte mRNA levels of TNF-α significantly higher in first-episode SCZ patients [4]. Based upon the results obtained in the present study we concluded that Cgs may contribute to increased blood levels of these cytokines in SCZ and are involved in disease-associated activated peripheral inflammatory responses. Our suggestion does not exclude the possibility that early reported genetic or other environmental factors may be also responsible for altered blood levels of pro-inflammatory and cytotoxic cytokines in SCZ. Regarding IL-10, the increased blood levels of this cytokine earlier reported in patients with SCZ may be caused by early reported genetic factors rather than by environmental [1]. Similar effects related to TNF-α and IL-10 were observed earlier upon studying the influence of type I Cgs on PBMCs. After suppression of the complement activation the reverse effect was detected, e.g., decrease in TNF-α production and increase in IL-10 production by PBMCs in the presence of type I Cgs [5]. While IL-10 is known as inhibitor of TNF-α expression, it seems that in SCZ this regulatory mechanism does not work, since the increased levels of both cytokines were detected in this pathology [1] detected in this pathology [1].

Cgs can induce production of pro-inflammatory cytokines by PBMCs via Fc receptors as it was demonstrated in case of circulating immune complexes for TNF-α [6]. In addition, our previous study revealed the presence of the C1q complement protein, and C3-derived opsonins, natural ligands of CR1 complement receptor, in Cgs isolated from the blood of SCZ patients [2]. Binding of C3b-containing Cgs to CR1 on monocytes will induce IL-1β release, and binding of C1q-containing Cgs to CR1 on PBMCs will induce the activation of the complement as it usually occurs in case of circulating immune complexes. Since activation of the complement stimulates the expression of chemotactic cytokines [7], we propose that the effects of Cgs towards IL-8 and MCP-1 production by PBMCs may be realized by a complement dependent mechanism. Hyperactivation of the complement cascade in SCZ was demonstrated in a number of studies [8], thus this complement-dependent mechanisms may be implicated in Cgs-induced effects both in vivo and in vitro. However, direct induction of chemokine expression by Cgs via C1qR receptors, as it was described for IL-8 in case of C1q-containing circulating immune complexes [9], cannot be excluded. Further investtigations will help to clear these issues.

In summary, we concluded that Cgs presented in the blood of SCZ patients are implicated in disease-associated alterations in the immune response through induction of the expression of pro-inflammatory and chemotactic cytokines by PBMCs.

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