Cytokine and immunogenetic profiles in Japanese patients with adult Still’s disease. Association with chronic articular disease


Section of Rheumatology, Department of Internal Medicine, Keio University School of Medicine, Tokyo 160-8582, Japan

Abstract

Objective. To determine cytokines and MHC class II alleles in Japanese patients with adult Still’s disease (ASD) and clarify the association between those profiles and chronic articular disease.

Methods. Of 35 patients with ASD (13 men, 22 women, mean age at onset 34.0 yr), 17 (49%) had chronic arthritis (> 6 months, chronic articular ASD) and 18 (51%) lacked chronic arthritis (systemic ASD). Cytokines and cytokine receptors in sera were measured by ELISA. Correlations of each cytokine with disease activity or C-reactive protein (CRP) were determined. MHC class II alleles were examined by polymerase chain reaction methods.

Results. In chronic articular ASD, female gender was more frequent and liver dysfunction and myalgia were rarer than in systemic ASD. In active disease, the white blood cell count was lower, but total IgG was greater in patients with chronic articular ASD than in those with systemic ASD. Tumour necrosis factor (TNF) α, soluble TNF receptor 2 and interleukin (IL)-18 were increased in both types of ASD, even in remission. Soluble IL-2 receptors, IL-4 and IL-18 levels were correlated with disease activity or CRP value only in chronic articular ASD. Interferon γ and IL-8 remained increased only in chronic articular ASD, even when disease activity, including IL-6 and CRP, was low. DRB1*1501 (DR2) and DRB1*1201 (DR5) alleles were more frequent in chronic articular than in systemic ASD, whereas DQB1*0602 (DQ1) was frequently observed in both types of ASD.

Conclusion. The present study suggests that ASD with chronic articular disease has distinct clinical, cytokine and immunogenetic profiles.

Key words: Adult Still’s disease, Cytokines, MHC class II alleles, Chronic arthritis.
Patients and methods

Patient selection and evaluation of their clinical findings

We studied 35 Japanese patients (13 men and 22 women; mean age at disease onset 34.0 yr, mean disease duration 8.9 yr) who were followed up in the Rheumatology Clinic at Keio University Hospital (Tokyo, Japan) between 1974 and 1995 and fulfilled the preliminary criteria for classification proposed by Yamaguchi et al. [14]. Briefly, five or more criteria are required for diagnosis, including two or more of the major criteria, which include fever (39°C or higher), arthralgia (lasting more than 2 weeks), typical rash, and leucocytosis with granulocytosis. Minor criteria include sore throat, lymphadenopathy and/or splenomegaly, liver dysfunction, and negative results of tests for rheumatoid factor and antinuclear antibodies. Patients with infection, cancer or other rheumatic diseases were excluded. In 35 patients, the frequency of arthritis of 12 areas (cervical spine, temporomandibular, shoulder, elbow, wrist, metacarpophalangeal (MCP), proximal interphalangeal (PIP), distal interphalangeal (DIP), hip, knee, ankle and metatarsophalangeal (MTP) and their durations were examined retrospectively. Arthritis in peripheral joints was defined as tenderness or swelling, and cervical, temporomandibular or root joint arthritis was defined as the presence of pain on passive motion. All individuals were classified as having either (i) chronic articular ASD—individuals with chronic arthritis involving at least one joint area, lasting longer than 6 months; or (ii) systemic ASD—individuals without chronic arthritis. Patients with arthritis occurring intermittently over a 6-month period were classed as having chronic articular ASD if they had arthritis lasting more than 6 months, and as having systemic ASD if the disease duration was shorter.

Disease flare-up was defined as two or more of the following, in the absence of other evident causes: fever of ≥ 39°C; arthralgia; a rash with features typically seen in juvenile RA; and leucocytosis (≥10,000/mm³) including at least 80% granulocytes. The following extra-articular findings of ASD, observed by rheumatologists, were compared between patients with systematic and chronic articular ASD: typical skin eruption; sore throat; liver dysfunction (abnormality of liver function tests); hepatitis; splenomegaly; lymphadenopathy; serositis; and myalgia. ASD-related laboratory parameters [white blood cell counts (WBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), total IgG and serum ferritin] were compared between the two types of ASD in the active phase.

Serum cytokine and soluble cytokine receptor determinations

Sera were collected from both types of patients in the active phase and in remission. The active phase and remission were defined respectively as the presence and absence of otherwise unexplained high fever. Control sera were collected from Japanese individuals who had no symptoms related to rheumatic disease. Sera were stored at −70°C. Enzyme-linked immunosorbent assay (ELISA) kits were used according to the manufacturer’s instructions (Medical Biological Laboratories, Nagoya, Japan) for the detection of the following cytokines or cytokine receptors: interleukin (IL)-4, IL-6, IL-8, IL-18, interferon (IFN)-γ; tumour necrosis factor (TNF)-α, soluble IL-2 receptor (sIL-2R), soluble TNF receptor 1 (sTNF-R1) and soluble TNF receptor 2 (sTNF-R2). Samples were assayed in duplicate wells, and the mean values of absorbance were used for data analysis. In addition, CRP values and cytokine concentrations in one or two serum samples selected randomly from each patient were plotted, and correlation diagrams were made.

Determination of MHC class II alleles

Genomic DNA extracted from peripheral blood granulocytes was amplified by the polymerase chain reaction (PCR) using primers specific for the second exon of the DRB1, DQB1 or DPB1 gene [15]. MHC class II alleles were determined on the basis of restriction fragment length polymorphisms of PCR-amplified products (Shionogi Biomedical Laboratories, Tokyo, Japan). Healthy controls, who had no symptoms related to rheumatic disease, served as controls; these were Japanese individuals living in the Tokyo area.

Statistical analyses

The χ² test was used for comparison of non-parametric data and Fisher’s exact test was used when numbers were small. For comparison of parametric data, Student’s t-test for independent samples was employed. Correlations were examined using the Spearman rank correlation test.

Results

Clinical pictures, joint manifestations and laboratory findings in ASD

All ASD patients were assigned to either the systemic or the chronic articular ASD group (Table 1); 18 patients (51%) had systemic and 17 (49%) had chronic articular ASD. No significant differences in mean age at onset and number of flare-ups were observed. In the chronic articular ASD group, most patients were women (14 patients, 82%). Liver dysfunction and myalgia were rare in patients with chronic articular ASD.

Polyarthritis, oligoarthritis or arthralgia at disease onset was not an indicator of chronic articular disease. In chronic articular ASD, however, 10 patients (59%) subsequently developed narrowing of intercarpal joint spaces or carpal ankylosis. All the patients with carpal ankylosis belonged to the chronic articular ASD group. Nine of 10 patients (90%) with carpal ankylosis had the polycyclic chronic articular pattern, and five of seven patients (71%) without carpal ankylosis had the monocyclic chronic articular pattern. The association of carpal joint damage with cytokines was not observed. In our
series, root joint arthritis at disease onset was more frequently observed in the systemic ASD group (50%) than in the chronic articular ASD group (24%), and no significant difference was found. While the WBC was greater in systemic ASD, the elevated level of total IgG was greater in chronic articular ASD.

Methotrexate (MTX) was used to treat 10 patients (56%) with systemic ASD and 12 (71%) with chronic articular ASD (not significant). The doses of MTX, corticosteroid therapy, and non-steroidal anti-inflammatory drugs were not significantly different between the two groups (data not shown).

Cytokine levels in ASD

Cytokine profiles are shown in Table 2. Unfortunately, paired sera obtained in both the active phase and remission were limited to 31 pairs (systemic ASD, 16; chronic articular ASD, 15). In systemic ASD, serum concentrations of IL-6, sTNF-R1, sTNF-R2 and IFN-γ were increased in association with signs of systemic disease activity, including elevated CRP; even in remission, sTNF-R2 still was higher than in healthy control subjects. IL-18 also remained increased even in remission. In chronic articular ASD, IL-6, sTNF-R1, sIL-2R, IL-4 and IL-18 were increased in association with disease activity, while sTNF-R1 and IL-18 in remission were still higher than in controls. sTNF-R2 showed a similar trend to sTNF-R1 in chronic articular ASD.

Although IFN-γ and IL-8 concentrations were not significantly different between the active phase and remission, both were increased compared with controls, even in remission. On the other hand, the concentration of IL-4 in remission was significantly less in the group with chronic articular ASD than in controls. In both types of ASD, the concentration of TNF-α, which was not associated with disease activity, was always higher than in controls. When compared in patients with the same disease activity, in the group with chronic articular ASD sIL-2R was significantly higher in the active phase and IFN-γ and IL-8 were higher in remission.

No significant correlations between increases in cytokines and extra-articular manifestations were observed in the present study.

Correlation between serum cytokine levels and acute-phase reactants

To evaluate the direct correlation of cytokine levels with CRP values, correlation diagrams were made for each cytokine. In both types of ASD, there were positive correlations between IL-6, sTNF-R1 or R2 and CRP (Fig. 1a). In patients with systemic ASD, sIL-2R, IL-4 and IL-18 were not correlated with CRP, whereas they were correlated in patients with chronic articular ASD (Fig. 1b). IFN-γ, TNF-α and IL-8 were not correlated with CRP in either type of ASD (data not shown).

### Table 1. Clinical and laboratory findings in patients with systemic and chronic articular ASD

<table>
<thead>
<tr>
<th></th>
<th>Systemic ASD (n = 18, 51%)</th>
<th>Chronic articular ASD (n = 17, 49%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age at onset (yr) (mean ± sd)</td>
<td>31.4 ± 13.2</td>
<td>36.8 ± 14.7</td>
<td>NS</td>
</tr>
<tr>
<td>Mean observation period (yr) (mean ± sd)</td>
<td>6.4 ± 6.2</td>
<td>9.2 ± 7.3</td>
<td>NS</td>
</tr>
<tr>
<td>Number of flare-ups</td>
<td>1.1 ± 1.5</td>
<td>1.9 ± 1.6</td>
<td>NS</td>
</tr>
<tr>
<td>Female gender</td>
<td>44%</td>
<td>82%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Typical skin eruption</td>
<td>94</td>
<td>88</td>
<td>NS</td>
</tr>
<tr>
<td>Sore throat</td>
<td>67</td>
<td>71</td>
<td>NS</td>
</tr>
<tr>
<td>Liver dysfunction</td>
<td>50</td>
<td>18</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>44</td>
<td>24</td>
<td>NS</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>56</td>
<td>29</td>
<td>NS</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>56</td>
<td>47</td>
<td>NS</td>
</tr>
<tr>
<td>Serositis</td>
<td>17</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Myalgia</td>
<td>61</td>
<td>12</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Joint manifestation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyarthritis</td>
<td>78</td>
<td>88</td>
<td>NS</td>
</tr>
<tr>
<td>Polymyalgia</td>
<td>11</td>
<td>12</td>
<td>NS</td>
</tr>
<tr>
<td>Root joint arthritis</td>
<td>50</td>
<td>24</td>
<td>NS</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>11</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Radiological joint alterations</td>
<td>0</td>
<td>59</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Laboratory data (mean ± sd)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC (u/ml)</td>
<td>22,025 ± 6846</td>
<td>17,553 ± 5515</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>96 ± 34</td>
<td>104 ± 41</td>
<td>NS</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>18.6 ± 12.4</td>
<td>16.0 ± 8.2</td>
<td>NS</td>
</tr>
<tr>
<td>Total IgG (mg/dl)</td>
<td>1805 ± 453</td>
<td>2295 ± 674</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>7451 ± 10,214</td>
<td>10,055 ± 23,022</td>
<td>NS</td>
</tr>
<tr>
<td>Use of anti-rheumatic drugs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of MTX</td>
<td>56%</td>
<td>71%</td>
<td>NS</td>
</tr>
<tr>
<td>Other anti-rheumatic agents</td>
<td>Salazosulphapyrazine 1</td>
<td>Auranofin 2</td>
<td></td>
</tr>
<tr>
<td>Gold sodium thiomalate 1</td>
<td></td>
<td>Azathioprine 1</td>
<td></td>
</tr>
</tbody>
</table>
Serum ferritin and ESR were not correlated with any cytokine (data not shown).

The longitudinal associations of clinical manifestations, CRP, serum ferritin level and cytokines in a patient with chronic articular ASD are shown in Fig. 2. This patient developed bilateral intercarpal joint-space narrowing and functional deterioration, even though she was taking prednisolone (PSL) and MTX or laboratory findings were not significantly correlated with these MHC class II alleles. No significant association of an increased cytokine concentration with a particular MHC class II was found in the present study.

**MHC class II alleles associated with ASD**

Thirty of the samples were tested. While four patients with chronic articular ASD had DRB1*1501 (DR2)/1201 (DR5), three others had DRB1*04051 (DR4)/0901(DR9) (data not shown). On the other hand, all patients with systemic ASD different from each other in their DRB1 alleles. Statistical analysis showed that DRB1*1501 or DRB1*1201 was more frequent in patients with ASD overall and in chronic articular ASD than in controls (Table 3). DQBI*0602 (DQ1) was more frequent in both types of ASD than in controls. As DRB1*1201 was not detected in patients with systemic ASD, the allele was more frequent in chronic articular than in systemic ASD. No significant difference was observed for other alleles. On the other hand, the other clinical or laboratory findings were not significantly correlated with these MHC class II alleles. No significant association of an increased cytokine concentration with a particular MHC class II was found in the present study.

**Discussion**

In the present study, we determined the association between cytokine and immunogenetic profiles and chronic articular disease in Japanese patients with ASD. Several reports regarding clinical manifestations in ASD have been published [2–7]. In several reports [3, 6, 7], the criteria of Medsger and Christy [4] were used. We used the criteria of Yamaguchi et al. [14], which were considered the most sensitive [16]; had used Medsger’s criteria [4] in our series, five patients (two with systemic and three with chronic articular ASD) would not have been diagnosed with ASD because they had either demonstrable rheumatoid factor or no articular symptoms. These patients, however, had no evidence of other diseases capable of causing high spiking fever and showed no erosive articular changes similar to those seen in RA, and had no evidence of a malignancy as a cause of fever.

Here, because we defined chronic arthritis as the existence of arthritis for > 6 months, a relatively high percentage of patients may have been allocated to the chronic articular disease group in comparison with previous reports from Southern China (27% [6]), Canada (36% [3]) and France (38.5% [5]). In these reports, patients whose disease course was dominated by articular disease were...
considered to have chronic articular ASD. Because arthritis is recognized in more than 70% of cases of ASD, the high rate of chronic articular disease depends on the criteria used to define chronic arthritis. Masson et al. reported that polyarticular onset or root joint arthritis at disease onset is one of the indicators of chronic articular disease. On the other hand, Pouchot et al. comment that root joint arthritis or oligoarthritis is the important prognostic indicator. These articular manifestations, however, were not the prognostic factor for chronic articular disease in our series. As we reported previously, chronic arthritis of the shoulder or hip joint is rare in Japan. We do not speculate about this discrepancy in the frequency of root joint destruction between Japanese and French patients. Wouters et al. have reported that HLA-DR6, which is rare in our series, may be associated with root joint arthritis [18]. Comparison of MHC class II alleles in French and Japanese patients with severe hip joint damage should be helpful, although there may be racial differences in the clinical manifestations of ASD.

Our results indicate that IL-6 is a useful marker of disease activity, as previously reported in ASD [10, 11]. IL-6 participates in the systemic inflammation of both types of ASD but may not be involved in chronic articular damage. Similarly, sTNF-Rs appeared to be associated with activity in both types of ASD, whereas the level of TNF-α remained high irrespective of activity. While elevated TNF-α has been described [10], elevations of TNF receptors have not been reported in patients with ASD. These results suggest that TNF-α–TNF receptor systems might be involved in ASD; additionally, macrophages, which produce TNF-α, are activated independently of disease activity. Interestingly, serum concentration of macrophage-colony stimulating factor was reported to be significantly increased in ASD [19], and macrophage activation syndrome (MAS) has sometimes been observed together with systemic juvenile RA (JRA) [20]. Because both sTNF-Rs are related to the coagulation abnormality induced by MAS in systemic juvenile systemic arthritis [21], determination of sTNF-Rs may be particularly important in the laboratory monitoring of ASD patients.

In the two types of ASD that we compared, sIL-2R, IL-4 and IL-18 showed different profiles in correlation diagrams. These cytokines may be involved in the pathogenesis of chronic articular ASD. sIL-2R cleaved from the surfaces of active T lymphocytes may be a useful marker in autoimmune diseases such as RA, systemic lupus erythematosus, Sjögren’s syndrome [22] and systemic JRA [23]. Schwarz-Eywill et al. reported a patient with ASD whose sIL-2R concentration was correlated with disease activity [24]. This result, along with the proliferation of T cells bearing the Vγ9/Vδ2
Both IL-8 and IFN-γ are induced by IL-18 [27], which indicates the participation of Th1-related pathways in chronic articular damage in ASD. Of note, IL-18, as well as sTNF-R2, was increased in remission even in systemic ASD. Recent reports indicate that IL-18 is markedly increased in sera from patients with ASD [28], suggesting that IL-18 has a central role in the clinical manifestations of ASD.

IL-8, a chemokine derived from macrophages, is involved in the recruitment of neutrophils in RA synovial tissue [29]. Notably, in chronic articular ASD, IFN-γ and IL-8 remained increased in serum even during remission. Anti-IFN-γ monoclonal antibody therapy sometimes ameliorates articular damage [30], suggesting that IFN-γ may have regulatory effects on joint damage. On the other hand, IL-8 may stimulate the migration of T cells or monocytes from the circulation into synovial tissues through epithelium [31]. To begin to elucidate these mechanisms, we are planning to examine local mRNA expression and the concentrations of IFN-γ and IL-8 in carpal lesions of patients with chronic articular ASD. As articular damage occurs independently of systemic activity, these cytokines may be candidate markers of chronic articular ASD and chronic articular damage.

Although MTX was used to similar extents in patients with systemic and chronic articular ASD and had little effect on cytokine profiles, we could not exclude the possibility that anti-rheumatic drugs affect the levels of these cytokines. Comparison between sera from patients who received the same doses of anti-rheumatic drugs may be informative.

The immunogenetic associations of ASD have been controversial. A few studies concerning MHC class II in ASD have been reported [3, 5, 18, 32]. While DR2 was observed frequently in our patients with ASD (53%), as previously reported [3], DR6, which has been associated with root joint involvement [24], was rare in our series. HLA-DRB1 alleles related to RA among Japanese (DRB1*0101 and 0405 [33]) were equally frequent in both types of ASD and in normal controls, arguing against a link between chronic articular ASD and RA. We strongly suggest that chronic articular disease is associated with certain immunogenetic backgrounds, although both genetic and environmental factors may be involved in ASD [34, 35].
We conclude that chronic articular ASD has immunogenetic and cytokine profiles distinct from those of systemic ASD. These laboratory findings should be useful markers of articular damage in ASD. Combination treatment with immunosuppressive agents such as cyclosporin A [36] or MTX [37] and biological agents such as TNF-α antagonists may be effective against chronic articular ASD.

References