Serum Levels of Interleukin 6 and Stress Related Substances Indicate Mental Stress Condition in Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To evaluate the influence of mental stress on the neuroendocrine-immune system in patients with rheumatoid arthritis (RA).

Methods. Twenty-two patients with RA and 8 patients with osteoarthritis (OA) who underwent total knee or hip arthroplasties under general anesthesia were enrolled in the study. The blood levels of interleukin 6 (IL-6) and other substances related to stress were measured just before administering anesthesia on the day of the operation when the patients lay on the operating table and roughly 30 min later when the patients were under general anesthesia without mental stress. These values were compared with those at the same time on the day before the operation, which were considered the control levels.

Results. In patients with RA, the levels of IL-6, cortisol, and epinephrine in the peripheral blood were significantly increased under mental stress, before anesthesia ($p < 0.01$). However, under general anesthesia, the IL-6, cortisol, and epinephrine were significantly decreased, compared with the levels before anesthesia ($p < 0.01$). Such changes were not apparent in patients with OA. The levels of other substances in the peripheral blood known to be related to stress, such as corticotropin-releasing factor, dopamine, and norepinephrine, showed no changes in patients with RA or OA.

Conclusion. In patients with RA, excessive mental stress should be eliminated to modify the interaction between the stress-immune system and stress-endocrine system as a method to better control disease activity. (J Rheumatol 2001;28:490–5)

Key Indexing Terms:
RHEUMATOID ARTHRITIS   MENTAL STRESS   INTERLEUKIN 6   CORTISOL

We have empirically known the close relationship between the mind and body since the dawn of history. Reports indicate that the immune system is influenced by mental condition and there is interaction between the endocrine and immune systems in the response to psychological stress[1]. Changes in the production of proinflammatory cytokines, such as tumor necrosis factor, interleukin 6 (IL-6), and interferon-γ, and negative immunoregulatory cytokines, such as IL-10 and IL-4, are involved in the homeostatic responses to psychological stress[2]. Especially in patients with rheumatoid arthritis (RA), it has been described that psychoimmune processes may be implicated in short term changes in RA disease activity[3], and that patients with RA have more stress at disease onset[4]. To assess the relationship between mental condition and physical response objectively, we measured the levels of substances that are believed to reflect the activity of the neuroendocrine-immune network in the peripheral blood and compared findings in patients with RA and a healthy control group.

The subjects were instructed to listen to mirthful laughter in the traditional Japanese art of narration (Rakugo) for about 1 h, and the levels of the aforementioned substances were measured before and after the Rakugo performance. The results revealed that the levels of cortisol and IL-6 and the CD4/CD8 ratio, which were higher in the RA group than in the control group, were significantly decreased after listening to the Rakugo performance[5,6]. In particular, the unexpected finding of a decrease in the level of IL-6, which is closely related to the disease activity in RA[7-9], was noteworthy.

Based on this result, we propose that mirthful laughter suppressed or blocked the interaction between stress and the immune system as one of the mechanisms of the decrease in the blood level of IL-6 in patients with RA. We believe that patients who are about to undergo surgery are under excessive mental stress due to anxiety or fear when they are on the operating table. Conversely, during the period between the administration of anesthesia and just before the operation, we assumed that the input of stress information to the cerebral cortex and limbic system is suppressed, and therefore the stress-immune and stress-endocrine interactions are suppressed or blocked.

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MATERIALS AND METHODS

Subjects. We studied 22 patients with RA and 8 patients with OA who had been hospitalized in the Department of Joint Disease and Rheumatism for total knee or hip arthroplasties under general anesthesia. We obtained informed consent for participation in the study from all the subjects. All subjects were female.

The RA subjects ranged in age from 52.2 to 70.8 years (average 61.5 yrs), while OA subjects ranged in age from 64.3 to 78.8 (average 71.5 yrs). The duration of disease in the RA subjects ranged from 9.3 to 28.3 years (average 18.8 yrs). According to Steinbrocker classification of the stages of RA, based on the radiographic findings in the joints, 7 subjects were classified into stage III and 13 into stage IV. The mean value of 4 items from the Lansbury index[10] was 48 ± 17.8. Among the RA subjects, 19 were taking 2.5 to 7.5 mg prednisolone, 13 were taking disease modifying antirheumatic drugs (DMARD), and 11 were taking 2.5 to 7.5 mg methotrexate (MTX), while only 3 of the OA subjects were taking nonsteroidal antiinflammatory drugs (Table 1).

Measurement items. Roughly 12 ml of peripheral blood was collected from each subject, and the blood levels of 6 variables, namely, IL-6, epinephrine (EP), norepinephrine (NE), dopamine, corticotropin releasing factor (CRF), and cortisol, were measured. The chemiluminescence enzyme immunoassay (CLEIA) method was employed for the measurement of IL-6 levels, high performance liquid chromatography for the measurement of EP, NE and dopamine levels, and radioimmunoassay for the measurement of the CRF and cortisol levels. A human IL-6 measuring cartridge from Fuji Rebio Inc. (Japan) was used for measurement of IL-6 and Gamma coat™ cortisol from Incstar Corp. (France) for cortisol.

Blood sampling. To establish the control level for each measurement item, peripheral blood samples were collected from the subjects at 8:30 AM (indicated as I) and 9:00 AM (indicated as II) on the day before the operation. Peripheral blood samples were also collected just before anesthesia was administered on the day of the operation when the subjects lay on the operating table. We determined their mental condition at this time by administering a questionnaire (Table 2) after the operation. In this mental-questionnaire survey, items numbers 1 to 5 are anxiety, worry, or fear, that is to say "mental stress." We classified the degree of mental stress into 4 ranks. The severe stress group is more than 4 items, moderate stress is 3 items, mild stress is 2 items, and little stress is one item or none.

It is considered that it takes about 30 minutes for the induction of general anesthesia and preparation of the patients for the operation; during this period, mental stress inducing stimuli to the cerebral cortex and limbic system are suppressed or blocked. A blood sample was collected again at around 9:00 AM, just before the commencement of the operation (indicated as IV).

Sevoflurane and propofol were used as the general anesthetic agents, and vecuronium bromide was used as the muscle relaxant. Antirheumatic drugs, including steroids and premedications for anesthesia, were all administrated on the morning before and on the day of the operation.

Statistical analysis. The Wilcoxon signed-ranks test was used to compare the level of each variable on the day of the operation, the level on the day before the operation being considered the control level.

Table 1. Characteristics of patients with RA and OA. Values are mean ± SD.

	OA	RA
Number of patients	8	22
Mean age, yrs	71.5 ± 7.2	61.5 ± 9.3
Duration of disease, yrs	ND	18.8 ± 9.5
C-reactive protein, mg/dl	< 0.4	2.4 ± 2.0
ESR, mm/h	18.4 ± 6.8	53.1 ± 25.4
Lansbury index, %	ND	48.0 ± 17.8
Corticosteroids	0	19
NSAID	3	19
DMARD	0	13
Methotrexate	0	11

DMARD: disease modifying antirheumatic drugs, NSAID: nonsteroidal antiinflammatory drugs.

Table 2. Questionnaire for patients who underwent total joint arthroplasty.

What did you feel while you were lying on the operating table and were about to be administered anesthesia? Please check items which correspond to your feelings.

1. [ ] Will the anesthesia work effectively?
2. [ ] Will the pain in my joints be relieved by the operation?
3. [ ] Will I be able to walk after the operation?
4. [ ] Will I be able to use my arms freely after the operation?
5. [ ] Will my movements in daily living become easier after the operation?
6. [ ] I will be comfortable after falling asleep and waking up later.
7. [ ] I will finally be relieved from pain in my joints.
8. [ ] I will finally be able to walk properly.
9. [ ] I will finally be able to use my arms freely.
10. [ ] My movements in daily living will finally become easier than they were before the operation.

RESULTS

In the mental-questionnaire survey, 25 from the total of 30 patients (including RA and OA) were "severe" and 5 patients were in the "moderate" group. There were none in the "mild" and "little" groups. These results show all the patients had mental stress of more than moderate grade, but among these patients only 5 (3 with RA and 2 with OA) were hopeful for a successful operation. In all 6 variables, there was no significant difference between the severe and the moderate group. Accordingly, it was determined that at around 8:30 AM, just before anesthesia was administered (indicated as III) all the patients were under excessive mental stress.

In the RA patients, at 8:30 AM just before administration of general anesthesia on the day of the operation (III), IL-6 levels in the blood were 54.5 ± 22.1 pg/ml, which represented a significant increase compared to control levels of 32.8 ± 15.9 pg/ml in blood samples collected at 8:30 AM on the day before the operation (I). However, at 9:00 AM, 30 minutes after administration of the general anesthetic and just before commencement of the operation (IV), the IL-6 levels in the blood were 25.3 ± 9.7 pg/ml, which represented a significant decrease compared to levels observed just before administration of the general anesthetic (III) and the control level at 9:00 AM on the day before the operation (II) (Figure 1).

The EP levels in the blood were 83.0 ± 42.1 pg/ml at 8:30 AM on the day of the operation (III), which represented a significant increase compared to control levels of 27.2 ± 7.7 pg/ml at the same time on the day before the operation (I). However, EP levels at 9:00 AM (IV) were 24.5 ± 9.4 pg/ml,

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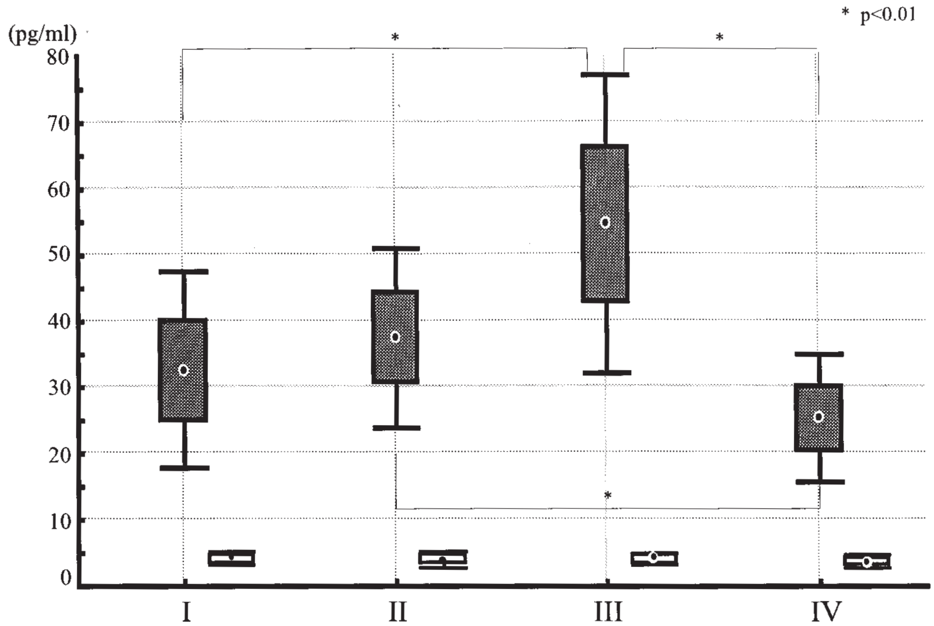

Figure 1. Upper shaded boxes indicate changes of IL-6 in peripheral blood in patients with RA. There were significant changes between Groups I and III, III and IV, and II and IV. Lower boxes indicate changes of IL-6 in patients with OA. There were no significant changes. I: 8:30 AM (the day before operation), II: 9:00 AM (the day before operation), III: 8:30 AM (just before anesthesia), IV: 9:00 AM (under anesthesia for 30 min). Bar and box show the mean ± SD and mean ± SE, respectively. *p < 0.01.

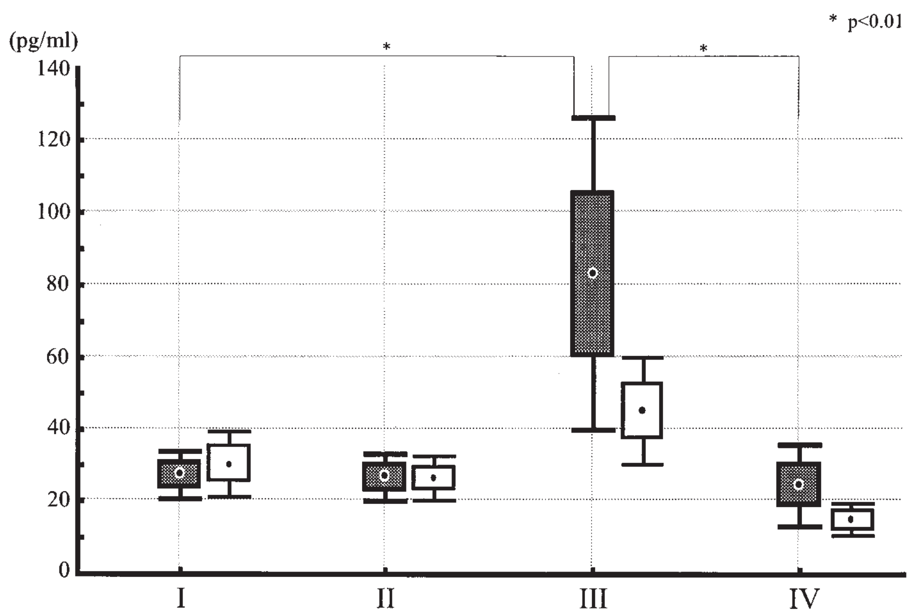

Figure 2. Shaded boxes indicate changes of epinephrine in peripheral blood in patients with RA. There were significant changes between Groups I and III, III and IV. White boxes indicate changes of epinephrine in patients with OA. There were no significant changes. Groups I, II, III, and IV represent the same subjects as in Figure 1. Bar and box show the mean ± SD and mean ± SE, respectively. *p < 0.01.

a significant decrease compared to EP levels at 8:30 AM on the day of the operation (III) and control levels at the same time on the day before the operation (II) (Figure 2).

The levels of cortisol in the blood were 8.8 ± 1.1 µg/dl at 8:30 AM on the day of the operation (III), which represented a significant increase compared to control levels of 7.1 ± 1.1 µg/dl at the same time on the day before the operation (I), but were significantly decreased to 7.6 ± 0.9 µg/dl at 9:00 AM on the day of the operation (IV) (Figure 3).

No significant differences were observed between the

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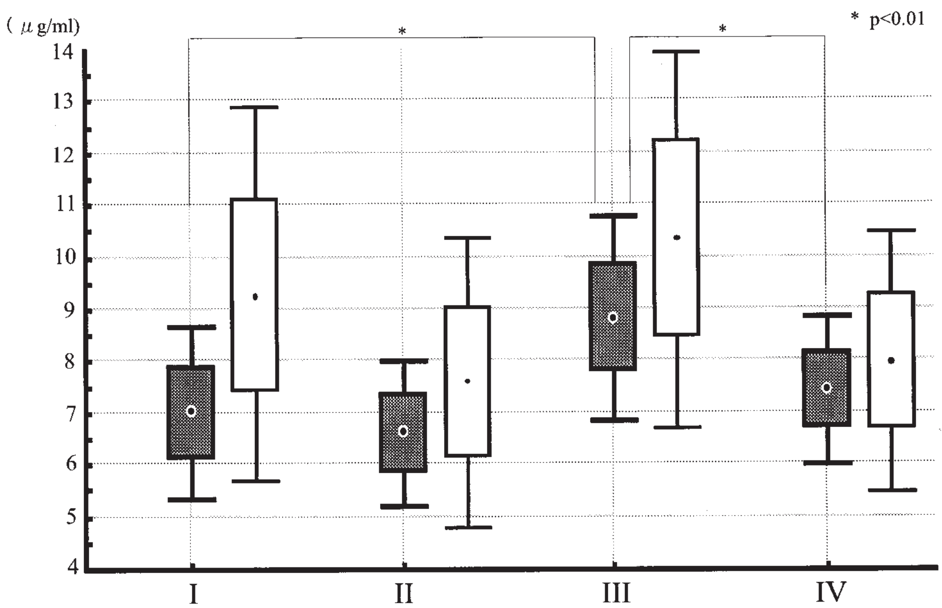

Figure 3. Shaded boxes indicate changes of cortisol in peripheral blood in patients with RA. There were significant changes between Groups 1 and II, III and IV. White boxes indicate changes of cortisol in patients with OA. There were no significant changes. Groups I, II, III and IV represent the same subjects as in Figure 1. Bar and box show the mean ± SD and mean ± SE, respectively. *p < 0.01.

levels of CRF, dopamine, and NE at 8:30 AM on the day of the operation (III) and the control levels on the day before the operation (I), or between levels observed just before administration of the general anesthetic at 8:30 AM (III) and those observed 30 minutes later (IV) (Table 3).

Meanwhile, in the OA subjects, no significant differences were observed between the levels of any of the 6 measured items including IL-6, cortisol, EP, CRF, dopamine, and NE at 8:30 AM on the day of operation (III) and the control levels on the day before the operation (I), or between the levels just before the administration of general anesthetic (III) and those determined 30 minutes later (IV) (Table 3).

Table 3. The changes of IL-6 and stress related substances in peripheral blood in patients with RA and OA. Values are mean ± SD.

	I	II	III	IV
IL-6, pg/ml				
RA	32.8 ± 15.9	38.5 ± 13.6	54.5 ± 22.1	25.3 ± 9.7
OA	3.5 ± 0.5	3.4 ± 0.7	3.5 ± 0.4	3.0 ± 1.2
Cortisol, μg/dl				
RA	7.1 ± 1.1	6.7 ± 0.9	8.8 ± 1.1	7.6 ± 0.9
OA	9.2 ± 1.8	7.5 ± 1.4	10.3 ± 1.8	7.9 ± 1.3
Epinephrine, pg/ml				
RA	27.2 ± 7.7	26.6 ± 8.4	83.0 ± 42.1	24.5 ± 9.4
OA	31.5 ± 4.5	27.6 ± 4.5	46.1 ± 7.7	16.2 ± 2.3
CRF, pg/ml				
RA	7.4 ± 0.7	7.3 ± 0.6	6.7 ± 0.9	6.6 ± 0.8
OA	9.5 ± 0.9	9.8 ± 1.0	10.5 ± 1.9	9.2 ± 1.5
Dopamine, pg/ml				
RA	15.5 ± 1.9	18.1 ± 1.9	18.5 ± 2.2	26.0 ± 4.6
OA	16.0 ± 3.9	16.2 ± 4.3	15.9 ± 3.0	22.9 ± 5.7
Norepinephrine, pg/ml				
RA	451.0 ± 49.6	498.1 ± 60.8	568.2 ± 90.0	475.0 ± 60.4
OA	426.8 ± 84.7	400.6 ± 59.7	468.6 ± 76.8	432.8 ± 84.1

I: 8:30 AM (the day before operation), II: 9:00 AM (the day before operation), III: 8:30 AM (just before anesthesia), IV: 9:00 AM (under anesthesia for 30 min).

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DISCUSSION

We performed experiments under conditions we believed reinforced, or conversely, adjusted and modified the interaction between the stress and immune systems in subjects with RA and OA. The results revealed that the levels of IL-6[7-9] (a mediator related to inflammation or the disease activity of RA), cortisol[11,12], and epinephrine[12,13] (a neuroendocrine transmitter) were markedly increased in RA subjects who lay on the operating table and were under excessive mental stress due to the impending general anesthesia and operation. However, in OA subjects, the levels of all of the aforementioned variables remained within the normal range and no significant changes were observed even in the subjects under mental stress. In the literature, a laboratory stress-activity model[14] was used to test the hypothesis that differences in stress reactivity between individuals with RA and those with OA are attributable to differences in the sensitivity of the neuroendocrine axis in regulating secretion of prolactin, adrenocorticotropic hormone, and cortisol, and in the patients with RA, stress activated the immune system, but not the consequent physiological downregulation.

Conversely, under the condition of general anesthesia, a significant decrease in the levels of the IL-6, cortisol, and EP was observed in the RA subjects. Anesthetics tend to depress neuronal firing and excitatory synaptic transmission, and potentiate synaptic inhibition[15], and we consider that anesthesia can suppress not only the activity of the cerebral cortex and limbic system but also the transduction of stress information to the immune and endocrine systems. It was described that there was no increase in the levels of TNF[16] or IL-6[16,17] that could be induced by anesthesia alone, and that the significantly decreased level of IL-6 was caused only by the release from the mental stress. Meanwhile, in the OA subjects, the levels of all the measurement items remained unchanged regardless of the absence of mental stress.

From these results, we consider that the neuroendocrine-immune system is in a state of disarray in patients with RA compared with that in healthy persons, and also that the interaction pathways between stress and the immune system are activated, thereby increasing the sensitivity of the subjects to mental stress and placing the immune system, including IL-6, under the strong influence of stress[18].

A significant increase in the levels of EP in the sympathetic nerve-adrenal medullary system, which is one of the most important stress reaction systems, was observed in patients with RA under mental stress; however, the levels of NE and dopamine remained unchanged. EP is a more sensitive indicator of mental stress than NE, which has a higher threshold[19] for reaction to mental stress. It has been reported that secretion of dopamine in the brain is increased under conditions of stress[20]. However, no correlation has been observed between stress and the levels of dopamine in the blood, which we believe explains the absence of significant changes in dopamine levels in blood under conditions of stress in this study.

In subjects with RA, a significant increase in the blood levels of cortisol, a hormone related to the hypothalamic-pituitary-adrenal axis, another stress-endocrine reaction system, was observed; however, no such changes were observed in the levels of CRF in the blood. It is well known that CRF production in the hypothalamus is increased by stress, which then stimulates the production of cortisol via the hypothalamic-pituitary-adrenal axis and increases the levels of cortisol in the blood[11]. However, there is also a report[21] that levels of CRF in the blood are unchanged under conditions of stress.

Another report indicates that lymphocytes and macrophages possess $ß_2$-adrenergic receptors on their surface[22], and that IL-6 production is reinforced when catecholamines act on these lymphocyte and macrophage receptors *in vitro*, explaining the significant changes in blood levels of IL-6 in patients with RA. Accordingly, the influence of EP and NE cannot be ignored[23-25]. In patients with RA, cells of the immune system are activated[26-31] and the secretion of EP is enhanced[12] in response to excessive mental stress; therefore the production of IL-6 is also enhanced *in vivo* via a mechanism similar to that in the experiments *in vitro*.

Our results suggest that in patients with RA excessive mental stress should be eliminated to adjust or modify the interaction between the stress-immune system and stress-endocrine system, as a method to better control disease activity.

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