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Changes in serum concentrations and in cytokine production by blood cell cultures of a patient with major depression – case report

Traditionally, both stress and depression have been associated with impaired immune functions and increased susceptibility to infectious and neoplastic diseases. However, over the years a large body of evidence suggests that major depression may be associated with signs of immunological activation, especially with increased circulating concentrations of cytokines such as IL-1β, IL-6, IFN-γ and positive acute phase proteins (2).

The data concerning the serum concentration of cytokines as well as cytokine production ability of blood cells in major depressed patients are somehow inconsistent and, for example, IL-1β increased production in blood cells of depressed patients was described by some authors, while others detected no changes in IL-1 production (1, 14) or described decrease in this cytokine production from mitogen stimulated PBMC cultures (15).

Several papers strongly stress that the immune system shows regularly recurring rhythmic variations at numerous frequencies: the circadian – for about 24 h, a week (circaseptan) or seasonal (circannual). Such rhythms have been described for numerous haematological and immune functions. Some of these, i.e. in the circaseptan frequency range, seem to be important for humoral and cell mediated immune functions and some with periods of 19 to 22 days are very important in cyclic neutropenia in animal models (5, 6). In our opinion, the above mentioned inconsistence in the results of cytokine production measurements are, at least in part, the results of blood sampling from patients who are at different stages of their biological rhythms.

In order to verify this hypothesis we monitored during several days the serum cytokine level and we also induced cytokine production in cell cultures obtained from blood of a patient suffering from major depression and one selected healthy subject.

MATERIAL AND METHODS

Two women were included in the experiments. A 53-year-old woman suffering from unipolar major depressive disorder was hospitalised due to the third illness episode since 1992 in the Clinic and the Department of Psychiatry, Medical University of Lublin from 2nd January 2001. The control was a 52-year-old woman, university worker, without a lifetime history of psychiatric disorders. The study was an add-on to the previous research project approved by Ethics Committee. Both women were diagnosed to be in good health after a physical examination, with no medical conditions involving the immune system and no current use of medication, except for the patient with major depression who received only 10 mg/day of diazepam for over a month. Because of the severity of illness she underwent anti-depressive therapy from the beginning of observation as indicated in Table 1. Both women were postmenopausal but did not receive any hormonal therapy and did not display any evidence of abnormal complete blood counts and levels of serum proteins. Their pattern in caffeine use was comparable (about 1 cup daily) and both did not smoke or consume any alcohol for several months.

Table 1. Demographic and clinical characteristics of subjects

	Depressed patient	Control subject
Demographic characteristics		
Age	53	52
Marital status	married	married
Clinical characteristics:		
4th Jan. 2000		
HAM-D	31	
Treatment		
4th Jan. 2000 diazepam 10 mg/day		
5th Jan. 2000 diazepam 10 mg/day, amitriptyline 50 mg/day		
8th Jan. 2000 diazepam 5 mg/day amitriptyline 100 mg/day		
10th Jan. 2000 diazepam 10 mg/day amitriptyline 125 mg/day		
11th Jan. 2000 diazepam 10 mg/day amitriptyline 175 mg/day	no changes in HAM-D	
12th Jan. 2000 diazepam 10 mg/day amitriptyline 200 mg/day		
8th Feb. 2000 amitriptyline 250 mg/day mianserin 60mg/day chlorprothixene 30 mg/day		
HAM-D	14	

The depressed subject met DSM IV criteria for major depressive disorder without psychotic features. Diagnosis was made by an experienced psychiatrist. The illness severity was assessed using 17-item version of HAM-D (4).

Blood samples of both women were taken on the same days in January. Following an overnight fast, blood samples were always taken in a glass tube without anticoagulant and in a glass tube with heparin (Heparinum, Polfa 20 U/ml), at 9 a.m. The blood without anticoagulant was allowed to clot at room temperature, and the serum was separated by centrifugation at 3000 rpm for 10 min. The serum was then collected and stored at -20°C before cytokine examination (not longer than 1 month).

Peripheral blood leukocytes (PBL) of the depressed patient and control were cultivated by a whole blood technique (7). Briefly, heparinized blood was diluted in Eagle's Minimal Essential Medium (MEM) supplemented with 2 mM L-glutamine, 100 U/ml of penicillin and 100 μg/ml of streptomycin to obtain a leukocyte density of $1x10^6$ cells/ml. The blood suspension was distributed (2 ml/well) into 24-well plastic plates (Falcon, Bedfors, MA) and induced to cytokine production with a mixture of PHA (Sigma, St. Louis, MO) 10 μg/ml with LPS from *E. coli* 0111:B4 (Sigma) 2 μg/ml and incubated at 37°C in 5% CO_2 in the air. Supernatants were collected after 24 h of incubation (blood samples induced for IL-1α, IL-1β, IL-6, IL-12 and TNF–α) or after 72 h (IFN-γ production) and stored at -20°C before cytokine assay, not longer than 1 month.

Cytokine concentrations were determined by ELISA technique using kits from Endogen Inc., Woburn, MA according to the manufacturer's instruction. The lower level of detection for IL-1α was 1 pg/ml, IL-1β 1 pg/ml, IL-6 1 pg/ml, IL-12 3 pg/m, TNF-α 5 pg/ml and IFN-γ 2 pg/ml. All assays were carried out at the same time using the same batch of reagents by a single researcher.

RESULTS

Table 1 presents the demographic data and clinical characteristics of the subjects studied. The depressed and control women did not differ in age and marital status, however, the depressed subject suffered from a severe depressive episode, as indicated by HAM-D scores.

Figure 1 shows the changes in serum cytokine levels over the detection limit. It can be seen that the serum IL-1β level was comparable to control during few days of observation but increased significantly forming one wave with a peak on day 5 of observation. IL-1β level seemed to normalize after therapy when HAM-D scores decreased significantly (Table 1).

One wave was seen when IL-6 level was measured in serum of depressed patient, and remained elevated after therapy. In serum of the control person IL-6 also formed waves with maximal cytokine level on day 1, 6 and 8 of observation.

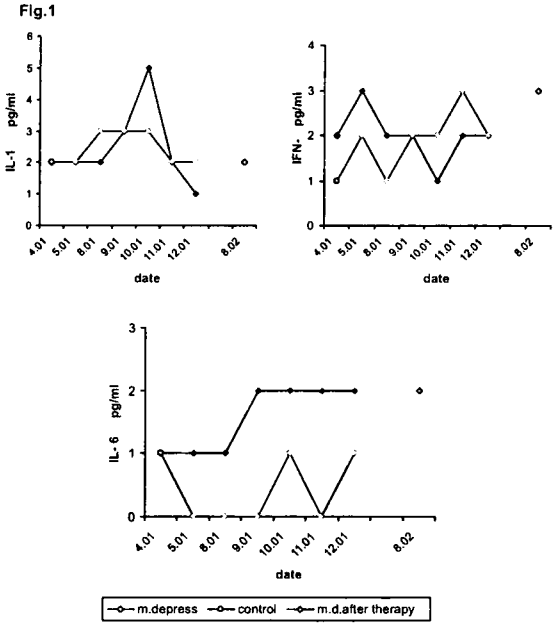

Fig. 1. Cytokine levels in sera of the depressed patient and control subject changing during the observation time

IFN-g was elevated during first days of observation in the depressed patient, and as other cytokines examined formed waves. Its concentration in serum of the depressed patient after therapy was also elevated. No IL-1α, IL-12 and TNF-α were detected in sera of both persons examined.

When blood cells were induced *in vitro* by a mixture of mitogens (PHA+LPS), the waves of cytokine concentrations seen in sera of both subjects were not so pronounced as in the sera (Fig. 2). However, the differences in the ability of blood cells to produce certain cytokine were more striking. The amount of IL-1α produced by blood cells of the depressed patients was about two times lower in comparison to control, but normalized after therapy. Similarly, the production of IL-1β in cells of the depressed patient was decreased in comparison to control and normalized after therapy. IL-12 level in the supernatant of blood cell cultures of the healthy control was nearly two times higher than that produced by blood cells of the depressed patient. Moreover, it was low in the depressed patient after therapy. However, some cytokines such as TNF-α, IL-6 and IFN-γ were produced at comparable amounts by blood cells of both subjects examined.

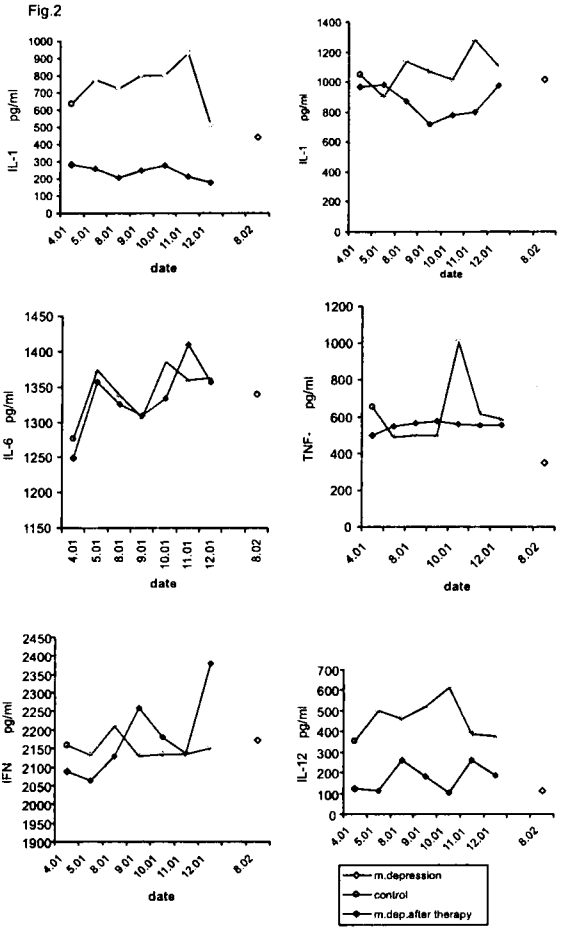

Fig. 2. Cytokine production by stimulated *in vitro* blood cells of the depressed patient and control subject

DISCUSSION

Maes et al. (12) suggested a role of both IL-1β and IL-6 in major depressive disorders. Both cytokines are mediators of the inflammatory response and additionally both cytokines were demonstrated to affect brain metabolism of serotonin, a neurotransmitter system found to be disturbed in depression (2). Moreover, antidepressants had no significant effect on serum IL-6 level but reduced serum sIL-6R (10). Our data presented in this paper, indicating elevated levels of IL-6 in serum and temporal increase serum concentrations of IL-1β in depressed patient are consistent with those of other authors (8) and seem to confirm the aforementioned suggestion concerning the

role of both cytokines in major depression, especially in induction of depressive symptoms and sickness behaviour. They may also contribute to the hyporeactivity of the hypothalamic-pituitary-adrenal (HPA) axis (2).

In our experiments we also observed elevated levels of other proinflammatory cytokines such as IFN-γ in serum of the depressed patient. The role of this cytokine in major depression is not so evident, however, the role in activation of the inflammatory response system (IRS) in depression is also postulated. Maes et al. (11) postulated that major depression is accompanied by increased production of IFN-γ, cytokine which is the product of Th1 lymphocyte subpopulation. Experimentally it was detected that blood cells of depressed patients produce elevated levels of this cytokine. Moreover, some of the antidepressants suppress the IFN-γ/IL-10 production ratio, as IL-10 is the inhibitory cytokine produced mainly by Th2 lymphocytes (9). In our experiments we observed an increased serum IFN-γ level changing with time, but in blood cell cultures stimulated *in vitro* by mitogens IFN-γ level was comparable in the depressed patient and in healthy control. It is worth mentioning that other authors did not observe any changes in m-RNA for IFN-γ, measured by RT-PCR in peripheral blood cells.

The reason of these discrepancies is not clear, however, when we measured a single cytokine level in the depressed patient we observed significant changes during the evolution of the depressive episode. One may speculate that the discrepancies in the literature concerning serum cytokine levels in major depression resulted from blood sampling at the time when a certain cytokine was near its peak or not. We have to stress that in our experiment blood was taken both from the patient and healthy control exactly on the same day and at the some morning hour (9 p.m.) to exclude differences in biological rhythms connected with circadian and circannual rhythms of the immune system.

When we measured cytokine production in the cultures of blood cells after *in vitro* stimulation with mitogens, rhythms in healthy control and in the depressed patient were, except IL-12, not so evident. However, it was easier to estimate if cytokine level produced in blood cell culture of the depressed patient was higher or lower in comparison to control. In our experiments IL-1α and IL-1β or IL-12 levels produced in blood cell cultures were lower in the patient with major depression in comparison to control, but IL-6, TNF-α and IFN-γ concentrations were comparable to control.

To the best of our knowledge this is the first report concerning decreased production of IL-12 in blood cell cultures of depressed patients. As IL-12 is known to induce IFN-γ production after antigen or mitogen stimulation by resting human Th1 lymphocytes in the presence of MHC II class positive cells (13), we expected elevated levels of this cytokine in serum of the depressed person, in whom elevated IFN-g serum level was detected. Using ELISA method (Endogen) in which active 70 kDa heterodimeric form of this cytokine was measured, we did not detect it in serum of the depressed person. But when its production in blood cell culture after mitogenic induction was measured, the IL-12 production was significantly diminished in comparison to control. These results may suggest that *in vivo* IL-12 may be overproduced in de-

pressed patients. The reasons why it was not detected by „ELISA kit" which we used by us may have been low sensitivity of this method or the fact that IL-12 is produced locally, mainly by monocytes, macrophages and B lymphocytes in lymphoid organs (3) and not present in circulation.

In conclusion, the results of our experiments suggest that major depression may be characterized by the increased circulating level of some proinflammatory cytokines, especially IL-6, IL-1β, and IFN-γ and decreased ability of blood cells after in vitro induction to produce IL-1α, IL-1β, and IL-12. Cytokine release in major depression and in control are characterized by fluctuations in circulating cytokine levels, as it was observed in healthy persons and described as biological rhythms in the immune system (6, 7). Moreover, the differences in the results of several authors, who described decreased, increased or not changed levels of cytokines in comparison to healthy control, can be caused by the fact that patients were examined at the time when a certain cytokine was near its peak or declined significantly.

We cannot exclude that the results of our experiments may have been influenced by several factors like antidepressants used in the therapy of the depressed person, the sensitivity of methods used for detection and titration of cytokines or a short time of observation. However, the fluctuations of circulating cytokines in the depressed patient and healthy control should be taken into consideration in future experiments.

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SUMMARY

The aim of the paper was to compare the periodical changes in serum cytokine levels and in cytokine production in short-term blood lymphocyte cultures of two persons: the patient with major depression and healthy control. In sera of both persons such cytokines as IL-1β, IFN-γ and IL-6 were detected, but IL-6 level in serum of depressed patient was higher than that observed in healthy control. In both persons examined serum cytokine level changed periodically during 9 days of observation showing rhythmic waves. When cytokines were induced in lymphocyte cultures periodical changes in their production were also observed, but IL-1α, IL-1β and IL-12 production was significantly lower in comparison to lymphocytes of healthy control. After 36 days of medical treatment cytokine levels in serum of patient normalized, except for IL-12. Our results suggest that inconsistence in the data from papers of different authors concerning cytokine production in major depression could result from periodical changes in their level and from the fact that patients were examined at the time when certain cytokine was near its peak or declined significantly. The rhythmic changes in cytokine level should be taken into consideration when the role of cytokines in major depression is examined.

Zmiany poziomu cytokin surowiczych oraz indukowanych w hodowli komórek krwi pacjenta chorego na depresję – opis przypadku

Celem naszych badań była porównawcza ocena okresowych zmian w poziomie cytokin występujących w surowicy oraz wytwarzanych przez hodowle limfocytów krwi obwodowej dwóch osób: pacjentki z ostrymi objawami depresji oraz kontrolnej osoby zdrowej. Spośród 6 cytokin badanych metodą ELISA u osoby chorej na depresją i zdrowej wykryto obecność IL-1β, IFN-γ oraz IL-6, ale u osoby chorej stężenie IL-6 było na ogół wyższe niż u zdrowej. U obydwu badanych osób poziom surowiczych cytokin zmieniał się okresowo w ciągu 9 dni obserwacji, wykazując rytmiczne wahania. Gdy cytokiny indukowano w hodowli limfocytów, także stwierdzono okresowe wahania w ich wytwarzaniu, ale poziom IL-1α, IL-1β oraz IL-12 był u osoby chorej na depresję wyraźnie niższy niż u osoby zdrowej. Po 36 dniach leczenia i znacznej poprawie stanu zdrowia (HAM-D przed i po leczeniu odpowiednio 31 i 14) zaobserwowano powrót indukowanego (lecz nie surowiczego) poziomu cytokin do normy. Wyjątek stanowiła IL-12. Wyniki naszych badań sugerują, że rozbieżności danych z piśmiennictwa dotyczące poziomu cytokin u chorych na depresję mogą wynikać z okresowych, rytmicznych wahań poziomu cytokin i w zależności od czasu pobrania krwi poziom cytokin u chorych na depresję może być wyższy lub niższy niż u kontroli. Te wahania powinny być brane pod uwagę także przy ocenie roli poszczególnych cytokin w patofizjologii depresji.