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ORIGINAL RESEARCH ARTICLE

Decreased *in vitro* production of interferon-gamma and interleukin-2 in whole blood of patients with schizophrenia during treatment

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A pattern of aberrations in the T-cell cytokine system that is typical for autoimmune disorders has also been reported in patients with schizophrenia, namely a decreased interleukin-2 (IL-2) production and increased levels of the soluble IL-2 receptor (sIL-2R). It has also been reported that the production of interferon-γ (IFN-γ) may be lowered. In a longitudinal design, we studied the production of both IFN-γ and IL-2 and their correlation in patients with schizophrenia during treatment and investigated whether associations exist between cytokine production and clinical variables. The production of IFN-γ and IL-2 was measured in equal numbers ($n = 29$) of patients with schizophrenia (DSM-IV) and controls who were matched for age and gender. Patients were measured 1 day after admission (T1), after 14 (T2) and 28 (T2) days of treatment. Psychopathology was assessed after these times. The production of both IFN-γ and IL-2 was significantly lower in patients than in controls throughout the whole investigation period (T1–T3). The productions of both cytokines were significantly correlated in controls ($r = 0.60$, $P \leq 0.001$) as well as in patients with schizophrenia (mean production T1–T3: $r = 0.71$, $P \leq 0.001$). No associations between cytokine measurements and psychopathology or age-at-onset could be found. Our findings of lowered and correlated IFN-γ and IL-2 production indicate that alterations in the cytokine system of patients with schizophrenia might resemble those in autoimmune disorders. It is suggested that these immunological abnormalities are associated with acute exacerbation, rather than with a clinical subtype of schizophrenia. *Molecular Psychiatry* (2000) **5,** 150–158.

Keywords: schizophrenia; immunology; cytokines; interferon-gamma; interleukin-2

Introduction

As early as 1937, the neuropsychiatrist Lehmann-Facius[1] pointed to the possible role of autoimmunity in the pathophysiology of schizophrenia. Even today this assumption is still supported by certain clinical features that can be observed in both schizophrenia and autoimmune diseases, such as type-1 diabetes, lupus erythematosus or multiple sclerosis. Both groups of disorders often have juvenile onset and can be triggered by psychosocial distress, infections, drug abuse and physical injury.[2] A considerable variability of course, often with acute disease episodes and subsequent reduced level of functioning is also observed in both groups of diseases.

Over the last decade, knowledge concerning the pathophysiology of autoimmune disorders has significantly increased as a result of research on the role of cytokines in these diseases. There is to date considerable evidence that an altered regulation of a number of cytokines exist not only at inflammatory sites, but also in the peripheral blood, especially with systemic autoimmune disorders. An *in vivo* activation of T cells in these disorders is typically reflected by increased serum levels of their products, namely interleukin-2 (IL-2) and the soluble interleukin-2 receptor (sIL-2R). Paradoxically, a decreased *in vitro* production of IL-2 upon mitogen stimulation is found in these patients. These common features of autoimmune disorders[2,3] have lately been supplemented by the demonstrations of a decreased *in vitro* production of interferon-gamma (IFN-γ) upon mitogenic stimulation.[4] IFN-γ is a 143-amino acid residue glycoprotein with multiple biological functions including potent anti-viral activity, stimulation of macrophage activity, modulation of Major Histocompatibilty Complex (MHC) class I/class II expression and regulation of a diversity of specific immune responses. It is produced predominantly by CD4+ (TH-1) cells and particularly by CD4+CD45R0+ memory-cell.[5] With respect to their immunological functions, both TH1-cytokines IL-2 and IFN-γ are related and the release of IL-2 is stimulated by IFN-γ. Both cytokines have largely immune-stimulatory effects which apparently exacerbate the inflammatory process in autoimmune disorders.

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Aberrant proportions of immune-competent cells, indicating immunological dysfunctions, have often been reported in schizophrenia since the early work of Bruce and Peebles,[6] although there are conflicting results concerning the precise nature of these changes.[7] The search for tissue-specific antibodies which can serve as markers for schizophrenia has as yet not been successful.[7] However, recent studies which have focused on the physiology of cytokine production have provided increasingly consistent results. Six different groups, including our own, have independently demonstrated that mitogen stimulated IL-2 production by peripheral blood mononuclear cells (PBMC) is decreased in patients with schizophrenia.[8–15] This is the most frequently confirmed finding in the immunology of schizophrenia and there has not as yet been a failure in demonstrating this phenomenon. In the cerebrospinal fluid (CSF) of drug-free patients, increased levels of IL-2 were found[16] and also significantly predicted the recurrence of psychotic symptoms in relapse-prone schizophrenics.[17] Elevated levels of the sIL-2R in peripheral blood, a sign that also indicates lymphocyte activation, were also repeatedly observed in schizophrenia.[14,18,19] Hence concerning cytokines and their role as indicators for T-cell activation, there are impressive parallels between autoimmune disorders and schizophrenia.

The few existing reports on IFN-γ production in schizophrenia, however, paint a rather incomplete picture. Whilst there is some evidence that the mean serum levels of IFN-γ in schizophrenics (except in one report)[20] resemble those of normal controls,[21–23] the production of IFN-γ after mitogenic stimulation was only mildly and on the whole not significantly decreased in schizophrenia,[14,24–26] although one study did find a significant decrease.[27] The lack of coherence in these results can be explained by the fact that studies varied considerably with respect to the use of bioassays, cell preparation techniques, and also recruitment of patients. Although in some studies cells were separated from the sera and subsequently stimulated by mitogens, our group used a whole blood assay[28] whereby the lymphocytes were incultured with as little deviation as possible from their physiological milieu, ie cell populations were present in their natural frequency and distribution. It is therefore likely that *in vitro* stimulation effects better reflect actual *in vivo* cytokine production by immunocompetent cells. Using the same methods as were used in our pilot study,[14] we have demonstrated a significantly reduced production of IFN-γ in an acutely ill subsample of a larger group of patients with schizophrenia.[15] Recently this finding could be confirmed in samples of hospitalized acutely psychotic schizophrenics[29] and in acutely schizophrenic members of multiplex families.[30] However, as in the majority of studies in this area, only a cross-sectional approach was employed, which did not yield any information about changes in cytokine production while patients were undergoing remission.[31]

Encouraged by our preliminary findings, patients undergoing treatment due to worsening of schizophrenic symptoms were investigated in this study and the production of IFN-γ and IL-2 by lymphocytes of patients and healthy age- and gender-matched controls were examined. It was our intention to investigate in a larger patient sample whether IFN-γ as well as IL-2 is reduced in acute schizophrenia. We chose a prospective longitudinal approach with three time-points for investigation, timed at 14-day intervals from one another. Thus a 1-month period of observation was covered between the times of acute illness and clinical remission, so as to investigate whether changes in interferon production occurred which correlated with reduction of psychotic symptomatology.

Methods

Recruitment

Patients Twenty-nine patients with schizophrenia were recruited from the Department of Psychiatry of the University of Luebeck School of Medicine. The Department of Psychiatry (DP) provides most of the psychiatric inpatient services for the 220 000 inhabitants of the city of Luebeck, a north German town near the Baltic Sea. The DP is the community mental health center for the whole city of Luebeck and the surrounding rural areas and its patient population is therefore representative for the whole region. Patients were included in the study after it was described to them orally and by a study guide, according to the University of Luebeck Ethics Committee regulations. The patients underwent a structured clinical interview. DSM-IV diagnoses were made by consensus at a staff conference of four psychiatric researchers (VA, DE, MR, CW) who reviewed the results of the interview, along with all available information, including clinical records and information from relatives. The initial diagnostic assessment at day 1 (T1) was reviewed at every point of investigation, ie after 14 days (T2) and 28 days (T3). Only subjects who met DSM-IV criteria throughout the whole assessment period were retained in the sample. Patients with any form of psychiatric comorbidity, including substance abuse, were excluded. All patients were monitored for somatic illness throughout the investigation period and were excluded if symptoms of infections or symptoms of systemic somatic illness were present. Furthermore, the level of C-reactive protein (CRP) was controlled to validate clinical symptoms of infections, and all patients with a CRP $\geq$ 0.5 ml dl^{-1} were excluded from the study. Only physically healthy patients were included. The patients had not been treated with immunosuppressive drugs or steroids during the 3 months which preceded blood sampling. By these criteria, originally 52 patients were recruited. Since only 29 of these completed 4 weeks of treatment (T1–T3), only these patients were included in the study. Seventeen of the patients were male and 12 were female. The mean age was 34.0 (SD $\pm$ 12.7) years of age, ranging from 17 to 62. The mean duration of illness was 101.3 (SD $\pm$ 102.3) months. Prior to admission, 19 patients were treated

with typical, eight with atypical neuroloeptic drugs, two were non-medicated. During their hospital stay, 25 patients were treated with standard neuroleptic drugs, and four were treated with clozapine. The following chlorpromazine equivalents were obtained: T1: 495.6 (±375.1) mg; T2: 508.9 (±330.5) mg; T3: 482.6 (±269.0) mg.

Controls Healthy non-psychiatric controls were recruited from the Blood Transfusion Service of the Department of Immunology and Transfusion Medicine at the University of Luebeck. These individuals were chosen after analysis of full medical history and examination, especially for psychiatric disorders, and additional blood testing by a qualified physician at the department. The blood was analyzed to ensure that the healthy donors had not been taking any medical or illegal substances. Each schizophrenic patient was matched with one control with regard to gender and age. The mean age of controls was 34.1 (SD ± 12.7) years of age, ranging from 19 to 60 years.

Clinical assessments
At the beginning and during psychiatric inpatient treatment (T1, T2, T3), the psychopathology of the patients with schizophrenia was measured by the Brief Psychiatric Rating Scale (BPRS).[32] Measurement by the Positive and Negative Syndrome Scale (PANSS)[33] was also taken at T1. Age of schizophrenia onset was defined as the age at which symptoms first appeared, as reported by the patient, the family or medical staff.

Immunological methods
Heparinized blood was drawn by venous puncture from patients and controls always at 8.00 am, in order to avoid diurnal variation effects. Although we did not record possible sleep disturbance patterns in patients with schizophrenia and controls, the clinically treated patients generally slept undisturbed after 7–10 days of treatment, due to sleep medication. After the blood was drawn, it was stored at 4°C and cultured in a whole-blood assay within 1–2 h according to a technique previously described.[26] In a 5-ml polystyrene tube (Greiner, Nuertingen, Germany), 100 μl of blood was added to 850 μl of Roswell Park Memorial Institute 1640 medium (Biochrom, Berlin, Germany) supplemented at a ratio of 1:10 with 2 mmol L-glutamine, 100 U of penicillin per ml, and 100 μg of streptomycin per ml (Gibco, Karlsruhe, Germany). For the induction of cytokines, phytohaemagglutinine (PHA, Burroughs Wellcome, Dartford, UK) was added at a concentration of 1.78 mitogenic units (0.1 mg ml^{-1}). Tubes were covered and incubated in a humidified atmosphere of 95% air/5% CO_2 at 37°C for 48 h (IL-2), or 96 h (IFN-γ) respectively. The supernatants were recovered and kept frozen at −80°C. Cytokine concentrations were determined by ELISA techniques, whereby recombinant cytokines were used as standards. We used ELISA kits supplied by BioSource International, Camarillo, USA (IL-2, IFN-γ) for the determination of cytokines, carried out according to the manufacturer's instructions.

Statistical analysis
As descriptive measures of cytokine production, the means (±SD percentile) are given, since the majority of the variables were normally distributed in patients (Kolmogorov–Smirnov test: IL-2/T1: $Z = 1.03$, $P = 0.23$; II-2/T2: $Z = 1.22$, $P = 0.10$; IL-2/T3: $Z = 0.91$, $P = 0.39$; IFN-γ/T1: $Z = 1.31$, $P = 0.07$; IFN-γ/T2: $Z = 1.51$, $P = 0.02$; IFN-γ/T3: $Z = 0.67$, $P = 0.77$) and in controls (IL-2: $Z = 0.92$, $P = 0.37$; IFN-γ: $Z = 0.98$, $P = 0.29$). Hence, intercorrelations between variables were examined by the use of Pearson's product-moment-correlation coefficient *r*. In order to correlate cytokine production variables with chlorpromazine equivalents, Spearman's Rank test was used (coefficient rho, ρ). The differences in cytokine production between the schizophrenic and healthy control samples at three time-points of investigation (T1–T3) were examined by Friedman two-way ANOVAs. Differences in mean cytokine production at the different time-points of investigation were tested by the *t*-tests for matched pairs and for independent samples. When the production of IFN-γ at T2, which was not normally distributed, was compared with the values of controls, the Wilcoxon test was used.

Results

Psychopathology
At admission (T1 = day of admission), the mean PANSS total score was 117.0 ± 28.0, the PANNS negative score was 32.4 ± 7.5 and the positive score 28.0 ± 9.3. The mean BPRS score at T1 was 69.4 ± 15.8. During 28 days of inpatient treatment (T2 = 14th day after admission, T3 = 28th day after admission), the mean BPRS total score improved significantly from T1 to T2 (60.4 ± 18.3) and T3 (53.9 ± 18.0; Friedman two-way ANOVA: $\chi^2 = 32.2$, df = 2, $P \leq 0.001$) corresponding to a mean reduction of 22.3% in psychopathological signs.

Immunological findings
The mean (±SD) *in vitro* production of IFN-γ after mitogenic stimulation was measured in patients with schizophrenia at T1 (2627 ± 3220 pg ml^{-1}), at T2 (4096 ± 5236 pg ml^{-1}) and at T3 (3217±2139 pg ml^{-1}) and compared to their age- and sex-matched controls (5718 ± 5265 pg ml^{-1}; Friedman two-way ANOVA: $\chi^2 = 19.4$, df = 3, $P = 0.0002$. When the IFN-γ productions in the schizophrenic sample at the different time-points were tested, they were not found to be significantly different from one another (Friedman two-way ANOVA: $\chi^2 = 3.6$, df = 2, $P = 0.17$). The IFN-γ productions of the patients with schizophrenia were tested *post hoc* against the controls at the single time points and were significantly lowered at T1 (*t*-test for matched pairs: $t = 2.97$, $P = 0.006$), T2 (Wilcoxon-test, $Z = -2.28$, $P = 0.02$) and T3 ($t = 2.70$, $P = 0.012$) (Figure 1).

Concerning IL-2, the mean (±SD) production in the patients with schizophrenia at T1 (90 ± 78 pg ml^{-1}), at T2 (166 ± 156 pg ml^{-1}) and at T3 (121 ± 86 pg ml^{-1}) was compared with their age- and sex-matched controls

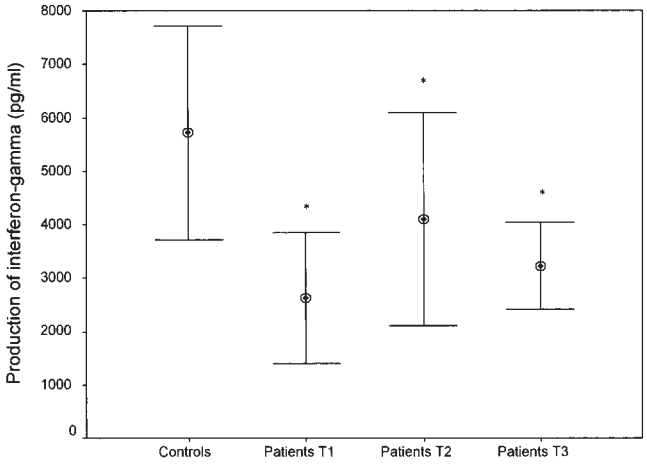

Figure 1 Production of interferon-γ of patients with schizophrenia at three treatment points (T1, admission; T2, after 14 days; T3, after 28 days) and healthy controls. Differences of the IFN-γ production (mean) of controls and schizophrenics were tested (significance is marked by *) at T1 ($t = 2.97$, $P \leq 0.006$), T2 ($Z = -2.3$, $P = 0.023$) and T3 ($t = 2.7$, $P = 0.012$).

(269 ± 222 pg ml^{-1}, Friedman two-way ANOVA: $\chi^2 = 21.2$, df = 3, $P = 0.0001$). When the means of the IL-2 productions by patients with schizophrenia were tested against one another, the result was also significant (Friedman two-way ANOVA: $\chi^2 = 8.3$, df = 2, $P = 0.02$). However, when the results at each of the time-points were tested *post hoc* against the controls, they were significantly lower at T1 (*t*-test for matched pairs: $t = -4.50$, $P \leq 0.001$) and at T3 ($t = -3.9$, $P = 0.001$), but not at T2 ($t = -1.93$, $P = 0.06$) (Figure 2).

Significant intercorrelations between the production of IFN-γ and IL-2 were detected, not only with the control subjects ($r = 0.60$, $P = 0.001$) but also in the schizophrenic sample, at all three time-points (Table 1). The productions of both cytokines at T2 were also significantly correlated with the productions at T3 (Table 1). This also held true when these results were controlled for the effects of multiple testing using the Bonferroni correction, albeit with a reduced significance level of $P \leq 0.003$. When each of the productions of IFN-γ and IL-2 were added up over the three time-points for every patient and then correlated, the result was highly significant ($r = 0.71$, $P \leq 0.001$). The distribution of the sums of the cytokine productions are demonstrated in Figure 3.

Table 1 Intercorrelations between production of interleukin-2 (IL-2) and interferon-γ (IFN-γ) in patients with schizophrenia at three treatment points

	IL-2T1	*IL-2T2*	*IL-2T3*	*IFN-γT1*	*IFN-γT2*	*IFN-γT3*
IL-2T1	–					
IL-2T2	0.09	–				
IL-2T3	0.48*	0.66***	–			
IFN-γT1	0.62***	0.07	0.29	–		
IFN-γT2	0.02	0.65***	0.62***	0.10	–	
IFN-γT3	0.24	0.55*	0.60**	0.22	0.49*	–

*$P \leq 0.01$; **$P \leq 0.001$; ***$P \leq 0.0001$.

The productions of cytokines were correlated with the respective chlorpromazine dosage equivalent to the neuroleptic intake of the patients and three points of treatment. The results were all non-significant: (T1) IFN-γ: $\rho = 0.24$, $P = 0.40$; IL-2: $\rho = -0.06$, $P = 0.83$; (T2) IFN-γ: $\rho = -0.16$, $P = 0.56$; IL-2: $\rho = -0.21$, $P = 0.44$; (T3) IFN-γ: $\rho = 0.24$, $P = 0.38$; IL-2: $\rho = 0.11$, $P = 0.68$.

Regarding the clinical features of the patients with schizophrenia, neither IFN-γ nor IL-2 production at admission were found to be correlated significantly

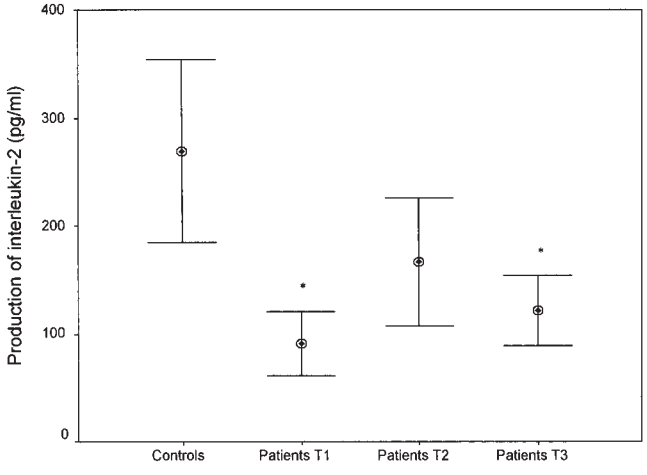

Figure 2 Production of interleukin-2 of patients with schizophrenia at three treatment points (T1, admission; T2, after 14 days; T3, after 28 days) and healthy controls. Differences of the IL-2 production (mean) of controls and schizophrenics were tested (significance is marked by *) at T1 ($t = -4.50$, $P \leq 0.001$), T2 ($t = -1.93$, $P = 0.06$) and T3 ($t = -3.9$, $P = 0.001$).

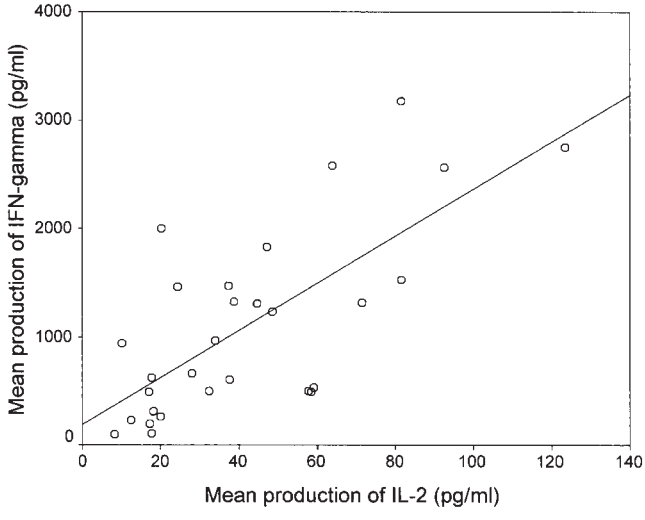

Figure 3 Scatterplot of mean production of IFN-γ and IL-2 over three treatment points. The intercorrelation between the mean production over three treatment points of both cytokines is significant ($r = 0.71$, $P \leq 0.001$).

with age, onset of illness, duration of illness, the BPRS total score, the PANSS positive score, and the PANSS negative score (Table 2). Furthermore, no significant correlations between both of the cytokine productions and these clinical variables could be found (Table 2).

Discussion

To our knowledge, this is the first study to date on cytokine production in schizophrenia in which a longitudinal design was employed, not only with respect to the production of IL-2, but particularly in the case of IFN-γ. Our results confirm the association between acute schizophrenia and low IL-2 production,[8–15] but also clearly confirm our earlier results[30] which showed a decreased IFN-γ production in acute schizophrenia. Deficient lymphocyte productions of both IL-2 and IFN-γ were also found throughout the time period of clinical remission, which were also significantly intercorrelated with one another at all three time-points of the investigation.

A decreased *in vitro* production of IL-2, together with an increased serum level of the shedded sIL-2R is a common characteristic of some autoimmune diseases. As an example, lowered IL-2 production was demonstrated for insulin-dependent (type I) diabetes mellitus,[34,35] which was not altered by insulin treatment.[35] This was also present only in affected children from monozygotic twin pairs who were discordant for the disease.[36] In rheumatoid arthritis, a lowered IL-2 production can be seen,[37] especially in the active disease when the joints become eroded.[38] Increased levels of sIL-2R have also repeatedly been reported[39] which were correlated with the rheumatic disease activity. Similar findings have been reported for systemic lupus erythematosus[40–43] and even for Grave's disease.[44,45] In multiple sclerosis, cytokines have also become a subject of intense interest,[46] whereby a decreased production of IL-2,[47,48] elevated serum levels of IL-2[49] and increased PBMC expression of high-affinity IL-2 receptors are all present which correlate with disease activity.[50]

Since the biological functions of IL-2 and IFN-γ are physiologically interconnected, it might be expected that there would also be an aberrant regulation of IFN-γ production in autoimmune disorders. Although fewer reports exist concerning this cytokine, a reduced *in vitro* production was found in type I diabetes,[51] even though IFN-γ seems to play an essential role in pancreatic β-cell destruction of[52] and diminishes β-cell responsiveness to glucose.[53] Also in rheumatoid arthritis, the *in vitro* production of IFN-γ is lowered,[4,54] although increased IFN-γ levels play a significant role at the inflammatory sites of the disease.[55,56] In multiple sclerosis, IFN-γ seems to promote the disease process.[57] An elevated production was found which preceded the manifestation of clinical symptoms,[58] although this was not seen in acute episodes or remissions.[48]

Despite the fact that the complex pathophysiological functions of TH1-cytokines in autoimmune disorders are not yet fully understood and that the above mentioned inconsistencies of data remain to be clarified, mounting evidence suggests a major role for these (and other) cytokines, not only as indicators of T cell activity but also as direct inflammatory agents. Considering the current knowledge about cytokine production in PBMCs and autoimmune disorders, some striking similarities exist between these diseases and schizophrenia. The data suggesting a decreased production of IFN-γ during acute schizophrenia directly support the idea that autoimmune activity plays an important role in pathogenesis.[2] The fact that this feature was observed only in the acutely ill, but not in the residual patients[15,30] is also worthy of note. Furthermore, as with type-I diabetes, where low IL-2 production is obviously acquired, and not inherited,[36] we found decreased IFN-γ production in the acutely schizophrenic, but not in unaffected first degree relatives within families showing multiple occurrence of

Table 2 Pearson's product-moment correlation coefficients *r* (*P*) of intercorrelations between production of interleukin-2 (IL-2), interferon-γ (IFN-γ) and clinical correlates in patients with schizophrenia at admission (T1), after 14 (T2) and after 28 (T3) days

	Production of:					
	IL-2 (T1)	*IL-2 (T2)*	*IL-2 (T3)*	*IFN-γ (T1)*	*IFN-γ (T2)*	*IFN-γ (T3)*
Age-of-onset	0.30 (0.11)	0.29 (0.13)	–0.13 (0.53)	–0.20 (0.31)	–0.07 (0.71)	–0.16 (0.40)
Duration of illness	0.03 (0.88)	–0.07 (0.71)	–0.01 (0.96)	0.01 (0.98)	0.06 (0.75)	0.18 (0.33)
PANSS positive	–0.14 (0.48)			–0.16 (0.40)		
PANSS negative	–0.09 (0.63)			0.02 (0.91)		
BPRS total score (T1)	–0.03 (0.88)			–0.18 (0.34)		
BPRS total score (T2)		–0.02 (0.93)			0.17 (0.38)	
BPRS total score (T3)			–0.21 (0.30)			0.22 (0.27)

Significant associations between age and cytokine production were found neither in patients with schizophrenia throughout three treatment points (see above), nor in control subjects (Pearson's product-moment correlations: IFN-γ: $r = 0.09$, $P = 0.63$; IL-2: $r = -0.13$, $P = 0.50$).

schizophrenia.[30] This finding also suggests that the deficit is associated with the active disease, rather than with a genetic predisposition.

Whilst our state of knowledge on the pathophysiological effects of cytokines in autoimmune disorders is rapidly increasing, the involvement of aberrant immune functions in the pathogenesis of schizophrenia is poorly understood. Experimental and clinical studies have provided some evidence that cytokines play an important role in the development of central nervous system (CNS) tissue.[59] IFN-γ activates embryonic microglia, which results in the activation of a soluble molecule that promotes the differentiation of cholinergic neuronal cell precursors in both the septal nuclei and adjacent basal forebrain and stimulates the development of cholinergic embryonic septal neurons.[60] These findings highlight the role of IFN-γ in the development of neurotransmitter systems. IL-2 modulates neuronal activity; IL-2 and its receptors have a widespread localization in gray matter areas of the CNS.[61] It is of considerable interest to schizophrenia research that IL-2 modulates dopamine release in the striatum by influencing the function of excitatory amino acids.[62,63] On the other hand, the number of studies is increasing that demonstrate lesions of hypothalamic structures lead to substantial disruptions of the immune system integrity.[64] However, considering that limited observations on the complex interactions of CNS tissue, neurotransmitters and cytokines are presently possible, caution must be exercised in interpreting psycho-immunological data, particularly since, despite considerable research, the mechanisms underlying the aberrant immune functions of PBMC from schizophrenics are as yet not understood at the cellular level.

Four hypotheses have been put forward as explanation for the cellular mechanisms underlying reduced TH-cytokine production in schizophrenia: (1) the lowered cytokine production is a trait phenomenon, resulting from a genetic predisposition; (2) cytokine production is 'down-regulated' by negative feedback after initial overproduction; (3) the cells are producing cytokines in excess *in vivo*, so that when they are isolated they have apparently become 'exhausted' and produce less *in vitro*; (4) cytokines produced *in vitro* are rapidly 'consumed' by receptors which are also overtly expressed after stimulation. This process might thereby bring about a false indication of a decreased cytokine production. Most of the empirical data from recent studies support the last two hypotheses. However, it would exceed the scope of this article to discuss these underlying mechanisms in closer detail. In future studies, with respect to evaluation of the last hypotheses, the IL-2 production should be analyzed more comprehensively through use of anti-CD25 monoclonal antibodies.[65] It should also be noted that studies of the messenger-RNAs for IL-2 and IFN-γ are currently under way in order to gain information at the intracellular level.

Few studies have been performed whereby the association between psychopathological symptoms of schizophrenia and cytokine production has been investigated. Cross-sectional studies have shown that negative symptoms of schizophrenia were significantly negatively correlated with both increased soluble IL-2-receptor concentration[66] and lowered IL-2 production in non-medicated schizophrenics.[67] In the latter study, a highly significant positive correlation was found between age-of-onset and IL-2 production. This is particularly important, since it suggests an association between lowered IL-2 production and the structural brain abnormalities which are supposed to be associated with these clinical features. However, earlier cross-sectional studies by our group[14,15] failed to show such correlations. Also in the present study, no correlations between negative or positive symptoms and production of IL-2 or IFN-γ could be observed at T1. There were also no correlations between the BPRS total score, or its subscores, and cytokine production at T1, T2, or T3.

The factors suspected of having a major confounding influence on the lowered cytokine production in schizophrenia include: (a) number of producer-cells; (b) production of counter-regulatory cytokines, especially IL-4 and IL-10; (c) medication; (d) non-specific stress; and (e) diurnal variation of cytokine production.

(a) In no study cited until now[14,15,29] could significant differences in the relevant lymphocyte populations be detected. The CD4CD45RO+ memory-cells were studied in particular by our group, since they are supposed to be the main producers of IFN-γ,[5] however without detecting differences between patients with schizophrenia and controls.[15] No differences were found in an extensive analysis of cell counts for leukocytes, lymphocytes, pan T cells, activated T cells and absolute numbers of B cells; however, a slight elevation of CD3/CD25+ cells carrying the IL-2 receptor and absolute/relative monocyte counts could be detected.[68]

(b) In earlier studies we reported that there was also no evidence for an influence of IL-4[15] or IL-10[29] on cytokine production. The production of IL-10 was in fact lowered in schizophrenia,[29] a finding which even suggests a malfunction in the physiological antagonism between the TH-1 and the TH-2 systems. On the other hand, Gazullo *et al*[26] found an increased IL-10 production in patients with paranoid schizophrenia. It seems possible that this difference is due to the fact that different laboratory techniques were used in this study, eg lymphocytes were separated and washed. This may also be of relevance for their non-detection of lowered IFN-γ and IL-2 production.

(c) The possible influence of antipsychotic medication has regularly been debated. In this study, no significant correlations between neuroleptic dosages and cytokines could be obtained. In one early study, Moises *et al*[27] did not find an *in vitro* effect of haloperidol, on either the proliferation of lymphocytes or on IFN-γ production. In two clinical studies lymphocyte subpopulations were not influenced by neuroleptic treatment.[69,70] Never-medicated first-episode patients with schizophrenia showed similar abnormalities in IL-2

production[65] as were found in medicated patients, and both IL-2 production and supernatant levels of sIL-2R remained unaffected by subsequent neuroleptic treatment.[10,13,71] It has preliminarily been reported that patients receiving haloperidol or risperidone produce less IL-2 than those receiving clozapine.[26] Interestingly, an *in vitro* study[72] has shown that both haloperidol and clozapine reduce lymphocyte proliferation and production of IL-2 and IFN-γ in a dose-dependent manner. Although the suppression effect was visible only above a threshold concentration of 1 μM, which is hardly reached in clinical treatment, there might still be an effect of long-term application on the immune system. An *in vivo* immune-suppressive effect of clozapine was recently confirmed by another study,[73] whereas these authors reported an immune stimulation *in vitro*, in disagreement with the results of Leykin *et al*.[72] In another study,[74] elevated levels of sIL-2R were observed only in patients with schizophrenia who were treated with the atypical neuroleptic compound clozapine. These findings, however, did not stand uncontradicted.[75] By employing a 'criss-cross' technique, we found no significant influence of the sera of medicated schizophrenic subjects on IL-2- or IFN-γ production by lymphocytes from normal individuals.[68] Hence the notion of a major *in vivo* influence by neuroleptic medication is not strongly supported by current findings, but can also not be completely ruled out.

(d) Until now, no evidence has been acquired which suggests an influential role for non-specific influence of stress on cytokine production in schizophrenia. Ganguli *et al* were unable to find correlations between anxiety and tension BPRS scores and cytokine production. Ambiguous results were reported concerning distress-related cortisol levels in schizophrenia. Whilst there are reports of increased levels associated with decreased IL-2 production,[2] other groups were unable to detect increased cortisol levels.[15,76] In the present study no correlations between cortisol levels and production of IL-2 or IFN-γ could be detected (data not shown).

(e) The possible influence of sleep disturbance on cytokine production has hardly been regarded in studies of schizophrenia and TH-2 cytokines. However this might be an important point since it is known that in healthy persons serum levels of IL-1β and IL-6 temporally increase during sleep.[77] The productions of TNF-α and IL-1β upon mitogen stimulation are diminished, and the production of IL-2 is enhanced during sleep.[78] Sleep deprivation seems to affect catecholamine and IL-1 levels, the levels of IL-2 remained unchanged.[79] For patients with schizophrenia, it was reported that reduced sleep is associated with increased serum levels of IL-1.[80] Although there is no direct evidence for the lowered production of IL-2 and IFN-γ to be associated with sleep disturbances in patients with schizophrenia, such an effect could not be ruled out in this study. Future work in this field should regard the possible effects of sleep disturbances and diurnal variation.

In conclusion, *in vitro* production of IL-2 and IFN-γ is significantly reduced and also intercorrelated in acute schizophrenia. These results support the main body of published evidence. An aberrant production of these TH-1 cytokines is not only found on admission, but is also likely to be seen throughout early stages of remission. Lowered cytokine production might represent a biological state marker for acuteness of schizophrenia, however caution is recommended with respect to the fact that we investigated patients with chronic schizophrenia, a limited follow-up interval, and the possible influence of neuroleptic medication. Since there are ambiguous results with respect to possible associations between cytokine production and clinical features, more research is needed to clarify whether cytokine production abnormalities can act as markers for a certain, also clinically characterized, subtype of the disease. The involvement of immunological abnormalities in the pathophysiology of schizophrenia is still largely unclear and remains to be explored.

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