In vivo type II T-helper cells shift in schizophrenia compared to sex- and age-matched healthy controls

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ABSTRACT – *Background and Objectives:* Over-production of the type II T-helper cells (Th2-shfit) has been suggested as a candidate mechanism for the etiology in at least one subgroup of schizophrenia. Hitherto, empirical evidence is derived mostly from in vitro cytokine production. Due to frequently undetectable serum levels of the major Th2 cytokine Interleukin-4 (IL-4), direct evidence, measured as a ratio between Th1/Th2 (type I/type II T-helper cells) characteristic cytokines, is rare. This study aimed at examining whether a serum Th2-shift occurs in schizophrenia. Th2-shift was defined as markedly decreased serum IFN-γ/IL-4 and/or IFN-γ/IL-10 and/or IL-2/IL-4 ratios, compared with healthy subjects.

Methods: Totally 74 subjects were recruited: 37 drug-free subjects with schizophrenia according to ICD-10 and DSM-IV as well as 37 age- and sex-matched healthy controls. Cytometric Bead Array, which enables a simultaneous measurement of 6 cytokines with the same volume of test sample, was used to assess serum Th1/Th2 ratios and cytokine levels. Non-parametric Mann-Whitney U test was utilized to detect the diversities in serum Th1/Th2 ratios and cytokine levels between both diagnostic groups.

Results: Subjects with schizophrenia showed significantly reduced serum IFN-γ/IL-4 and IFN-γ/IL-10 ratios if compared to healthy controls. If both sexes analyzed separately, males with schizophrenia had significantly reduced serum IFN-γ/IL-10 ratios, while female patients showed markedly decreased serum IFN-γ/IL-4 ratios.

Conclusions: A clear Th2-shift was observed in schizophrenia. Males and females with schizophrenia seemed to have different profiles of Th2-shift. Th1/Th2 ratios appeared to play different roles in the pathology of males and females with schizophrenia.

Introduction

Immune dysfunction is thought to link to the pathomechanism of schizophrenia[1]. Epidemiological studies relating to antibody titers against various viruses indicate that viral infection could be the cause of immune dysfunction in one subgroup of schizophrenics[2]. Several viral infections have been associated with the risk of this disorder[3-5]. During antiviral immunity, accurate control of the balance between the type I and type II T-helper cells (Th1 and Th2) is pivotal for optimizing immune response[6]. As a result of host defense against diverse viral infections and protective mechanisms from autoimmunity, Th1/Th2 imbalance was often implicated[7-9]. Th1 cells mainly produce interferon-gamma (IFN-γ), interleukin-2 (IL-2), and tumor necrosis factor-a (TNF-α), while Th2 lymphocytes predominantly release interleukin-4 (IL-4), interleukin-10 (IL-10), and interleukin-6 (IL-6)[10-12].

In vivo serum data reflect the possible cumulative effects of diverse biological systems. Although they show that subjects with schizophrenia had comparable serum IL-2[13-16], TNF-α[17-23], IL-4[16], IL-10[24], and IFN-γ[13, 25-27] levels with healthy controls, much more empirical findings concerning the major Th1 cytokine, IFN-γ, in vitro production[16, 28-35] and Th2 cytokine, IL-6, serum, plasma, and CSF levels[16,17, 36-46] revealed under-production of Th1-cytokines, however, over-production of Th2-cytokines in schizophrenia. They suggest a possible Th1/Th2 imbalance in schizophrenia. Th2-shift was therefore speculated as a potential cause of schizophrenic symptoms in at least one subgroup of schizophrenia[47-49].

Empirical studies examining individual Th1/Th2 cytokines in schizophrenia are plenty, while those investigating the ratios between the in vivo levels of major Th1/Th2 cytokines are rare. Due to the inhibitory effects of IL-10[50] and IL-4[51] on Th1 cells, IFN-γ[52] on Th2 cells as well as the role of IL-4[53] in Th2 and IFN-γ[54] in Th1 development, IFN-γ/IL-4[55] and IFN-γ/IL-10 ratios[55] have been frequently used to examine Th1/Th2 immunity. Up to date, only two studies[56, 57] examined IFN-γ/IL-4 ratios in schizophrenia; one found unaltered in-vitro IFN-γ/IL-4 ratios, while the other showed increased in-vivo ones. In this study, we attempted, based on evidence from studies on individual Th1/Th2 cytokines, to examine whether in vivo Th2-shift, defined as remarkably reduced serum IFN-γ/IL-4 and/or IFN-γ/IL-10 ratios, occurred in schizophrenia since they are regarded as indicators of Th1/Th2 balance[58,59] due to their roles in the differentiation and development of the Th1/Th2 systems. In addition, IL-2 and IL-4 may regulate Th1/Th2 balance and activity in brain function[60]. Thus, the IL-2/IL-4 ratios were also examined.

Methods

Participants

Thirty-seven subjects with schizophrenia and 37 healthy controls were recruited. Each group consists of 14 females and 23 males. After the aim of the study fully explained, they had given their written informed consent for participating in this study. The obligatory inclusion criteria for all participants were: free of any severe medical diseases, acute allergies, inflammatory disorders, autoimmune diseases, and clinically apparent infections. These were controlled through conducting thoroughly laboratory and medical examinations. Further crucial criteria for subjects with schizophrenia in-

cluded: (1) neuroleptic-free for at least 7 days, (2) a schizophrenia diagnosis in accordance with ICD-10[61] and DSM-IV[62], (3) no history of psychotropic substance addiction or abuse except nicotine, and (4) no personality disorders according to DSM-IV. Two other important inclusion criteria for healthy controls to fulfill were (1) no psychiatric disorders and (2) no first-degree biological relatives who had (had) any psychiatric diseases. The Positive and Negative Syndrome Scale (PANSS)[63] was used to assess the psychopathology of subjects with schizophrenia. Symptom severity of subjects with schizophrenia was measured by using the Clinical Global Impression (CGI)[64].

Cytokine assessments

Serum of each subject was drawn between 8-9 AM, then immediately centrifuged at 6°C with a speed of 3200 ´ g for 10 minutes, finally frozen at –80°C until analysis. The Human Th1/Th2 Cytokine Cytometric Bead Array (CBA) Kit-II (Becton Dickinson Pharmingen, USA) was applied to detect Th1/Th2 cytokines including IFN-γ, IL-2, TNF-α, IL-4, IL-10, and IL-6. CBA is an ELISA-variant, and is measured by flow cytometry. CBA is as specific and sensitive as ELISA. Both methods share many similarities, including the working mechanism – the antibody-cytokine-antibody sandwich principal. The main reason to choose CBA, instead of conventional ELISA, was the comparability among distinct cytokines measured is higher in CBA than that in ELISA since diverse cytokines of the same individual can be detected with the same test sample. That is, there is no inter-assay variance among distinct cytokines of the same subject. It is,

therefore, particularly proper to scrutinize IFN-γ/IL-4, IFN-γ/IL-10, and IL-2/IL-4 ratios. The whole procedure to measure cytokines was conducted as the manual describes except the volumes of serum, capture bead mixture, and phycoerythrin (PE) detection reagent. Instead of 50 ml, 100 ml of each was used. In addition, the bottom standard was diluted to 2 levels lower than that described in the manual to detect serum IL-4 and IL-2 levels in all cases. According to the information given by the manufacturer, the intra-assay coefficients of variance for IL-2, IL-4, IL-6, IL-10, TNF-a, and IFN-γ are 4-5%, 2-5%, 4-6%, 4-5%, 6-8%, and 3-4%, while those inter-assay ones are 7-9%, 5-11%, 7-13%, 6-11%, 8-12%, and 8-11%, correspondingly.

Experimental design and statistics

The independent variable was diagnostic group (schizophrenia vs. healthy controls). The major dependent variables contained serum Th1/Th2 ratios and various cytokine levels. The discrepancies between both diagnostic groups in age, onset age, hospitalization length, illness duration, the scores of PANSS scales, and those of CGI were examined by using student T-tests. Spearman correlations were applied to examine the associations between Th1/Th2 cytokines/ratios, psychopathology, and symptom severity, whereas Pearson correlations were used to check the relationships of Th1/Th2 cytokines/ratios to hospitalization and illness duration. Non-parametric Mann-Whitney U tests were conducted to detect the differences in serum Th1/Th2 ratios and cytokine levels. The data were evaluated by conducting SPSS (Version 11.5).

Results

Demographical and clinical data

Totally, 74 subjects (age range: 18 – 60 years old) had participated into this study. Thirty-seven of them were subjects with schizophrenia. The remaining 37 were healthy controls. Each group contained 23 males and 14 females. On average, the patient and control groups were 31.65 (SD = 11.22) and 31.70 (SD = 11.13) years old, correspondingly. No remarkable differences in age were shown (t = -0.02, df = 72, p = 0.98). Similarly, between both male and both female subgroups demonstrated likewise no diversities (male: t = -0.01, df = 44, p = 0.99; female: t = -0.02, df = 26, p = 0.99). The mean ages of both male subgroups were 31.13 (SD = 11.27) and 31.17 (SD = 11.10), while these for female patients and controls were 32.50 (SD = 11.53) and 32.57 (SD = 11.53) years old, respectively. Among the 23 males with schizophrenia, 15 were paranoid schiz-

ophrenia, 4 disorganized schizophrenia, 3 catatonic schizophrenia, and 1 schizoaffective disorder. Among the 14 female subjects with schizophrenia, there were 12 paranoid schizophrenia, 1 disorganized schizophrenia, and 1 catatonic schizophrenia according to DSM-IV. Data regarding onset age, hospitalization length, illness duration, PANSS, and CGI scores of some patients were missing; thus, results in these regards concerned only one part of the subjects with schizophrenia. The means for the PANSS positive scale (PANSS P), the negative scale (PANSS N), the general psychopathology scale (PANSS G), the CGI scores at admission (CGI-A) and at discharge (CGI-D) as well as differences between admission and discharge (CGI-diff) in subjects with schizophrenia are summarized in Table 1. Both males and females with schizophrenia were similar in terms of psychopathology, symptom severity, hospitalization, onset age, and illness duration (PANSS P: t = -1.54, df = 23, p = 0.14; PANSS N: t = 0.91, df = 23, p =

Table 1

Clinical data of subjects with schizophrenia. Summary of the means (M) and standard deviations (SD) of the clinical data, psychopathology, symptom severity in subjects with schizophrenia

	Male + female		Male		Female	
	M	SD	M	SD	M	SD
Onset age	26.50	9.61	26.20	10.65	27.00	8.16
Hospital	8.19	6.02	8.17	6.60	8.23	5.10
Ill_dur	5.67	8.68	4.80	8.44	7.11	9.37
PANSS P	22.40	5.45	21.07	5.55	24.40	4.90
PANSS N	26.24	10.04	27.73	10.95	24.00	8.54
PANSS G	49.12	14.12	51.47	14.84	45.60	12.88
CGI-A	5.73	0.78	5.69	0.79	5.80	0.79
CGI-D	3.62	1.58	3.38	1.54	4.00	1.63
CGI-diff	2.12	1.40	2.31	1.58	1.80	1.03

Note: Onset age: years old; Hospital = hospitalization length (weeks); ill_dur = illness duration (years); PANSS = the Positive and Negative Syndrome Scale; PANSS P = The Positive Scale of PANSS; PANSS N = the negative Scale of PANSS; PANSS G = The General Psychopathology Scale of PANSS; CGI = the Clinical Global Impression of Severity; CGI-A = the CGI score at admission; CGI-D = the CGI score at discharge; CGI-diff = the CGI score difference between admission and discharge.

0.37; PANSS G: t = 1.02, df = 23, p = 0.32; CGI-A: t = -0.35, df = 24, p = 0.73; CGI-D: t = -0.98, df = 24, p = 0.34; CGI-diff: t = 0.91, df = 24, p = 0.37; hospitalization: t = -0.23, df = 34, p = 0.98; onset age: t = -0.19, df = 22, p = 0.85; illness duration: t = -0.62, df = 22, p = 0.54).

Th1/Th2 ratios and cytokines

After doubling the volumes of the reagents and serum samples and lowering the bottom standard, the cytokine levels of all subjects were detectable. The data regarding serum Th1/Th2 ratios and cytokine levels in subjects with schizophrenia and healthy controls are summarized in Table 2.

Subjects with schizophrenia vs. healthy controls

At individual serum cytokine levels, subjects with schizophrenia as a whole group had lower average serum IFN-γ, IL-2, and IL-4 levels, but higher average serum IL-6, IL-10, and TNF-α levels. However, the means of all Th1/Th2 ratios examined in subjects with schizophrenia were lower than those of their healthy counterparts. Non-parametric Mann-Whitney U tests revealed that schizophrenic subjects had significantly lower serum IFN-γ/IL4 and IFN-γ/IL10 ratios, but showed markedly higher IL-6 levels than did healthy controls (IFN-γ/IL4: Z = -2.01, p = 0.05; IFN-γ/IL10: Z = -2.35, p =

Table 2
Serum Th1/Th2 cytokine levels and ratios. Summary of the means (M) and standard deviations (SD) of serum Th1/Th2 cytokines and ratios in subjects with schizophrenia (SCH) and healthy controls (CON)

M	Male + female		Male		Female	
(SD)	SCH(N=37)	CON(N=37)	SCH(N=23)	CON(N=23)	SCH(N=14)	CON(N=14)
IL-2/IL-4	0.65	0.72	0.73	0.66	0.51	0.83
	(0.30)	(0.45)	(0.34)	(0.38)	(0.17)	(0.54)
IFN/IL-4	13.18*	18.51	14.85	18.94	10.44*	17.80
	(7.64)	(11.45)	(8.42)	(12.61)	(5.37)	(9.63)
IFN/IL-10	14.80*	19.71	14.99*	20.89	14.48	17.78
	(7.91)	(10.25)	(8.59)	(11.35)	(6.95)	(8.14)
IFN	39.27	50.54	40.99*	57.81	36.44	38.61
	(21.39)	(28.56)	(25.03)	(32.73)	(13.89)	(14.08)
TNF-α	1.86	1.77	1.77	1.82	1.99	1.68
	(0.48)	(0.49)	(0.36)	(0.57)	(0.62)	(0.33)
IL-2	1.92	1.96	2.00	2.08	1.78	1.77
	(0.65)	(0.95)	(0.76)	(1.12)	(0.42)	(0.55)
IL-4	3.28	3.40	3.02	3.78	3.73*	2.77
	(1.14)	(2.17)	(1.06)	(2.40)	(1.15)	(1.62)
IL-6	3.60*	2.48	2.74	2.69	5.02*	2.14
	(5.42)	(1.61)	(1.03)	(1.88)	(8.71)	(1.01)
IL-10	2.83	2.77	2.88	3.07	2.75	2.28
	(1.23)	(1.51)	(1.27)	(1.80)	(1.19)	(0.64)

Note: IFN = IFN-γ; * p ≤ 0.05.

0.02; IL-6: Z = -2.34, p = 0.02). Apart from these marked diversities mentioned above, none of the rest serum Th1/Th2 parameters examined in this study achieved a statistic significance level (IL-2/IL-4: Z = -0.44, p = 0.66; IFN-γ : Z = -1.82, p = 0.07; IL-2: Z = -0.35, p = 0.73; TNF-α: Z = -0.89, p = 0.37; IL-10: Z = -0.79, p = 0.43; IL-4: Z = -0.41, p = 0.68).
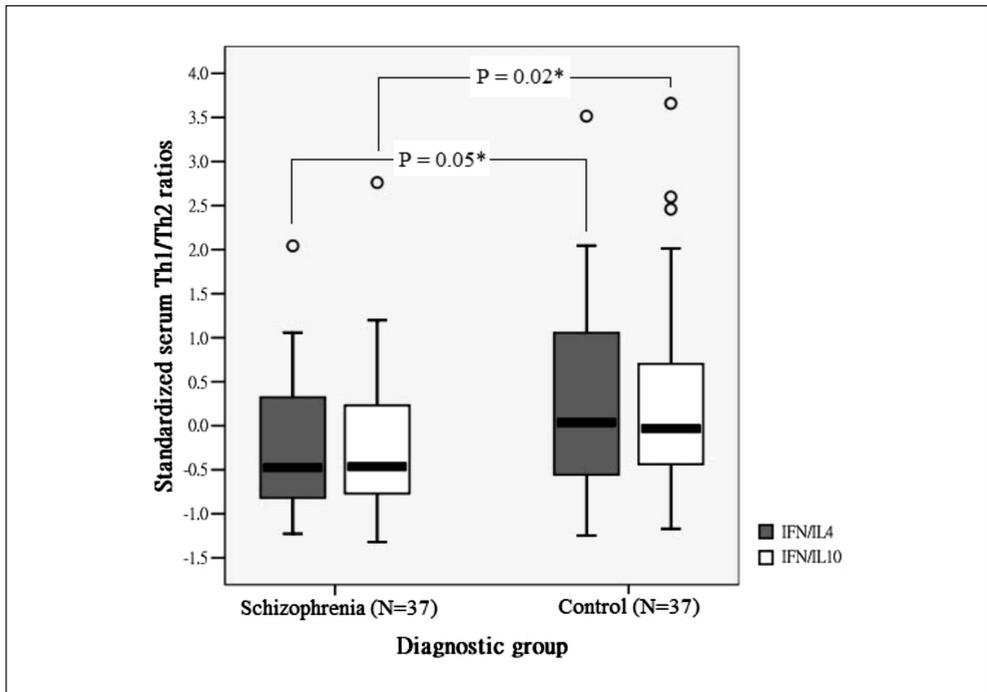

Figure 1. Significantly reduced serum Th1/Th2 ratios in schizophrenia. Box-plots of the standardized serum Th1/Th2 ratios in subjects with schizophrenia and healthy controls show the medians, quartiles, and outliers (IFN/IL4 = IFN-γ/IL-4 = gray box, N = 37; IFN/IL10 = IFN-γ/IL-10 = white box, N = 37; the box = inter the 25th and 75th percentile range; cross-box line = the median; lower bar = the 25th percentile; upper bar = the 75th percentile ; O = outliers; * p ≤ .05)

Males with schizophrenia vs. male controls

If compared to male healthy controls, males with schizophrenia had significantly lower serum IFN-γ levels and IFN-γ/IL-10 ratios (IFN-γ : Z = -2.19, p = 0.03; IFN-γ/IL-10: Z = -1.99, p = 0.05). However, both male subgroups had similar IL-2/IL-4, IFN/IL-4 ratios, TNF-a, IL-2, IL-4, IL-6, and IL-10 levels (IL-2/IL-4: Z = -0.80, p = 0.42; IFN-γ/IL-4: Z = -0.89, p = 0.37 ; IL-2: Z = -0.41, p = 0.68; IL-4: Z = -1.29, p = 0.20; IL-6: Z = -1.33, p = 0.18; IL-10: Z = -0.04, p = 0.97).

Females with schizophrenia vs. female controls

Nevertheless, the comparisons between both female subgroups demonstrated that female schizophrenia had significantly higher serum IL-4 and IL-6 levels, but had markedly lower IFN-γ/IL-4 ratios than did healthy

females (IL-4: Z = -2.21, p = 0.03; IL-6: Z = -1.98, p = 0.05; IFN-γ/IL-4: Z = -2.11, p = 0.04). But both female subgroups were comparable in terms of IL-2/IL-4, IFN-γ/IL-10 ratios, IFN-γ, TNF-a, IL-2, and IL-10 levels (IL-2/IL-4: Z = -1.42, p = 0.15; IFN-γ/IL-10: Z = -1.15, p = 0.25 ; IFN-γ: Z = 0.00, p = 1.00 ; TNF-a: Z = -1.24, p = 0.22; IL-2: Z = -0.28, p = 0.78; IL-10: Z = -1.06, p = 0.29).

Th1/Th2 ratios and clinical symptoms

The correlations between Th1/Th2 ratios and psychopathology as well as symptom severity are summarized in Table 3. According to the data available in this study, none of the Th1/Th2 ratios significantly correlated with any scores of PANSS or CGI in schizophrenia as a whole group. If only male schizophrenia considered, then the IL-2/IL-4 ratios were positively associated with both the PANSS negative syndrome subscale (R = 0.55, p = 0.04) and the general psychopathology subscale (R = 0.54, p = 0.04), while the IFN-γ/IL-10 ratios were positively related to the CGI scores at discharge (R = 0.52, p = 0.04). In female schizophrenia, none of the Th1/Th2 ratios significantly correlated with any scores of PANSS scales. However, the IFN-γ/IL-10 ratios in females with schizophrenia were negatively associated with the CGI scores at admission (R = -0.75, p = 0.01) and at discharge (R = -0.79, p = 0.006), but were positively correlated with the CGI score differences/changes between admission and discharge (R = 0.64, p = 0.05).

Table 3
Correlations between Th1/Th2 ratios and psychopathology. Summary of correlations between Th1/Th2 ratios and psychopathology (measured with PANSS) and symptom severity (measured with CGI) in schizophrenia

	PANSS (N=25)			CGI (N=26)		
Male + Female	Positive	Negative	General	Admission	Discharge	Difference
IL-2/IL-4	0.00	*0.35*	*0.34*	0.27	0.18	-0.10
IFN/IL-4	-0.34	0.14	0.17	-0.07	0.09	-0.14
IFN/IL-10	-0.11	0.10	0.07	-0.22	-0.03	-0.03
	PANSS (N=15)			CGI (N=16)		
Male	Positive	Negative	General	Admission	Discharge	Difference
IL-2/IL-4	0.08	0.55*	0.54*	0.17	0.10	-0.02
IFN/IL-4	-0.30	0.22	0.16	0.06	0.41	-0.38
IFN/IL-10	-0.10	0.21	0.08	0.21	0.52*	*-0.47*
	PANSS (N=10)			CGI (N=10)		
Female	Positive	Negative	General	Admission	Discharge	Difference
IL-2/IL-4	0.40	-0.08	-0.08	0.51	0.45	-0.26
IFN/IL-4	-0.23	-0.10	0.07	-0.25	-0.33	0.37
IFN/IL-10	-0.46	-0.13	0.06	-0.75**	-0.79**	0.64*

Note: PANSS = The Positive and Negative Syndrome Scale; Positive = Positive Scale; Negative = Negative Scale; General = General Psychopathology Scale; CGI = The Clinical Global Impression ; Admission = at admission; Discharge = at discharge; Difference = difference between at admission & discharge; IFN = IFN-γ.

Th1/Th2 ratios and illness duration/hospitalization

However, none of the Th1/Th2 ratios and serum cytokine levels examined in this study significantly correlated with illness duration and hospitalization length, regardless of patients' genders. Due to the space limit of this report, detailed results in these regards are available on request.

Conclusions

A clear in vivo Th2-shift was observed in our drug-free patients with schizophrenia, showing significantly reduced serum IFN-γ/IL-4 and IFN-γ/IL-10 ratios. At the individual cytokine levels, schizophrenia as a whole group also showed significantly higher serum IL-6 levels than did healthy controls. If both sexes analyzed separately, the Th1/Th2 dysfunction profiles of male and female schizophrenia were different. Male subjects with schizophrenia revealed significantly lower serum IFN-γ/IL-10 ratios and IFN-γ levels, whereas female subjects with schizophrenia showed markedly lower serum IFN-γ/IL-4 ratios, but had significantly higher serum IL-4 and IL-6 levels.

At individual cytokine levels, our findings generally correspond to the majority of relevant literature showing increased serum IL-6[22,40-43,45,65-70], unaltered serum IL-2[13,18,26,71], TNF-α[17-20,22,43,71], IFN-γ[13,27,57], IL-4[24,70], and IL-10[24] levels in schizophrenia as a whole group. It's noteworthy that although recent evidence showing intense exercise transiently increases IL-6[72], blood samples of our subjects were collected between 8-9am without any exercise. Therefore, increased serum IL-6 levels in our schizophrenic subjects were not results of exercise. Decreased

serum IFN-γ levels in our schizophrenic males could be genetically determined since Freudenreich et al.[73] included almost only (86.7%) males with schizophrenia and found significantly reduced IFN-γ mRNA in schizophrenia. Increased serum IL-4 levels in our schizophrenic females might be related to the pathology since IL-4 is able to regulate brain activity[60]. However, IL-4 could also influence the pathology through its impact on IFN-γ/IL-10 and IL-2/IL-4 balance since serum IFN-γ/IL-10 balance were related to symptom severity in females and IL-2/IL-4 ratios to negative symptoms/psychopathology in males with schizophrenia, but not it per se. Diversities between some other studies[15,18,20,21,24,26,41,68,71,74] and ours may be raised by different gender distributions and/or average ages since (1) gender dimorphism during immune responses was observed[75] and (2) cytokine levels could vary in age-related manner[20,76]. The subjects of those studies were either not exactly sex- and/or age-matched or averagely much older than ours. Due to limited space and the main focus of this study, more detailed comparisons at individual cytokine levels won't be described here.

Studies directly examining Th1/Th2 ratios in schizophrenia are rare. So far no findings regarding serum IL-2/IL-4 ratios in schizophrenia were reported. Although serum IL-2/IL-4 ratios were found unchanged in our subjects with schizophrenia, they were related to negative symptoms and psychopathology in males, but not in females, with schizophrenia. The reason why our subjects with schizophrenia showing unaltered serum IL-2/IL-4 ratios could be that our patients were relatively selective and not predominated with negative symptoms since (1) persistent negative symptoms result in long-term disability[77] and (2) those patients participated in our study must have

relatively good responsiveness to give their consents and to be interviewed. Balance between IL-2 and IL-4 could be involved in general psychopathology in males with schizophrenia since IL-2 is likely, as shown in previous studies, associated with positive/negative symptoms[74,78,79].

So far, only Avgustin[56] and Kim[57] examined IFN-γ/IL-4 ratios in schizophrenia. In contrast to our finding, Avgustin found no differences in in-vitro IFN-γ/IL-4 ratios between schizophrenia and healthy controls, while Kim reported higher plasma IFN-γ/IL-4 ratios in schizophrenia. Although in-vitro production IFN-γ/IL-4 ratios are related to in-vivo serum IFN-γ/IL-4 ratios, both could be different due to distinct biological systems involved and interactions occurred and are thus not necessarily similar. This is likely the main reason why we had different result from that of Avgustin. Contrary to that study of Kim, we applied a modified Cytometric Bead Array to measure cytokines (using flow cytometry) and did not have missing data. The results of Kim's study might be the outcomes of selection because they had some subjects showing cytokine levels under the detectable limits and excluded into the analyses. Another reason might be different male/female ratios in both studies due to gender dimorphism during immune responses[75], a role of sex hormones in regulating immunity including Th1/Th2 balance[80], and significantly higher IFN-γ/IL-4 ratios in men[81]. Besides, the subjects with schizophrenia in Kim's study were possibly predominated with negative symptoms since significantly higher IFN-γ/IL-4 ratios were recently observed in depression[82]. This is the first study reporting significantly reduced serum IFN-γ/IL-4 in schizophrenic females. However, no links between serum IFN-γ/IL-4 ratios and psychopathology or symptom severity were found. Maybe significantly reduced IFN-γ/IL-4 ratios are rather trait markers than state markers for schizophrenic females; they might be the basis of IFN-γ/IL-10 imbalance in schizophrenia.

Up to date, we are the first group examining serum IFN-γ/IL-10 ratios and found a marked reduction in schizophrenia. Although serum IFN-γ/IL-10 ratios were not associated with psychopathology or symptom severity in schizophrenia as a whole group, they were markedly related to symptom severity at discharge in both genders with schizophrenia. Nevertheless, their associations to symptom severity in both genders were opposite; serum IFN-γ/IL-10 ratios were positively associated with symptom severity at discharge in schizophrenic males, but negatively with that in females. It seems that the more Th1 shift (higher IFN-γ/IL-10) in schizophrenic males, but the more Th2 shift (lower IFN-γ/IL-10) in schizophrenic females at admission, the worse their symptoms would be at discharge. Serum IFN-γ/IL-10 ratios seem to be able to predict treatment outcomes in schizophrenia. In addition, serum IFN-γ/IL-10 ratios were also negatively linked to symptom severity at admission and positively associated with differences/changes in symptom severity at discharge in females with schizophrenia. That is, Th2 shift appeared to be related to symptom severity at admission and to be able to predict treatment effects in female schizophrenia.

Both sexes of schizophrenia appeared to have different profiles of Th1/Th2 imbalance. Th1/Th2 imbalance seemed to have different meanings for both genders of schizophrenia. Sex differences in schizophrenia have been attributed to estrogen[83] more than one decade ago. Several indirect findings have suggested testosterone as a possible treatment modality for schizophrenia[84]. Questions remain how serum IFN-

γ/IL-10 and IL-2/IL-4 ratios interact with sex-hormones to interfere with patients' functioning and negative symptoms/psychopathology and how they are impacted by diverse neuroleptics. Whether or not are serum IFN-γ/IL-4 ratios related to the pathology in female schizophrenia and if they are, then how? Whether or not are those altered Th1/Th2 ratios schizophrenia-specific? Further studies are demanded since our findings involved only a relatively small number of subjects.

Acknowledgements

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