Mirthful laughter differentially affects serum pro- and anti-inflammatory cytokine levels depending on the level of disease activity in patients with rheumatoid arthritis

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Objectives. To examine the effect of mirthful laughter in rheumatoid arthritis (RA), we evaluated the levels of serum cytokines before and after patients experienced mirthful laughter.

Methods. Forty-one patients with RA and 23 healthy subjects were enrolled. They listened to ‘Rakugo’, a traditional Japanese comic story, to induce mirthful laughter. We measured serum IL-6, IL-1β, TNF-α, IL-4 and IL-1 receptor antagonist (IL-1Ra) concentrations before and after patients listened to the story. The RA subjects were divided into two groups. One was designated the ‘difficult-to-control RA’ group (CRP ≥ 1.0 mg/dl); The other group was regarded as the ‘easily controlled RA’ group (CRP < 1.0 mg/dl).

Results. The basal levels of serum IL-6 and TNF-α in the RA patients were significantly higher than those in the healthy group. After experiencing mirthful laughter, the levels of serum IL-6 decreased significantly in the RA group but not in the healthy subjects. Interestingly, the level of serum TNF-α decreased only in the easily controlled RA group. Serum IL-4 concentration in the RA group was significantly higher than that in healthy subjects before the story. After the story, the level of serum IL-4 significantly decreased in the RA group, especially in the difficult-to-control RA group. Serum IL-1Ra concentration was statistically higher in the RA group than that in healthy subjects before the story, and a further increase was observed after the story, especially in the easily controlled RA group.

Conclusions. Our findings suggest that mirthful laughter affects the levels of serum pro- and anti-inflammatory cytokines differentially, depending on the RA disease activity.

KEY WORDS: Rheumatoid arthritis, Cytokines, Mental condition, Stress, Mirthful laughter.

Rheumatoid arthritis (RA) is an autoimmune disease of unknown aetiology characterized by progressive destruction of joints. Recent advances in the field of immunology have revealed that pro-inflammatory cytokines play a primary role in mediating the pathological process underlying inflammation and tissue destruction in RA [1, 2].

From the earliest days of medicine, it was believed that the mind plays an important role in physical illness. Consistent with this notion, we have sometimes noticed that disease activity is influenced by mental condition in RA patients. Furthermore, several reports have demonstrated that stressful life events precede the onset of RA and exacerbate the disease activity of RA [3, 4].

We have already demonstrated that psychoimmune processes may be implicated in short-term changes in RA disease activity and raise the possibility that the mental condition of RA patients is easily influenced by several forms of stress.

In this study, to examine the effects of mirthful laughter on the immune response in RA patients, we investigated the serum levels of pro-inflammatory cytokines and also those of anti-inflammatory cytokines that are thought to suppress inflammation and joint destruction in RA [5–7].
were selected as pro-inflammatory cytokines, and IL-4 and IL-1 receptor antagonist (IL-1Ra) as anti-inflammatory cytokines [11, 12]. In this study, we defined that if the visual analogue scale (VAS) value for joy was longer than 50 mm, participants enjoyed this performance.

Several studies have suggested that C-reactive protein (CRP) is a good surrogate measure of disease activity in RA [13–15], and has been known to predict erosive damage of joints. Therefore, we divided the RA subjects into two groups according to the level of serum CRP as an indicator of the disease activity. One group was designated the ‘difficult-to-control RA’ group and the CRP of patients in this group was ≥1.0 mg/dl (range 1.0–4.78 mg/dl). The other group was designated as the ‘easily controlled RA’ group and patients in this group had a CRP of <1.0 mg/dl.

### Statistical analysis

Data are presented as mean ± s.e.m. The Wilcoxon signed rank test was used to compare each parameter before and after laughter. The Mann–Whitney U-test was employed to compare each parameter before and after laughter.

### Results

#### Effects of mirthful laughter on the serum concentration of pro-inflammatory cytokines

In our previous study, serum concentration of IL-6 was dramatically decreased after experiencing mirthful laughter, and this could not be explained on the basis of diurnal variation [9, 10]. This result prompted us to determine the effect of mirthful laughter on other pro-inflammatory cytokines, such as TNF-α. Mirthful laughter was induced by listening to ‘Rakugo’, a traditional Japanese comic story, as described previously [9, 10]. All subjects experienced mirthful laughter except for four participants. As previously described, a significant reduction in the level of serum IL-6 was observed in RA patients after the story (Fig. 1E). The levels of serum IL-6 in healthy subjects were not affected by mirthful laughter (Fig. 1E). In contrast, mirthful laughter had no effect on the level of serum IL-1Ra in the RA group, although serum IL-1Ra concentration in the easily controlled RA group was significantly higher than that in the easily controlled RA group (Fig. 1F). We then divided the RA patient group into two groups according to the level of serum CRP and compared their measurements. A significant decrease in the level of serum IL-4 was observed only in the difficult-to-control RA group (Fig. 1G). Interestingly, a significant increase in the level of serum IL-1Ra was observed only in the easily controlled RA group, although serum IL-1Ra concentration in the difficult-to-control RA group was significantly higher than that in the easily controlled RA group (Fig. 1H). These results indicate that mirthful laughter also differentially affected serum anti-inflammatory cytokines in RA, depending on the level of disease activity.

#### Discussion

The influence of stress on cytokine production is generally not well understood; however, increasing research evidence indicates that interaction between the stress system and the immune system plays a pivotal role in the aetiology and progression of autoimmune diseases such as RA [17–21]. Consistent with previous reports, our study demonstrated that abnormal levels of serum pro-inflammatory cytokines, such as IL-6 and TNF-α, and also the levels of serum anti-inflammatory cytokines, such as IL-4 and IL-1Ra, in RA patients were present in RA patients at baseline. After laughter, these molecules were differentially modulated depending on the disease activity of RA. These results suggest that not only the immunological process but also psychological condition regulates the production of several cytokines in RA. In this study, especially in the difficult-to-control RA group, the face scale score [22] was significantly higher than that in the easily controlled RA group before the mirthful laughter. After the laughter, face scale scores in both groups significantly decreased (data not shown). These results suggest that the mental condition of RA patients appears to be differentially influenced depending

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**Table 1. Comparison of patient characteristics between patients with RA and healthy subjects and between the easily controlled RA group (CRP <1 mg/dl) and the difficult-to-control RA group (CRP ≥1 mg/dl)**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients</th>
<th>Mean age (yr)a</th>
<th>Duration of disease (yr)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>41</td>
<td>62.9 ± 1.5</td>
<td>18.4 ± 1.8</td>
</tr>
<tr>
<td>CRP &lt;1 mg/dl</td>
<td>23</td>
<td>64.0 ± 2.6</td>
<td>20.3 ± 2.4</td>
</tr>
<tr>
<td>CRP ≥1 mg/dl</td>
<td>18</td>
<td>61.4 ± 2.6</td>
<td>16.0 ± 2.5</td>
</tr>
<tr>
<td>Healthy subjects</td>
<td>23</td>
<td>62.3 ± 2.3</td>
<td>not done</td>
</tr>
</tbody>
</table>

\(a\)Mean ± s.e.m.

**Table 2. Profile of the drug therapies used by our RA patients (number of patients)**

<table>
<thead>
<tr>
<th>Drug</th>
<th>RA</th>
<th>CRP &lt;1 mg/dl</th>
<th>CRP ≥1 mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prednisolone</td>
<td>36</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>DMARDs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methotrexate</td>
<td>23</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Salazosulphapyridine</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mizoribine</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Bucillamine</td>
<td>16</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>32</td>
<td>18</td>
<td>14</td>
</tr>
</tbody>
</table>

Prednisolone: 36/47

DMARDs: Methotrexate: 23/16

Salazosulphapyridine: 2/2

Mizoribine: 2/1

Bucillamine: 16/9

NSAIDs: 32/14
on RA disease activity. The difficult-to-control RA group is under more stress than the easily controlled RA group and mirthful laughter can be used as a means to improve mental condition. We considered that uninhibited, spontaneous laughter induced by 'Rakugo' enhances the sense of well-being to a much higher degree than just mild amusement. Therefore, the levels of serum cytokines in RA patients seem to be greatly affected when patients engage their mind with the story. Moreover, our previous study showed that general anaesthesia reduced the levels of serum proinflammatory cytokines in RA patients but not OA patients. Taken together, these results strongly suggest engagement of the mind with the story or that deep sleep may have an anti-inflammatory effect in RA patients.

Several studies have suggested that the hypothalamic–pituitary–adrenal (HPA) axis is an important anti-inflammatory and anti-stress pathway that is impaired in RA patients [23, 24]. In addition, dysregulation of this interaction between neuroendocrine and immune mechanisms has been shown to contribute to the initiation and/or perpetuation of inflammatory diseases such as RA.

In this study, the levels of serum IL-6 decreased after patients experienced mirthful laughter, especially in the difficult-to-control RA group. Cronbach’s α

\[ \alpha = 0.85 \]

Though the levels of serum IL-6(C), TNF-α(D), IL-4(G) and IL-1Ra(H) were measured in these groups as described above.

\[ P \text{ value } < 0.05, \text{ before versus after experiencing mirthful laughter, } * \text{ value } < 0.05, \text{ for comparison of patients group and healthy subjects, or 'easily controlled RA' group and 'difficult-to-control RA' group.} \]

**FIG. 1.** Effects of mirthful laughter on the levels of serum pro-inflammatory cytokines and anti-inflammatory cytokines. Mirthful laughter was induced by listening to “Rakugo” (a traditional Japanese comic story). Blood samples were collected before and after the induction of mirthful laughter. Serum concentrations of IL-6(A), TNF-α(B), IL-4(E) and IL-1Ra(F) were measured by ELISA. RA subjects were divided into two groups. One group was “difficult-to-control RA” group (CRP ≥ 1.0 mg/dl). The other was regarded as “easily controlled RA” group (CRP < 1.0 mg/dl). The levels of serum IL-6(C), TNF-α(D), IL-4(G) and IL-1Ra(H) were measured in these groups as described above.

\[ * \text{ value } < 0.05, \text{ for comparison of patients group and healthy subjects, or 'easily controlled RA' group and 'difficult-to-control RA' group.} \]
RA group. This observation may reflect the improvement of mental condition and modulation of HPA axis by experiencing mirthful laughter that resulted in the reduction of IL-6. Thus, the effects of mirthful laughter might be remarkable, especially in the difficult-to-control RA group, since it is thought that such patients are under much more psychological and physical stress in daily life compared with patients with easily controlled RA.

Interestingly, in contrast to the concept described above, the level of serum TNF-α decreased only in the easily controlled RA group after the laughter. Therefore, the regulation of TNF-α by the HPA axis may be different from that of IL-6. In contrast with IL-6, TNF-α may play a minor role in the HPA axis. Therefore, changes in the levels of serum TNF-α may not have been easily observed because of the high serum TNF-α concentration in the difficult-to-control RA group. Alternatively, several studies have demonstrated that TNF-α is produced not only in peripheral lymphocytes but also in several brain sites [25]. Therefore, the discordant link between TNF-α and IL-6 might reflect the differential production of TNF-α and IL-6 in the central nervous system. Further study is needed to resolve this issue.

In addition, we demonstrated differential effects of mirthful laughter not only on pro-inflammatory cytokines but also on anti-inflammatory cytokines. It has been shown that IL-4 inhibits the production of pro-inflammatory cytokines and Th1-type cytokines [5, 26]. Marked changes in the level of serum IL-4 were not seen in the easily controlled RA group or in healthy subjects. However, the level of serum IL-4 decreased significantly in the difficult-to-control RA group after the laughter. It is unclear whether this is the result of a decrease in the number of IL-4-producing Th2 cells or whether it is related to decreased IL-4 production from existing Th2 cells. In any case, these results may reflect the normalization of the anti-inflammatory mechanism.

It has been shown that dysregulation in the production of IL-1Ra and IL-1β contributes to the pathogenesis of RA [27]; therefore, an increase in the production of IL-1Ra may be beneficial in RA. Recent clinical trials have proved beneficial effects of IL-1Ra in the treatment of RA [28, 29]. In contrast to the remarkable reduction in serum IL-4 levels in the difficult-to-control RA group, the levels of serum IL-1Ra in the easily controlled RA group increased significantly after the laughter. In this study there was a marked reduction in serum IL-4 levels in the difficult-to-control RA group and the levels of serum IL-1Ra in the easily controlled RA group increased significantly after patients experienced mirthful laughter. These observations suggest that the regulation of anti-inflammatory cytokines in RA patients depends on the severity of disease.

In summary, we observed differential effects induced by mirthful laughter on the levels of serum pro-inflammatory cytokines and anti-inflammatory cytokines depending on the activity of RA. These findings suggest the possibility that the various immunomodulatory responses to mental condition depend on the disease activity of RA. Therefore, psychological support should be considered indispensable for the treatment of RA.

Key messages

- RA patients are under physical and psychological stresses caused by deformity of joints, pain, and other complications.
- Mirthful laughter has been shown to improve their psychological condition.
- Mirthful laughter differentially affects serum cytokine levels depending on the level of disease activity in RA.

The authors have declared no conflicts of interest.

References


