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Expression of anti-cardiolipin antibodies and inflammatory associated factors in patients with schizophrenia

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ABSTRACT

Numerous evidences have implicated a connection between schizophrenia and autoimmune disorders. However, the precise relationship and underlying mechanism are still obscure. To further identify the association between autoimmune disorders and schizophrenia, the mRNA expressions of various cytokines and Toll-like receptors (TLRs) in monocytes are examined by using RT-PCR. Additionally, ELISA and zymography were performed to determine the anti-cardiolipin antibody (aCL) and MMP9 activity in serum form schizophrenic patients. Notably, significantly increased interleukin (IL)-6 and IL-10 mRNA were observed in schizophrenic patients, whereas the significant reductions of TLR-3 and TLR-5 mRNA were detected. Moreover, significantly increased aCL antibody and higher frequency of positive-MMP9 activity were detected in serum from patients with schizophrenia. Meanwhile, no significant association was found between each of the medication and aCL activity. These findings demonstrated the autoimmune related phenomena in schizophrenic patients and further suggested a connection between schizophrenia and autoimmune disorders.

1. Introduction

Schizophrenia (SZ) is an exhausting sickness. Along with the behavioral and mental deterioration observed in patients with schizophrenia, many neuro-imaging and postmortem findings reflect significant neuro-degenerative process (Lieberman, 1999). Recently, a study indicated that history of any autoimmune disease was associated with a 45% increase in risk for schizophrenia (Eaton et al., 2006). Although the mechanism underlying is still obscure, increasing evidences have been associated autoimmune disorders with schizophrenia.

Previously, schizophrenia has been proposed to be an autoimmune disorder in which antibodies are elevated against specific self-antigen in brain (Bergen et al., 1980; Heath et al., 1989; Henneberg et al., 1994). In a clinical study, anti-brain antibodies were detected in sera and cerebrospinal fluid from 26 out of 54 schizophrenic patients but in none of 27 healthy controls (Pandey et al., 1981). Another study indicated that increased CD5 positive B-lymphocytes in schizophrenic patients were observed and recognized to play a role in the pathogenesis of schizophrenia (McAllister et al., 1989). Moreover, autoantibodies against platelet or nicotinic acetylcholine receptors in patients with schizophrenia were also reported (Shinitzky et al., 1991; Mukherjee et al., 1994). Significant increases of various natural autoantibodies, including antinuclear, anti-double-stranded DNA, anti-Sm, and anti-single-stranded DNA autoantibodies, were significantly more frequent in schizophrenic patients than in normal subjects (Sirota et al., 1993). These reports did suggest the association between schizophrenic and autoimmune disorders.

Evidences have indicated that IL-6 is a key mediator of various autoimmune diseases such as SLE and RA (Cronstein, 2007) and targeting IL-6 might be a promising therapy of autoimmune diseases (Fujimoto et al., 2008). Apparently, similar phenomena have been reported in schizophrenia patients. Various cytokines are also recognized to be

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involved in the development of schizophrenia. Abnormal cytokine profile and increasing activated CD4± and CD16± natural killer cells were observed in schizophrenic patients (Theodoropoulou et al., 2001). Another study indicated the elevated level of IL-6 in schizophrenia (Naudin et al., 1996) as well as the increased levels of IL-2 and IL-8 in serum from neuroleptic-free schizophrenia was reported in other studies (Ganguli et al., 1994; Zhang et al., 2002). These evidences suggested that IL-6 play a role between autoimmune diseases and schezophrenia.

Toll-like receptors (TLRs) have been known to play important roles in innate immunity and associated with autoimmune diseases (Abdollahi-Roodsaz et al., 2007; Barrat and Coffman, 2008). Genetic variations in TLR5 and TLR9 have been associated with the disease activity of autoimmune disease such as SLE (Hawn et al., 2005; De Jager et al., 2006). A recent study demonstrated that TLR-7 is specifically required for the production of RNA-reactive autoantibodies and the development of glomerulonephritis in pristane-induced murine lupus mice (Savarese et al., 2008). Another study indicated that TLR9 knockout mice revealed exacerbated autoimmune disorders including activated lymphocytes, plasmacytoid dentric cells, increased serum IgG and IFN-alpha whereas TLR7 knock out mice revealed ameliorated disease and failed to generate antibody against Smith (Sm) Ag (Christensen et al., 2006). However, the relationships between autoantibody and innate immunity such as Toll-like receptors (TLRs) in patients with schizophrenia are still unknown. In the current study, we firstly reported the decreased mRNA expression of TLR-3 and TLR5 in monocytes from schizophrenic patients, which is associated with the increased anti-cardiolipin antibodies and mRNA expression of IL-6 and IL-10, and suggested a connection between schizophrenia and autoimmune disorders.

2. Materials and Methods

2.1. Patients and monocytes isolation

Twenty-two healthy individuals and forty-six volunteer in-patients from a psychiatric unit participated in this study, approved by Institutional Review Board (IRB), Tsao-Tun Psychiatric Center, Department of Health, Executive Yuan, Taiwan. Patients were recruited if they experienced psychosis (hallucinations or delusions) during or just prior to admission. All patients willing to volunteer were accepted without exclusion and diagnosis was made by a single board certified psychiatrist (Huei-Huang Tsai). The majority of patients were on neuroleptic medication. The monotyes were isolated from the peripheral blood monocyte cells (PBMC) of healthy and schizophrenic subjects by using Histopaque-1077 (Sigma Chemicals, Poole, Dorset, UK) according to the manufacture's instructions.

2.2. RT-PCR

All studies were carried out in a designated PCR-clean area. RNA was extracted from isolated cells using a Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Total RNA was isolated from the monocytes of healthy individuals and schizophrenic subjects. RNA samples were resuspended in diethyl pyrocarbonate (DEPC)-treated water, quantified, and then stored at −80 °C until used. RNA concentration and purity were determined by a spectrophotometer by calculating the ratio of optical density at wavelengths of 260 and 280 nm. The first-strand of cDNA for RT–PCR was synthesized from the total RNA (2 μg) using the Promega RT–PCR system (Promega, Madison, Wisconsin, USA). Specific primer sets are listed in Table 1. The amplification was performed in a 50 μl reaction volume containing 1×reaction buffer (Promega), 1·5 μM of MgCl2, 200 μM of dNTPs, 1 μM of each primer and 2·5 units of Taq DNA polymerase (Promega, Madison, WI, USA) using a Perkin-Elmer Gene Amp PCR system 2400. Each cycle consisted of denaturation at 95 °C for 1 min, annealing at 60 °C for 45 s and amplification at 72 °C for 45 s. The RT–PCR-derived DNA fragments, obtained by 25 PCR cycles, were subjected to electrophoresis in a 4% acrylamide gel. Following staining with ethidium bromide, the gels were photographed. The specific RNA level of every sample was expressed as the product's intensity and the cDNA encoding glyceraldehyde-3-phosphate -dehydrogenase (GAPDH) was amplified as internal control.

2.3. ELISA

The serum samples were collected from healthy and schizophrenic subjects. The antibodies against cardiolipin were measured with commercial QUANTA Lite ACA IgG III enzyme-linked immunoabsorbent assay kits (INOVA Diagnostic Inc., San Diego, CA, USA). Anti-human IgG-HRP conjugate was used as the secondary antibody and the color developed with chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB). The enzymatic reaction is directly proportional to the amount of antibodies present in the sample.

Table 1
Primer pairs used for RT-PCR.

Gene type	Sequence	Size
IL-12	F 5'-CAGACCCAGGAATGTTCCCA-3' R 5'-TCTTGAACTCCACCTGGTAC-3'	310 bp
IL-10	F 5'-GGACAACATACTGCTAACCGAC-3' R 5'-AAAATCACTCTTCACCTGCTCC-3'	256 bp
IL-13	F 5'-ATGCATCCGCTCCTCAATCC-3' R 5'-TCTTCTCGATGGCACTGCAG-3'	286 bp
IFN-γ	F 5'-CTTGGCTGTTACTGCCAGGA-3' R 5'-TTCCTTGATGGTCTCCACAC-3'	240 bp
IL-6	F 5'-ATGAACTCCTTCTCCACAAGCGC-3' R 5'-GGATCAGGACTTTTGTACTCATC-3'	454 bp
IL-5	F 5'-GCTAGCTCTTGGAGCTGCCT-3' R 5'-CTATTATCCACTCGGTGTTC-3'	370 bp
TGF-ß	F 5'-CTGCTACCGCTGCTGTGGCT-3' R 5'-GGGTGCTGTTGTACAGGGCG-3'	211 bp
TLR-4	F 5'-ATGATGTCTGCCTCGCGCCT-3' R 5'-CCCAGGGCTAAACTCTGGAT-3'	349 bp
TLR-1	F 5'-ATGACTAGCATCTTCCATTTTGCC-3' R 5'-GCCAAACTCTTTGCATATAGGC-3'	408 bp
TLR-3	F 5'-ATGAGACAGACTTTGCCTTG-3' R 5'-TCTGGCACAATTCTGGCTCC-3'	279 bp
TLR-5	F 5'-ATGGGAGACCACCTGGACCTTC-3' R 5'-TCTTACTACTTCCCAGGTCCAAG-3'	319 bp
TLR-2	F 5'-ATGCCACATACTTTGTGGATGG-3' R 5'-CCTCTGTAGGTCACTGTTGCTA-3'	221 bp
TLR-9	F 5'-ATGGGTTTCTGCCGCAGCGC-3' R 5'-CTGGTGACATTGCCACGGGG-3'	200 bp
CD14	F 5'-CGTCCTGCTTGTTGCTGCTG-3' R 5'-CCAGTAGCTGAGCAGGAACC-3'	309 bp
TLR-6	F 5'-ATGTCTCAGAACTACATCGCTG-3' R 5'-GATGGGCAGGGCCTTGAAATCAT-3'	240 bp
GAPDH	F 5'-ATGGGGAAGGTGAAGGTCGG-3' R 5'-TGGTGAAGACGCCAGTGGAC-3'	310 bp

IL indicates interleukin. IFN indicates interferon. TGF indicates tumor growth factor. TLR indicates toll-like receptor. CD indicates cluster of differentiation. GAPDH indicates glyceraldehyde-3-phosphate dehydrogenase. F indicates forward primer. R indicates reverse primer.

2.4. Gel Zymography

MMP-9 and MMP-2 activities were analyzed by gelatin zymography. Ten microliters of diluted serum was separated on an 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) gel polymerized with 1 mg/ml gelatin. Gels were washed once for 30 mins in 2.5% Triton X-100 to remove the SDS and then soaked in the reaction buffer containing 50 mM Tris-HCl, 200 mM NaCl, 10 mM CaCl2, and 0.02% (w/v) Brij 35 (Sigma, St. Louis, MO; pH 7.5) for 30 mins. The reaction buffer was changed to a fresh one, and the gels were incubated at 37 °C for 24 hrs. Gelatinolytic activity was visualized by staining the gels with 0.5% Coomassie brillant blue and quantified by densitometry (Appraise; Beckman-Coulter, Brea, CA).

2.5. Statistical analysis

All the statistical analyses were performed using SPSS 10.0 software (SPSS Inc, Chicago, IL). Three independent experiments were repeated. Statistical analyses were performed using the one-way ANOVA or Chi-square. $P<0.05$ was considered statistically significant.

3. Results

3.1. Up-regulation of IL-6 and IL-10 mRNA in monocytes from schizophrenic patients

Forty-six volunteer patients with schizophrena and 22 healthy subjects agreed to donate peripheral blood and the analyses. Schizophrenic subject age at time of enrollment ranged from 24 to 61 years (average 40±10.42). The gender of the participants was all male and the racial background was 100% Taiwanese. The age of 22 healthy subjects was range from 21 to 55 (average 33±5.88). No significant differences of various biochemistry and blood-cell parameters between schizophrenic and healthy subjects were observed (Table 2). To investigate the mRNA expression of cytokines in monocytes from healthy and schizophrenic subjects, RT-PCR was performed to

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Table 2
Characteristics of healthy controls and schizophrenic (SZ) subjects.

	SZ subjects (N = 46)	Normal subjects (N = 22)
Age	40 ± 10.42	33 ± 5.88
Gender		
Male	46	22
Female	0	0
Age of first episode	30 ± 9.81	-
Years of illness	10 ± 6.30	-
CGI score		
6	1	-
5	4	-
4	7	-
3	27	-
2	7	-
Glucose	89.2 ± 15.70	88.6 ± 11.2
Bilirubin	10.3 ± 3.49	11.3 ± 2.7
Uric Acid	5.5 ± 1.75	6.4 ± 1.1
Creatinine	0.9 ± 0.17	0.9 ± 0.1
Aspartate aminotransferase	23.0 ± 7.94	21.0 ± 4.1
Alanine aminotransferase	24.0 ± 14.27	22.7 ± 7.4
Cholesterol	165.9 ± 34.18	176.6 ± 20.2
Triacylglycerol	112.3 ± 75.98	114.2 ± 43.2
Bili-D	0.1 ± 0.05	0.1 ± 0.1
Bili-T	0.7 ± 0.36	1.0 ± 0.2
r-Glutamyl Transpeptidase	24.1 ± 23.10	22.0 ± 11.1
Alkaline phosphatase	65.4 ± 22.38	58.6 ± 15.1
Red blood cell	465.4 ± 45.0	483.2 ± 30.2
Hemoglobulin	14.3 ± 1.2	14.8 ± 0.74
Ht	48.0 ± 9.2	42.8 ± 2.7
White blood cell	7287.3 ± 2230.7	5909 ± 1100
Platelet	230.1 ± 57.4	250.8 ± 44.3
Segmental cell	58.0 ± 11.9	55.9 ± 6.6
Lymphocyte	31.1 ± 11.0	33.2 ± 5.7

CGI indicates clinical global impression.

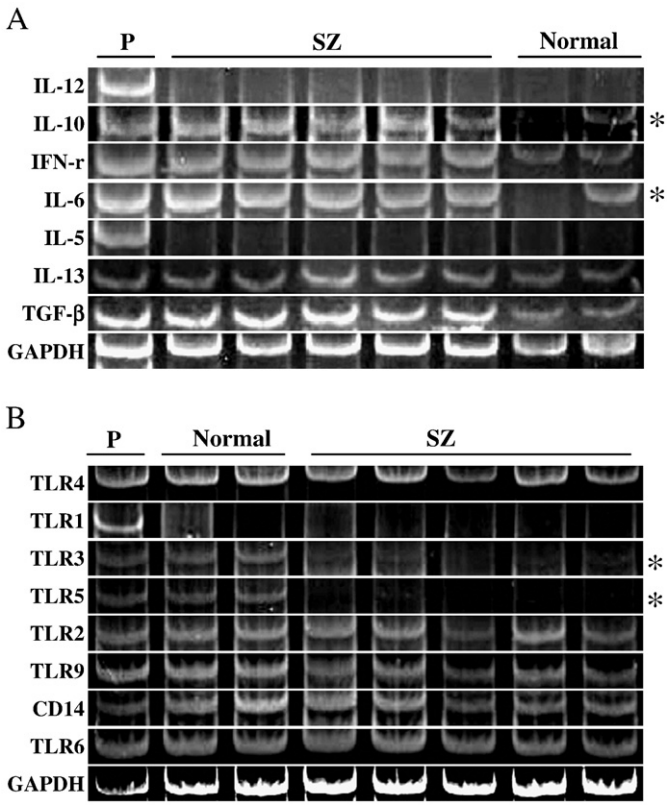

Fig. 1. Expression of various cytokines and Toll-like receptors. The mRNA of monocytes from schizophrenic and normal subjects were isolated and extracted for analysis. The mRNA expressions of (A) cytokines and (B) TLRs were detected by RT-PCR. P, SZ, and Normal indicate positive, schizophrenia, and healthy controls, respectively. Three independent experiments were performed and similar results were observed.

determine the mRNA expression of IL-5, IL-6, IL-10, IL-12, IL-13, IFN-γ, and TGF-β (Fig. 1A). Significant difference in IL-6 and IL-10 mRNA expression between healthy and schizophrenic subjects was observed (Table 3). Five out of 22 healthy subjects (22.7%) express IL-6 mRNA whereas 43 out of 46 (93.5%) schizophrenic subjects express IL-6 mRNA. Ten out of 22 healthy subjects (45.5%) express IL-10 mRNA whereas 41 out of 46 (89.1%) schizophrenic subjects express IL-10 mRNA. No significant difference in IL-5, IL-12, IL-13, IFN-γ, and TGF-β mRNA expression was detected.

3.2. Down-regulation of TLR-3 and TLR-5 mRNA in monocytes from schizophrenic patients

To study the association between innate immunity and schizophrenic, expression of various TLR mRNA were determined. Fig. 1B revealed the RT-PCR results of TLR-1, TLR2, TLR-3, TLR4, TLR-5, TLR-6, TLR-9, and CD14. Significant difference in TLR-3 and TLR-5 mRNA expression between healthy and schizophrenic subjects was observed (Table 4). Sixteen out of 22 healthy subjects (72.7%) express TLR-3 mRNA whereas only 15 out of 46 (32.6%) schizophrenic subjects express TLR-3 mRNA. All 22 healthy subjects (100%) express TLR-5 mRNA whereas 3 out of 46 (6.5%) schizophrenic subjects express TLR-5 mRNA. No significant difference in TLR-1, TLR2, TLR4, TLR-6, TLR-9, and CD14 mRNA expression was detected.

3.3. Increased binding activity of anti-cardiolipin antibody and MMP9 activity in serum from schizophrenic patients

To further verify the connection between schizophrenia and autoimmunity, aCL antibody was detected in serum from healthy and schizophrenic patients. ELISA was performed to detect the binding activity of antibody against cardiolipin and the result was shown in Fig. 2A. Notably, binding activity of aCL antibody was significantly higher in serum of schizophrenic patients as compared to those from healthy subjects (Fig. 2A). Moreover, MMP9 activity was also detected, which is known as an inflammatory indicator and has been associated with autoimmune diseases. Fig. 2B shows the representative results of zymography. Significantly higher frequency of positive MMP9-activity was detected in serum from patients with schizophrenia as compared to those from healthy subjects (Table 5). Additionally, to verify whether the medications have influences on cardiolipin autoantibody development in patients with schizophrenia, statistic assay was performed. All patients were taking at least one and some were taking several antipsychotic medications (Table 6). Notably, no significant associations between any of these neuroleptic medications and aCL antibody development were found.

Table 3
Presence of different cytokine mRNA in control individuals and schizophrenic patients.

Gene type	Control (n = 22)	Patients (n = 46)	χ^2	df	P-value[a]
IL-12	0/22 (0)	0/46 (0)	0	1	-
IL-10	10/22 (45.5)	41/46 (89.1)	12.8992	1	<0.001*
INF-γ	21/22 (95.5)	45/46 (97.8)	0.05275	1	>0.05
IL-6	5/22 (22.7)	43/46 (93.5)	32.56	1	<0.001*
IL-5	0/22 (0)	0/46 (0)	-	1	-
IL-13	19/22 (86.4)	42/46 (91.3)	0.04	1	>0.05
TGF-β	21/22 (95.5)	45/46 (97.8)	0.05275	1	>0.05

Numbers in parentheses are percentages.
χ^2 indicates the Chi-Square value.
df indicates the degree of freedom.
[a] Chi-Square was performed to determine the P-value.
* indicates statistically significant deviation.

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Table 4
Presence of different TLR mRNA in control individuals and schizophrenic subjects.

Gene type	Control (n = 22)	Patients (n = 46)	χ^2	df	P-value[a]
TLR4	21/22 (95.5)	46/46 (100)	0.1435	1	>0.05
TLR1	0/22 (0)	0/46 (0)	-	1	-
TLR3	16/22 (72.7)	15/46 (32.6)	8.1068	1	<0.001*
TLR5	22/22 (100)	3/46 (6.5)	51.98877	1	<0.001*
TLR2	22/22 (100)	44/46 (95.6)	0.050856	1	>0.05
TLR9	22/22 (100)	46/46 (100)	-	1	-
CD14	22/22 (100)	46/46 (100)	-	1	-
TLR6	22/22 (100)	46/46 (100)	-	1	-

Numbers in parentheses are percentages.
χ^2 indicates the Chi-Square value.
df indicates the degree of freedom.
[a] Chi-Square was performed to determine the P-value.
* indicates statistically significant deviation.

4. Discussion

Schizophrenia has been associated with a variety of immune abnormalities than heretofore suspected. Irregular levels or activity of lymphocytes, cytokines, or cytokines receptors, or symptoms of autoimmune disease were observed in patients with schizophrenia, which may play a role in the etiology of schizophrenia (Fudenberg et al., 1983; Ganguli et al., 1989; Smith, 1991; Gilmore and Jarskog, 1997; Eaton et al., 2006). However, the underlying psychopathologic mechanisms of schizophrenia and the underlying mechanism in immune regulation are still obscure. In current study, we reported the increased mRNA expression of IL-6 and IL-10, MMP-9 activity, and reduced TLR-3 and TLR-5 mRNA in monocytes from schizophrenic patients and suggested a connection with the increased activity of anti-cardiolipin antibody.

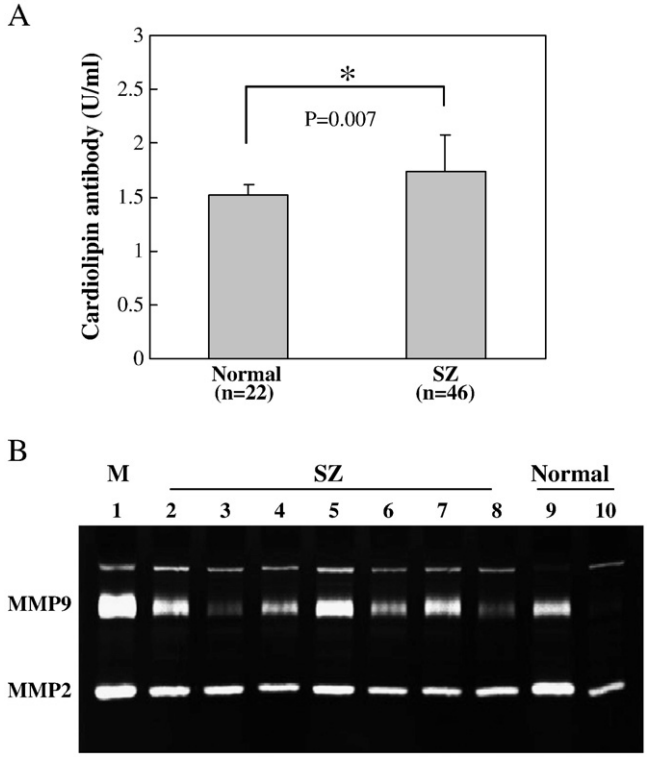

Fig. 2. Detection of anti-cardiolipin antibody and MMP9/2 activity. The serum from schizophrenic and normal subjects was collected and (A) the reactivity of anti-cardiolipin antibody was detected. (B) The figure shows representative results of MMP9/2 zymography. M, SZ, and Normal indicate standard MMP9/2 marker, schizophrenia, and healthy controls, respectively. Three repeated experiments were performed and similar results were obtained.

Table 5
Frequency of MMP9 activity in serum of control individuals and schizophrenic patients.

	Control (n = 22)	Patients (n = 46)	P-value[a]
MMP9	7/22 (31.2)	39/46 (84.8)	<0.001*

Numbers in parentheses are percentages.
[a]Chi-Square was performed to determine the P-value.
(*) indicates statistically significant deviation.

Cytokines are known to play crucial roles in many physiological and pathological processes including schizophrenia and autoimmune disorders. Significantly increased serum levels of IL-6 and IL-8 were also founded in patients with schizophrenia (Naudin et al., 1996; Ganguli et al., 1994; Zhang et al., 2002). Meanwhile, increased levels of IL-4, IL-6, and IL-10 were reported in CSF of schizophrenic patients (Zhang et al., 2002; Muller and Schwarz, 2006) that has been demonstrated to be associated with the pathological development of autoimmune diseases (Cronstein, 2007). In current study, similar results were observed that elevated expression of IL-6 and IL-10 mRNA was observed in monocytes from schizophrenic patients. Moreover, significantly higher binding activity of aCL antibody and frequency of MMP9-positive was founded in schizophrenic patients, which have been known as important indicators for autoimmune diseases such as SLE (Sammaritano and Gharavi, 1992; Ainiala et al., 2004; Robak et al., 2006; Hsu et al., 2008). Indeed, these findings implied an association between schizophrenia and autoimmune disorders and may provide a more credible detection in patients with schizophrenia although the precise mechanism underlying still needs further investigations.

Recently, toll-like receptors (TLRs) are known to play crucial roles in innate immunity and the development of autoimmune disorders (Hawn et al., 2005; Abdollahi-Roodsaz et al., 2007; Barrat and Coffman, 2008). A recent study indicated that TLR-7 is important for the production of

Table 6
Neuroleptic medications.

Medication	Number of subject	Serum aCL positive	P
Anxiedin	4	2	0.507
Apa-Risdol	14	4	0.966
Apo-Divalproex	6	0	0.164
Apo-Haloperidol	5	1	0.715
Ativan	8	2	0.831
Betamac	3	1	0.905
Biperiden	2	0	0.438
Cebotval	5	2	0.687
Clopine	22	4	0.363
Clozaril	8	5	0.123
Dogmatyl	3	0	0.338
Domilium	3	0	0.338
Eszo	16	4	0.737
Etumine	3	1	0.905
Euglucon	2	0	0.438
Eurodin	2	1	0.648
Fluanxol	2	0	0.438
Gendergin SR	2	1	0.348
Haldol	2	1	0.648
Halin	2	2	0.175
Inderal	17	4	0.586
Lodopin	5	0	0.208
Luvox	10	2	0.585
Mezapin	6	1	0.577
Rivotril	6	1	0.577
Seroquel	3	0	0.338
Silence	3	1	0.905
Solian	3	0	0.338
Surin	2	2	0.175
Switane	14	8	0.054
Tegretol	2	0	0.438
Uspen	3	1	0.905

All patients in the study received pharmaceutical administration at the time of serum collection. No significant association was found between each of the medication and aCL activity.

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RNA-reactive autoantibodies and the development of glomerulonephritis (Savarese et al., 2008). In a gene-knocked lupus-prone mice, the deficiency of TLR9 gene led to exacerbated autoimmune disorders such as activated lymphocytes, plasmacytoid dentric cells, increased serum IgG and IFN-alpha (Christensen et al., 2006). Although irreverent innate immunity has been reported in patients with schizophrenic such as increasing activated CD4+/CD16+ natural killer cells in schizophrenic, no study reported the profiles of TLRs in patients with schizophrenic (Theodoropoulou et al., 2001). However, no study of TLRs expression in schizophrenic patients was reported. In current study, we firstly reported the reduced expression of TLR-3 and TLR-5 mRNA in monocytes from patients with schizophrenia as well as the significantly increased binding activity of anti-cardiolipin antibody that has been known to play crucial roles in various autoimmune disorders. These findings may provide a clue in understanding the association of abnormal innate immunity in schizophrenic patients.

Several observational studies have suggested a link between the use of antipsychotics drugs and the phenomena of autoimmune disorders, such as increased thrombosis and aCL antibodies. Indeed, clozapine have been reported to induce venous thromboembolism in psychiatric patients (Brenner and Metz, 1995; Hägg et al., 2000). Recently, the association between conventional antipsychotics and venous thrombosis has been strengthened by an epidemiological research (Hägg and Spigset, 2002; Liperoti et al., 2005). Additionally, the correlation between the treatment of neuroleptic drugs and serum level of aCL, especially clozapine, has also been reported (Knudsen et al., 2000; Shen et al., 2009). A higher serum clozapine level is associated with an increased level of aCL antibodies in schizophrenia patients (Shen et al., 2009). Although these epidemiological data support an association among the treatment of neuroleptic drugs and increased thrombosis and aCL antibodies in psychiatric patients, the biological mechanisms involved in the pathogenesis are still obscure. In the current study, no significant correlations between any of the neuroleptic medications and increased aCL antibody level were found, including clozapine. Relatively, predominant autoimmune phenomena including increased MMP-9 activity and mRNA of IL-6 and IL-10 were reported in this study. Therefore, these findings demonstrated the autoimmune related phenomena in schizophrenic patients and suggested further connections between schizophrenia and autoimmune disorders. Although the influence of medications in psychiatric patients cannot be neglected and needed further investigations, this study did provide an alternative understanding for schizophrenic patients in developing the autoimmune disorders.

Contributors

Authors TCH conceived this study, drafted the manuscript, and performed the performed statistical analyses. CCT performed the RT-PCR and Zymography. YCT performed the Zymography. HHC provided the data necessary for our analysis. SHK, SHC and CYH provided material support and encouragement for this work. BST designed the study and provided material support, and drafted significant portions of the manuscript. All authors contributed to and have approved the final manuscript.

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Funding for this study was provided by Department of Health (DOH), Taiwan, ROC and had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Conflict of interest

There are no conflicts of interest.

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