Serum Level of Interferon-γ in Autoimmune Diseases

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Interferon-γ (IFN-γ) has not only the function of induction of antiviral state of cells like α or β type of interferons (Rubin and Gupta 1980) but also many immunoregulatory functions. IFN-γ is produced by activated T cells following exposure to non-specific mitogens or specific antigens (Kelly et al. 1987) and also by natural killer (NK) cells (Hanada et al. 1982). It is reported that IFN-γ is associated with the regulation of various immune responses such as the expression of HLA-DR antigen (Basham and Merigan 1983), intercellular adhesion molecule-1 (ICAM-1) (Buckle and Hogg 1990) and the growth or differentiation of B cells (Romagnani et al. 1980; Leibson et al. 1984). In autoimmune diseases, where T cell abnormality and polyclonal B cell activation was observed, there are several reports on abnormalities in the IFN-γ production (Tsokos et al. 1988) and the serum IFN-γ level (Hooks et al. 1982; Ytterberg and Schnitzer 1982). However, there is no consensus on the role of IFN-γ in the pathogenesis of autoimmune diseases. Some of the differences in these findings might have resulted from different methods, patient conditions, or medications. Kim et al. (1987) have reported that the serum IFN-γ level detected by radioimmunounoassay is increased

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in systemic lupus erythematosus (SLE). In order to study the significance of the serum IFN-γ level, we determined it by radioimmunoassay, and compared it with clinical findings, surface antigens on peripheral mononuclear cells (PMNC) and the NK cell activity.

**Methods**

**Patients**

The patients studied attended clinics of Kinki University School of Medicine. Sixty-four patients with SLE (16, active, 48, inactive) who met with ARA's criteria (Tan et al. 1982), 15 with mixed connective tissue disease (MCTD), 13 with Sjögren's syndrome and 11 with rheumatoid arthritis (Arnet et al. 1988) were selected. Eleven sex- and age-matched healthy donors served as controls. The patients were diagnosed as MCTD when they had Raynaud's phenomenon, swelling of fingers, positive test for anti-nRNP antibody (> × 1,000) and two or more symptoms or signs of SLE, scleroderma (Subcommittee of ARA 1980) or myositis (Bohan and Peter 1975). The patients were diagnosed as Sjögren's syndrome when they had one or more of the following findings: keratoconjunctivitis sicca, mononuclear cell infiltration in salivary gland, and abnormal shadow in sialogram. Patients with SLE were diagnosed as having active disease if they had three or more of the following items: fever (> 37°C), arthralgia, skin rash, oral stomatitis or hair loss, leucopenia (≤ 4,000/mm³), erythrocyte sedimentation rate ≥ 30 mm/hr, hypoalbuminemia (≤ 3.5 g/100 ml), hypocomplementemia (CH50 ≤ 20 unit/ml), and positive LE test. Sixty patients with SLE, all of the MCTD patients, 3 patients with the Sjögren's syndrome, and 3 patients with rheumatoid arthritis (RA) were taking prednisolone (5-30 mg/day). All of the RA patients were taking non-steroidal anti-inflammatory drugs. None of the patients were complicated apparently by infectious diseases such as respiratory, urinary or intestinal infections at the time of determination of the serum IFN-γ.

**Assay of IFN-γ**

Peripheral blood from fasting subjects was drawn between 7 and 10 p.m., and the serum sample was frozen at -70°C until IFN-γ levels were assessed. The level of IFN-γ was determined by a solid phase indirect immunoradiometric assay (Centocore Malvern, PA, USA). In brief, B1 monoclonal antibody-coated polystyrene beads, used as a solid phase immuno-adsorbent for IFN-γ were incubated with 200 μl of the serum in an assay plate at a room temperature for 2 hr. The beads were washed with water and incubated with a solution containing ¹²⁵I-labeled B3 monoclonal antibody at room temperature for 2 hr. After washing, radioactivity of the beads were counted with a γ counter. IFN-γ concentration was determined by a standard curve made using natural IFN-γ diluted with normal sera. Preliminary experiments using aggregated IgG showed that there was no influence of rheumatoid factor on the IFN-γ level.

**Assay of anti-DNA antibody**

Titer of anti-DNA antibody was measured by a modification of Farr's method. Briefly, 5 μl of the serum was mixed with 95 μl of ¹⁴C-labeled DNA (200 ng/ml in 10 mM Tris-EDTA; New England Nuclear, Boston, MA, USA) and incubated for 30 min at 37°C, subsequently overnight at 4°C, incubated with saturated ammonium sulfate for 60 min at room temperature, and then centrifuged at 7,000 × g for 1 min. Radioactivity of 75 μl of the supernatant was counted with a liquid scintillation counter, and the percent DNA binding was calculated. Each assay was done in duplicate.

**Assay of serum complement activity (CH₅₀)**

Serum complement activity was measured by use of hemolytic activity of the serum.
One CH50 unit was defined as the serum volume which was needed for 50% hemolysis of 5 x 10⁸ sheep erythrocytes sensitized with hemolysin in 7.5 ml gelatin-veronal buffer (pH 7.4).

Surface antigen on peripheral mononuclear cells

Peripheral mononuclear cells were isolated from freshly drawn heparinized blood by centrifugation on a Ficoll-Paque gradient (Pharmacia, Piscataway, NJ, USA). 1 x 10⁶ cells were suspended in 2 ml of phosphate buffered saline (PBS) treated with 10 μl of Fluorescein isothiocyanate labeled anti-CD4, CD8, and HLA-DR monoclonal antibodies (Becton Dickinson, CA, USA) for 60 min at 4°C. After washing, surface antigens on the mononuclear cells were identified by Spectrum III (Ortho Diagnostics, Westwood, MA, USA). The anti-HLA-DR antigen was specific for the HLA-DR epitope (27.36 Kd).

NK cell activity

NK cell activity of the mononuclear cells was measured by ⁵¹Cr release assay. Briefly, 5 x 10⁵ mononuclear cells in RPMI-1640 (GIBCO, Grand Island, NY, USA) were supplemented with 5% fetal calf serum, 10 mM HEPES and 1 x 10⁴ of ⁵¹Cr-labeled K562 cells, and incubated at 37°C for 5 hr. NK cell activity was determined as percentage of specifically released radioactivity in the supernatant.

Statistical analysis

The significance of the correlation between two parameters was analyzed by χ² test and F test.

RESULTS

Level of the serum IFN-γ

The mean level of IFN-γ (units/ml) was as follows: normal, 0.19±0.11 (s.d.); active SLE, 0.60±0.27; inactive SLE, 0.25±0.14; MCTD, 0.35±0.27; RA, 0.31±0.21; Sjögren’s syndrome, 0.29±0.13 (Fig. 1). IFN-γ level of active SLE

![Fig. 1. Serum level of interferon-γ (unit/ml). Shaded area represents normal range defined as the mean±2s.d. of normal donors.](image-url)
patients was higher than that of normal donors significantly ($p < 0.01$).

**Relationship between the serum IFN-γ level and the clinical findings in SLE**

Percentage of SLE patients whose IFN-γ level was higher than the mean + 2S.D. value of the normal donors was significantly higher in the cases with fever, skin rash and proteinuria ($p < 0.01$, $p < 0.01$ and $p < 0.05$, respectively) than in the cases without these findings, while there was no significant correlation between the

<table>
<thead>
<tr>
<th>Clinical findings</th>
<th>A. SLE</th>
<th>B. MCTD</th>
</tr>
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<tbody>
<tr>
<td>Fever</td>
<td>+ 14</td>
<td>10 (71.4)</td>
</tr>
<tr>
<td></td>
<td>- 50</td>
<td>7 (14.0)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>+ 9</td>
<td>4 (44.4)</td>
</tr>
<tr>
<td></td>
<td>- 55</td>
<td>13 (23.6)</td>
</tr>
<tr>
<td>Skin rash</td>
<td>+ 16</td>
<td>10 (62.5)</td>
</tr>
<tr>
<td></td>
<td>- 48</td>
<td>7 (14.6)</td>
</tr>
<tr>
<td>CRP</td>
<td>+ 11</td>
<td>7 (63.6)</td>
</tr>
<tr>
<td></td>
<td>- 18</td>
<td>6 (33.3)</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>+ 34</td>
<td>11 (32.4)</td>
</tr>
<tr>
<td></td>
<td>- 26</td>
<td>2 (7.7)</td>
</tr>
</tbody>
</table>

**C. Sjögren’s syndrome**

| Clinical findings | C. | | D. RA |
|-------------------|----|----|
| Stage             | ≥ II | 6 | 1 (17.7) | Fever | + | 2 | 1 (50.0) |
|                   | I | 5 | 2 (40.0) | - | 11 | 3 (27.3) |
| Class             | ≥ II | 6 | 1 (17.7) | Lymphadenopathy | + | 4 | 0 (0) |
|                   | I | 5 | 2 (40.0) | - | 9 | 3 (33.3) |
| RAHA$^b$          | ≥ ×80 | 8 | 2 (25.0) | CRP | + | 5 | 0 (0) |
|                   | < ×80 | 3 | 1 (33.3) | - | 8 | 3 (37.5) |
| CRP               | + | 6 | 1 (17.7) | - | 5 | 2 (40.0) |

$^*$Number and percentage of the patients whose serum IFN-γ level was higher than the mean + 2S.D. of the normal donors are shown. Statistical analysis of the difference between two parameters was done by $\chi^2$ test. n, number of the patients tested.

$^b$RAHA, hemagglutination test for rheumatoid factor.

*p < 0.05, **p < 0.01.
Fig. 2. Relationship between the serum IFN-γ level and CH50 in 50 (active, 8, inactive 42) patients with SLE. Statistical analysis of correlation was done by F test.

Fig. 3. Relationship between the serum IFN-γ and the positive rate of HLA-DR antigen on peripheral mononuclear cells in 25 (active, 6, inactive, 19) patients with SLE. Statistical analysis of correlation was done by F test.
serum $\gamma$-IFN level and arthralgia or C-reactive protein (CRP) (Table 1). There was an inverse correlation between the serum IFN-\(\gamma\) level and CH\textsubscript{50} which were measured on the same day in 50 patients with SLE (active, 8; inactive, 42; \(r = -0.614, p < 0.01\), Fig. 2), and there was no significant relationship between the serum IFN-\(\gamma\) level and the titer of anti-DNA antibody (data not shown). There was significant correlation between the serum IFN-\(\gamma\) level and the rate of positive cells for HLA-DR antigen (\(n = 25\); active, 6; inactive, 19; \(r = 0.743, p < 0.01\), Fig. 3), while there was no significant correlation between the serum IFN-\(\gamma\) level and the number of CD4- or CD8- positive mononuclear cells (data not shown), or NK cell activity of PMNC in 17 patients with SLE (active, 3; inactive, 14; Fig. 4).

**Relationship between the serum IFN-\(\gamma\) level and the clinical findings in MCTD**

Positive rate of serum IFN-\(\gamma\) was increased significantly when patients had fever, arthralgia, and positive test for CRP (\(p < 0.05, p < 0.05\) and \(p < 0.05\), respectively), and there was no correlation between the serum IFN-\(\gamma\) level and skin rash or proteinuria (Table 1B).

**Relationship between the serum IFN-\(\gamma\) level and the clinical findings in RA and Sjögren's syndrome**

There was no significant correlation between the serum IFN-\(\gamma\) level and stage, class, rheumatoid factor or CRP in RA, and also no correlation between the serum IFN-\(\gamma\) level and fever, lymphadenopathy or CRP in Sjögren's syndrome (Table 1C, D).
The serum IFN-γ level showed no definite relation to the dosage of prednisolone that the patients took (Table 2).

**Discussion**

The present study showed that the serum level of IFN-γ are increased in patients with various autoimmune diseases.

In the patients with SLE, the serum IFN-γ level was increased in active cases with fever, skin rash or proteinuria, and there was an inverse correlation between the serum IFN-γ level and the serum complement activity. Therefore, it was thought that the serum IFN-γ level can provide an index for disease activity in SLE. Kim et al. (1987) have also reported that the serum IFN-γ level was higher in the SLE with erythema than those without it.

Biological functions of IFN-γ in peripheral circulation is unknown, though many in vitro functions of it have been described, including the induction of class II antigen on various cell surface. The serum IFN-γ that we can detect might be only diluted one that derived from local lesions, or there still exists a possibility that circulating lymphocytes produce it. Since the serum level is determined not only by production but by catabolism, we must also study the degradation of IFN-γ during the reaction with the target cells including B cells. We found no relationship of the serum IFN-γ level to the rate of the peripheral mononuclear cells positive for CD4 and CD8, or to the NK cell activity of them. And it has been reported that the production of IFN-γ by T cells is reduced in SLE (Tsokos et al. 1988). Therefore, it is difficult to say that the increase of the serum IFN-γ level is the result of the increase of production of IFN-γ by T cells or NK cells. Since IFN-γ is known to induce HLA-DR antigen on the surface of various cells,

**Table 2. Relationship between the serum IFN-γ level and the dosage of prednisolone**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Dose of prednisolone (mg/day)</th>
<th>Number of the patients</th>
<th>Serum IFN-γ level (units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>4</td>
<td>0.45 ± 0.32</td>
</tr>
<tr>
<td>SLE</td>
<td>≤ 10</td>
<td>45</td>
<td>0.35 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>&gt; 10, ≤ 30</td>
<td>15</td>
<td>0.49 ± 0.30</td>
</tr>
<tr>
<td>MCTD</td>
<td>≤ 10</td>
<td>15</td>
<td>0.35 ± 0.27</td>
</tr>
<tr>
<td>RA</td>
<td>0</td>
<td>8</td>
<td>0.28 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>≤ 10</td>
<td>3</td>
<td>0.31 ± 0.26</td>
</tr>
<tr>
<td>Sjögren's syndrome</td>
<td>≤ 10</td>
<td>10</td>
<td>0.28 ± 0.20</td>
</tr>
</tbody>
</table>

1 mean ± s.d.
the fact that there exists a positive correlation between the serum IFN-\(\gamma\) level and the rate of the peripheral mononuclear cells positive for HLA-DR antigen is thought suggestive for the action of circulating IFN-\(\gamma\).

In the patients with MCTD, the serum IFN-\(\gamma\) level was increased in the cases with fever, arthralgia, and positive test for CRP. Therefore, the serum IFN-\(\gamma\) level can be also an index of disease activity as in SLE.

We found no relationship between the serum IFN-\(\gamma\) level and symptoms or laboratory findings in RA or Sjögren’s syndrome. Therefore, the serum level of IFN-\(\gamma\) was not thought to reflect the disease activity in these diseases apparently. This might be due to the fact that the main lesions in RA or Sjögren’s syndrome are relatively localized in synovial membrane or salivary gland as compared to SLE or MCTD.

It is known that interferons are induced in viral infections. And there is a hypothesis that viral infections are associated with the pathogenesis of SLE. However, the patients that we studied had no apparent complications of infectious diseases. Therefore, the increase of the serum IFN-\(\gamma\) level is unlikely to be associated directly with viral infections.

We reported elsewhere that IFN-\(\gamma\) inhibited the growth of the resting B cells induced by IL-4, and that this suppression was less in SLE than in normal control (Funauchi et al. 1991). We also reported that IFN-\(\gamma\) enhances the antibody production of activated B cells which was induced by T-cell factors or IL-2. These facts suggest that IFN-\(\gamma\) may exaggerate the polyclonal B cell activation in SLE and that IFN-\(\gamma\) may be one of the factors to sustain the pathogenesis of SLE. Therefore, the increase of the serum IFN-\(\gamma\) level is thought to be associated with the activity of SLE.

We found no relationship between the dosage of adrenocorticosteroids and these data. However, the influence of adrenocorticosteroids should not be forgotten, because they are known to affect the T cell functions or HLA-DR antigen expression induced by IFN-\(\gamma\) (Shen et al. 1986).

These data suggest that IFN-\(\gamma\) is associated with the pathogenesis of autoimmune diseases such as SLE or MCTD, and that the serum IFN-\(\gamma\) level can be one of the indices for the disease activity in SLE and MCTD. IFN-\(\gamma\) in synovial membrane or salivary glands should be also studied in rheumatoid arthritis or Sjögren’s syndrome.

References


