行政院國科會專題研究計畫成果報告

雙極性情感疾病患者之躁症狀態的免疫調節作用

Immunological Regulation in Bipolar Patients with Manic Episode

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摘要

目前有關躁症的免疫變化研究不多且結果不一致，由於細胞激素以及其受體在體內的變化可能與精神病患其精神—生理反應有關，而國內尚無相關之研究報告，故本研究乃就急性躁期前後之免疫力及細胞激素受體的變化加以研究。結果顯示PHA（刺激T淋巴細胞）（$p<0.01$）與PWM（刺激T淋巴細胞與B淋巴細胞）（$p<0.05$）之增生指數，於躁期均有明顯高於緩解期；SIL-2R於躁期血清濃度明顯高於緩解期（$p<0.05$），sIL-6R則均無變化。而緩解期則均無異於健康對照組。此外IFN-γ, IL-2, 和 IL-4濃度於躁期均有明顯異於緩解期. 本研究發現躁鬱症病患僅在急性躁期之細胞免疫力明顯活化，躁期症狀之嚴重度與免疫功能之改變有關；急性躁期僅sIL-2R升高而非sIL-6R，此現象不同sIL-2R與sIL-6R均升高的精神分裂症與重鬱症，顯示躁鬱症之精神—生理機轉不同於其他的重大精神疾病。

關鍵詞：雙極性情感疾患、急性躁期、細胞免疫力、細胞激素

Abstract

Whether patients with bipolar disorder have activation or reduction of immunity during a manic episode remains unclear. The data demonstrated lymphocyte proliferation to PHA and the plasma sIL-2R levels, but not sIL-6R, of bipolar patients were significantly higher in acute mania than in consequent remission. These elevations were not due to differences in medication status. Only in acute mania were the plasma sIL-2R levels of patients significantly higher than control subjects. A positive correlation between the changes of manic severity and plasma sIL-2R levels was observed. Furthermore, IFN-γ, IL-2, and IL-4 levels in acute mania were significantly different than patients in remission. Remitted bipolar patients and normal controls did not differ in any of these measures. These data suggest cell-mediated immunity activation in bipolar mania was supported and may be through a specifically state-dependent immune response.

Key word: bipolar disorder, mania, cell-mediated immunity, cytokine

Introduction

Macrophages and their products (cytokines) might play an integral role in the pathophysiology of certain psychiatric disorders including schizophrenia and depressive disorder (Fricchione et al 1996). Bipolar disorder is a mood disorder which manifests psychosis in manic episode. Additionally, an increased prevalence of thyroid autoantibodies (Haggerty et al 1990)

and immune-related diseases (Tsai et al 1997b) were reported in bipolar disorder patients. However, reports on the assessment of immunity in mania are limited and remain controversial.

The comparisons of the immune status between healthy controls and bipolar patients in acute mania as well as consequent remission have not been done. In an attempt to clarify the pathophysiology of bipolar disorder, we evaluated the lymphocyte proliferation to different mitogens and the plasma levels of sIL-2R, sIL-6R and and TH1 (IFN-g, IL-2) and TH2 (IL-10, IL-4) cytokines among bipolar patients in acute mania and remission. The questions to be addressed are: (1) does immune activation occur in a manic episode?, and (2) is there any immune modulator related to the severity of mania in bipolar disorder?

Materials and Methods

Subjects

Acute in-patients of Taipei City Psychiatric Center, meeting DSM-III-R (American Psychiatric Association 1987) diagnostic criteria of bipolar disorder were invited to participate. The severity of manic symptoms was rated on the Young Mania Rating Scale (YMRS, Young et al 1978). Eleven men and 12 women with manic episode took part in the study. The mean age of patients was 30.6 $\pm$ 8.9 (SD) years (range = 17-44). Mean duration of illness was 8.3 $\pm$ 6.0 (SD) years. The mean YMRS score of acute mania at the time of blood withdrawn for study was 34.3 $\pm$ 4.7 (SD) points (range = 26-40). The mean score of YMRS in the remission period was 3.5 $\pm$ 3.8 points (range = 0-12), which indicates clinically significant improvement in manic symptoms.

Age- and gender-matched healthy control subjects were recruited. The mean age of control subjects was 26.8 $\pm$ 3.9 years (range = 19 — 40 years). The written informed consent were obtained when the blood was drawn. Plasma was collected and frozen at -70 until used.

Lymphocyte proliferation assay

The $1x10^5$ peripheral blood mononuclear cell (PBMC) per well were incubated in the 96-well microculture plate. Based on previous reports (Darko et al 1991), phytohemagglutinin (PHA) (5 μg/ml), concanavalin A (Con A) (5 μg/ml) and pokeweed mitogen (PWM) (5 μg/ml) (Sigma) were added to the plates. After 72 hours of incubation, the cells were labeled for 18 hour with [methyl-^{3}H]-Thymidine (1 μCi/well) (NEN). Cells were then harvested by cell harvester (Skatron Instruments) and the samples were counted for radioactivity using beta counter (Beckman, LS 6500). The data of each sample were done by triplicated-well and represented by mean dpm $\pm$ SD.

Plasma concentrations of sIL-2R and sIL-6R

According to the manufacturer's instruction, the plasma levels of sIL-2R and sIL-6R were measured in duplicate using commercial enzyme-linked

immunosorbent assays (ELISA) purchased from BioSource International, Camarilla, CA and R&D Systems, Inc. Minneapolis, MN, respectively. *Cytokines Concentration Analysis*

IFN-γ, IL-2, and IL-4 concentrations were determined by enzyme-linked immunosorbent assay (ELISA) which purchased from BioSource International, Camarillo, USA and IL-10 kit were from Endogen Inc, Cambridge, MA, USA.

Statistic Analysis

A nonparametric distribution was found for the immune-inflammatory variables of bipolar patients and controls. Hence, the Wilcoxon rank sums test was used to analyze the differences between bipolar patients and controls. The relationships between variables were assessed by means of Spearman's correlations. Difference between acute mania and consequent remission in bipolar individuals were assessed by means of paired *t* test.

Results

The plasma sIL-2R levels of bipolar patients in acute mania were significantly higher than in consequent remission ($p < 0.025$) and those of control subjects ($p < 0.001$) (Table 1). However, in neither acute mania nor remission was there a significant difference in plasma sIL-6R concentrations between bipolar patients and control subjects. In bipolar patients, there was a significantly positive correlation between the Δ sIL-2R (mania minus remission values) and Δ YMRS scores (mania minus remission values) ($r = 0.50$, $p = 0.02$), but not between the Δ sIL-6R and Δ YMRS scores ($r = 0.03$, $p = 0.88$).

Con A- and PHA-induced lymphocyte proliferation of bipolar patients were higher in acute mania than in consequent remission, but only the response to PHA reached statistical significance ($p < 0.025$) (Table 1). In addition, patients in acute mania exhibited mild higher lymphocyte response to PHA than control subjects. Comparisons between remitted bipolar patients and control subjects showed no significant difference in mitogen-induced lymphocyte proliferation, plasma levels of sIL-2R and sIL-6R.

With respect to the production of TH1 (IFN-g and IL-2) and TH2 (IL-10 and IL-4) cytokines after stimulation with PHA, significant levels were observed in patients with acute mania than in remission except IL-10 (Table 2).

Discussion

Based on comparison between acute mania and consequent remission of bipolar individuals, a major finding of this study is that manic episode is accompanied by significantly higher PHA-induced lymphocyte proliferation as well as elevated plasma sIL-2R levels. Stimulation of lymphocytes by Con A tended to be increased in acute mania, although this result did not reach significance. Most studies of immunity in mood disorders (Kronfol and House 1988; Hickie et al 1993;

Maes et al 1992) have found changes in cellular (T-cell-mediated) immunity rather than humoral (B-cell-mediated) immunity. Since, increased plasma sIL-2R levels are an unequivocal index of T cell activation (Caruso et al 1993). Furthermore, when compared with age- and sex-matched controls in this study, such indexes of immunity change were noted only in manic state. These results indicate that activation of cell-mediated immunity occurs in acute mania. With the significant reduction in YMRS scores during remission period, our data support that immune activation in bipolar patients represents a state-dependent effect (Rapaport 1994).

Our results did not agree that elevated sIL-2R and sIL-6R levels are specific for mania (Maes et al 1995a), but consistently demonstrated that there is an association between acute psychotic disorders and altered immunity. Another major finding of this study is that plasma sIL-2R levels but not sIL-6R was significantly elevated in bipolar mania, despite considerable evidence suggesting that increased sIL-2R levels are commonly seen in schizophrenia characterized by activation of the T lymphocytic (CD4) arms of CMI (Hornberg et al 1995; Maes et al 1994, 1995a, 1996; Rapaport et al 1989, 1993, 1994; Rapaport and Lohr 1994) and depression (Maes et al 1991, 1995b; Sluzewska et al 1996). Moreover, there is a positive correlation between the changes of manic severity and plasma sIL-2R levels, but not sIL-6R. Consequently, this finding may be an evidence that the immune modulators in bipolar mania may be different from those in acute schizophrenia (Maes et al 1994, 1995a) and major depression (Maes 1995b, Sluzewska et al 1996), both having increased in vivo production of sIL-2R and sIL-6R.

Acute mania patients showed significantly lower levels of IFN-γ in comparision to the patients in controls. This is correspond to findings of other groups in schizophrenic psychosis (Rothermundt et al. 1996). Furthermore, IL-2, the other major cytokine secreted by TH1 is investigated. IL-2 plays a central role in the afferent phases of T-cell dependent immune responses and NK cells (Lederer and Abbas,1995). Conflicting results on IL-2 were reported by several groups in patients with depression. Our study demonstrated higher, but not statistical difference, of IL-2 production in patients with acute mania as compared to the controls. We also demonstrated that the production of IL-10 after stimulation is increased in acute mania when compare to controls. IL-10 (a TH2-cytokine) is the most potent suppressor of IFN-γ production (Howard and O'Garra 1992; Mosmann and Moore 1991). Together with IL-4, IL-10 represents the strongest antagonist of IFN-γ. Thus, it could be expected that a reduced production of IFN-γ in acute mania might be due to an increased production of IL-10.

Taken together, in this study bipolar patients in acute mania are characterized by T lymphocyte activation as evidenced by elevated PHA-induced lymphocyte proliferation as well as higher plasma sIL-2R but not sIL-6R levels. These elevations

were not due to differences in gender, age, nor medication status. Furthermore, a positive relationship between the changes of manic severity and plasma sIL-2R levels was observed. The IFN-γ is significantly decreased whereas the IL-10 and IL-4 were remain no statistical difference as controls. These findings support that cell-mediated immune activation represents state-dependent characteristics in bipolar mania. It is suggested that the immune modulators may vary in different psychotic disorders.

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Table 1. Immunological Variables in Normal Controls and Bipolar Patients

Category	Bipolar disorder (N=23)		Control subjects (N=23)	Comparison					
	Acute mania(A) mean (SD)	Remission(B) mean (SD)	(C) mean (SD)	A vs. B [a]		A vs. C [b]		B vs. C [b]	
				t,	*p*	*Z*,	*p*	*Z*,	*p*
Blank (10^3 × dpm)	1.1 (0.5)	1.1 (0.4)	1.0 (0.4)	−0.17, ns		−0.50, ns		1.33, ns	
PHA (10^3 × dpm)	206.8 (98.1)	160.5 (71.9)	190.0 (56.2)	2.50,<0.025		0.30, ns		1.51, ns	
Con A (10^3 × dpm)	123.8 (80.3)	106.6 (72.0)	130.6 (89.5)	0.96, ns		0.54, ns		1.37, ns	
PWM (10^3 × dpm)	37.0 (27.9)	36.7 (33.0)	36.5 (26.3)	0.29, ns		0.27, ns		0.69, ns	
sIL-2R (pg/ml)	261.2 (224.1)	194.7 (222.0)	81.8 (156.1)	2.43,<0.025		3.37,<0.001		1.99, <0.1	
sIL-6R (ng/ml)	44.5 (10.3)	46.3 (10.1)	42.6 (7.0)	−0.91, ns		0.43, ns		1.05, ns	

[a] paired *t* test, df = 44

[b] Wilcoxson rank sums test

Table 2. TH1 and TH2 cytokines in Normal Controls and Bipolar Disorder Patients

Category	Bipolar disorder (N=23)		Control subjects (N=23)	Comparison				
	Acute mania(A) mean (SD)	Remission(B) mean (SD)	(C) mean (SD)	A vs. B [a] *p*	A vs. C [b] *Z*,	*p*	B vs. C [b] *Z*,	*p*
IFN-γ	791.81 (542.76)	778.14 (512.09)	3222.9 (2150.19)	<0.0001	—	<0.0001	0.50,	1.33, ns
IL-2	1093 (1272)	1136 (1162)	587.3 (611)	<0.001	0.30, ns		1.51, ns	
IL-10	494.5 (462.7)	428.4 (367.0)	370.9 (249.6)	ns	0.54, ns		1.37, ns	
IL-4	114.6 (174)	109.8 (135.4)	86.29 (108.5)	0.0003	0.27, ns		0.69, ns	

[a] paired *t* test, df = 44

[b] Wilcoxson rank sums test