Decreased T Cell Response to Mitogen and Increased Anti-cytoplasmic Antibody in Drug-Free Schizophrenic Patients

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ABSTRACT

Background: Apart from genetic and environmental factors, activation of autoreactive mechanisms has been proposed to play a role in the pathogenesis of schizophrenia. In recent years, considerable work has been carried out to understand the role and contribution of the immune system in this disease. **Objective:** To investigate the T cell response to phytohaemagglutinin (PHA) and determine the serum levels of anti-nuclear antibody (ANA), anti-cytoplasmic antibody (ACA), and circulating immune complexes (CIC) in schizophrenic patients. **Methods:** A total of 30 drug-free schizophrenic patients and 42 healthy controls were enrolled in this study. T cell proliferation in response to PHA was measured using Methyl Thiazol Tetrazolium test. ANA and ACA were measured by indirect immunofluorescence. CIC concentration was determined using poly ethylene glycol precipitation assay. **Results:** Mean PHA response was 1.96 ± 0.83 in patients and 3.72±1.39 in healthy controls ($p < 0.001$). ANA and CIC concentrations were not significantly different between two groups. In addition, ACA was detected only in patients**.** **Conclusion:** Increased production of ACA together with lower T cell response to mitogens in our patients provides evidence for the involvement of autoimmune mechanisms in the pathogenesis of schizophrenia.

Keywords: Schizophrenia, T Cell Proliferation, ANA, ACA, CIC

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INTRODUCTION

As a severe psychiatric illness, schizophrenia is accompanied by several purturbations in the immune system. Increased levels of IL-1, IL-2, IL-6 and their soluble receptors (sIL-2R, sIL-6R), decreased production of IL-2 by lymphocytes, increased levels of nonspecific and specific autoantibodies against CNS in serum and CSF of schizophrenics, and increased number of monocytes in serum and CSF of these patients are the most consistent findings in schizophrenia, although there is a lot of immunological controversy in this illness.

Schizophrenia is a heterogeneous disease with an unknown aetiology. Nevertheless, there are several hypotheses related to its aetiology from which some are immunological. Autoreactivity is the first of these which was proposed by Lehmann-Faccius (1937). In addition, the role of viral infections focuses on activation of macrophages, over response of T-lymphocytes, and association of some HLA alleles with the occurrence of this disease.

Etiological heterogeneity of schizophrenia, methodological differences and the influence of confounding factors such as antipsychotics, alcohol/drug abuse, smoking and hospitalization are among the potential factors responsible for controversial results. In fact in most early studies these factors were usually left unnoticed (1, 2).

A decrease in T-cell response to mitogen have previously been reported in schizophrenia (3-6). However, no significant differences in T cell proliferation between the schizophrenics and normal population have been reported by others (7-9).

Inconsistent results on the level of serum ANA in schizophrenic patients have been published (6, 10-16). Although increased serum level of CIC in schizophrenic subjects was reported by some investigators (17-20), others found no significant difference in CIC concentration between the schizophrenics and normal controls (21). The aim of the present study was to evaluate some immunological variables including proliferative response of T cells to mitogen, serum ANA (Anti Nuclear Antibody) level, presence of ACA (Anti Cytoplasmic Antibody), and CIC (Circulating Immune Complexes) level in schizophrenic patients and compare them with corresponding values in normal subjects.

MATERIALS AND METHODES

Subjects. Thirty patients suffering from schizophrenia including 26 men and 4 women between 22 and 48 years of age who referred to the clinic of Razi psychiatry hospital between June and October 2004, and 42 healthy volunteers including 36 men and 6 women from Tehran Blood Transfusion Organization were matched for age, sex and smoking status .The mean age of the patients and healthy subjects were 36.77 ± 5.99 and 36.86 ± 6.6, respectively .There was no significant difference in age and sex distribution between the two groups .Mean duration of illness was 13.75 ± 7.31 years and mean age of onset was 22.67 ± 7.29 years in the patient group. Out of 30 patients, 20(69%) were classified as paranoid, 2(6.9%) as catatonic, 4(13.8%) as undifferentiated and 3 (10.3%) as residual schizophrenia. One patient could not be classified in any of schizophrenia subgroups .Among the patients, 13 (44.8%) were smokers and 16 (55.2%) non-smokers. Smoking status of one patient was unknown. On the other hand, within the control population, 15 (35.7%) were smokers and 27 (64.3%)

non-smokers. There was no significant difference between the two groups in regard to the history of smoking.

The patients were diagnosed by a psychiatrist according to axis I of DSM-IV (The fourth edition of the Diagnostic and Statistical Manual of Mental Disorders) criteria. None of the patients was diagnosed with axis II of DSM-IV (mental retardation or personality disorders). The exclusion criteria for the patients were taking neuroleptic or any other medications for at least two weeks, having long-acting injections during the past two months, having any accompanying somatic illness, and abusing alcohol and drugs. Exclusion criteria for the control group were having any psychiatric disorder and receiving medications potentially affecting the immune system for a period of 2 weeks prior to sampling.

Preparation of Peripheral Blood Mononuclear Cells (PBMCs). 5cc of heparinized blood was taken from each subject. Blood samples were then diluted 1:1 with RPMI-1640 culture medium and layered on Ficoll-Hypaque (1.077) and centrifuged at 400g for 20 minutes at 24°C. The interface was washed twice with RPMI-1640, each time centrifuging at 280 g for 20 minutes. Cell suspension was diluted to 5×10^5 cells/ml with RPMI-1640 supplemented with 10% FBS and antibiotics.

T Cell Proliferation in Response to PHA. MTT test was performed as described previously (22). Briefly, PBMC (in a concentration of 5×10^5 cells/ml) together with 2% PHA (Gibco, Germany) were cultured in flat bottomed 96 well sterile cell culture plates in a total volume of 200µl.

After 72 hours of incubation at 37°C in an incubator with 5% CO_2, 20 µl of a MTT solution (5mg/ml PBS) was added to each well. After 4 hours of incubation, 100µl of an acidified SDS solution (10% SDS in 0.01 N HCL) were added to each well. The plates were incubated for another 16 hours. Optical densities of the wells were read at 570 nm against the reference wavelength of 630 nm by an ELISA reader (Behring, Germany).

Stimulation Index for each test was calculated as: $$SI\% = \frac{OD_{(T)} - OD_{(B)}}{OD_{(C)} - OD_{(B)}}$$

Indirect Immunofluorescence. Indirect immunofluorescent assay was performed using 1:20, 1:40 and 1:160 diluted sera of the subjects. Mouse liver sections were fixed on slides as antigen and FITC (Fluorescein-Iso Thio Cyanate) conjugated with anti-human-antibody (DAKO, Denmark) was used as described before (23). ACA and ANA were detected by IF microscope (Zeiss, Germany).

CIC Concentration Assay by PEG Precipitation Test. As described in literature (24), 0.22 ml of 1:3 diluted sera in borate buffer (6.8gr boric acid + 9.4gr borax (6H2O) + 4.38grNaCl, in a total volume of 1000 ml with distilled water) were added to 2 ml PEG solution (4.16gr% PEG in borate buffer). The same volume of identically diluted sera was added to 2 ml of borate buffer. All tubes were incubated in dark and kept at room temperature. After 60 minutes of incubation, optical densities of all tubes were read against the blanks at 340 nm in a spectrophotometer (Pharmacia, Sweden). Standard curve was obtained using different concentrations of 5, 2.5, 1.25, 0.625, 0.3125 and 0.15625 mg/ml aggregated human gamma-globulin (Sclavo, Italy). The data obtained using the standard curve were then multiplied by 3 (dilution index of the sera) to represent CIC concentration.

Data Analysis. Data were analysed by SPSS ver. 11.5 software. Independent Student t-test, Pearson Correlation test, Mann-Whitney test, Chi-Square test, and Fisher's exact

test were applied. Results were then reported as Mean ± Standard Deviation (SD). Differences with the P value of less than 0.05 were considered significant.

RESULTS

The mean SI among patients was 1.96417 ± 0.830684, while the control population had a mean of 3.72971 ± 1.398237. Analysis of these data by Independent t-test showed a significant decrease in SI as compared with healthy subjects ($p< 0.001$).
Analysis of ANA level using Mann-Whitney test showed no significant difference between the two groups ($p = 0.661$). Mean CIC concentration in patient group was 4.838451 ± 2.48259228 while in the control subjects its value was 6.413372 ± 3.9370669 and no significant difference was found between the two populations using Independent t-test ($p= 0.102$). ACA was negative in 24 patients and positive in 6, while it was negative in all of the healthy subjects. Analysis of these data using Fisher's exact test showed a significant difference between the case and the control group ($p=0.004$).
Effects of the sex, age, age of disease onset, duration of illness, smoking status and disease subgroup based on the immunological indices were also evaluated. The only significant correlation was a positive relation between the ANA level and the age of onset ($p< 0.001$). No significant difference was found between paranoid and non-paranoid categories in the SI, CIC, ANA or ACA (Table 1).

Table 1. Comparison of different Immunologic parameters between the case and control groups

Groups	SI (%)		CIC (mg/ml)		ANA (Titer)	ACA (%)	
	Mean	S.D	Mean	S.D.	Mean Rank	Neg.	Pos.
Case	1.964	0.831	4.838	2.483	37.3	80.0	20.0
Control	3.730	1.398	6.413	3.937	35.93	100.0	0.0
P value	<0.001		0.102		0.661	0.004	

SI: Stimulation Index, CIC: Circulating Immune Complexes, ANA: Anti-Nuclear Antibody ACA: Anti-Cytoplasmic Antibody

DISCUSSION

In this study a decrease in T cell response to mitogen was found in patients compared with normal subjects. Decreased T cell proliferation and higher ANA levels and an HLA B8/DR3 genotype in schizophrenic patients was previously reported (6). Decreased proliferative response of T lymphocytes to PHA in patients receiving anti-schizophrenic medications as compared with normal population has also been reported (8). Also, a report of decreased production of IL-2 in response to PHA in drug-naïve schizophrenics as compared with healthy individuals is presented (9).
Contrary to these reports, no difference in T cell proliferative response to mitogens in schizophrenic patients and normal subjects or between the medicated and drug-free patients were reported by some investigators (7). The decreased T cell proliferation in response to mitogens can be considered as an intrinsic characteristic of schizophrenia, since it has been reproduced by almost all studies. The existing controversy could be attributed to either the confounding effects of antipsychotic drugs or the heterogeneity among studied populations.

In this study, increased production of ACA in the patient group was also noticed. This result along with the lower proliferative response of T cells to mitogens could still support the hypothesis for the role of autoimmunity in etiology of schizophrenia, although the type of target autoantigen(s) remains a major challenge and drawback. In our study, no significant difference was found in the concentration of CIC between the schizophrenics and the healthy individuals. However, increased production of CIC has been noticed in some other investigations (17, 19, 25).This could be attributed to either the confounding effects of neuroleptics or the etiological/genetic heterogeneity of the disease. Thus, it could be hypothesized that CIC concentration might also be related to the nature of the autoantigen(s) involved.

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