Clinical and pathological differences between Mikulicz’s disease and Sjögren’s syndrome


Objective. Mikulicz’s disease (MD) has been included within the diagnosis of primary Sjögren’s syndrome (SS), but represents a unique condition involving enlargement of the lachrymal and salivary glands and characterized by few autoimmune reactions and good responsiveness to glucocorticoids. We have previously described elevated immunoglobulin (Ig) G4 in the serum of four patients with MD. In this paper, we accumulated more MD cases and undertook clinical and histopathological analysis of these patients to clarify differences between MD and SS.

Methods. We diagnosed seven patients with MD according to the following criteria: (i) visual confirmation of symmetrical and persistent swelling in more than two lachrymal and major salivary glands; (ii) prominent mononuclear infiltration of lachrymal and salivary glands; and (iii) exclusion of other diseases that present with glandular swelling, such as sarcoidosis and lymphoproliferative disease. We summarized the clinical and serological characteristics (IgG subclasses and IFN-γ/IL-4 ratio) of seven patients with MD, compared with SS with glandular swelling (SSw) and without glandular swelling (SSo). After steroid administration, we analysed changes in IgG subclasses in SSw and SSo. Labial salivary gland specimens in MD, SSw and SSo were stained with anti-IgG4 antibodies.

Results. The concentration (±s.d.) of IgG4 was 1169.7 ± 892.2 mg/dl in MD, 24.4 ± 7.0 mg/dl in SSw (P < 0.005) and 82.6 ± 189.7 mg/dl in SSo (P = 0.005). The IFN-γ/IL-4 ratio was 0.392 ± 0.083 (0.78 ± 0.23/2.14 ± 0.31 IU/pg) in MD, 0.004 ± 0.002 (0.20 ± 0.07/57.02 ± 14.05 IU/pg) in SSw (P < 0.05) and 0.012 ± 0.009 (0.58 ± 0.86/116.24 ± 207.65 IU/pg) in SSo (P < 0.05). The concentration (±s.d.) of IgG4 in MD decreased to 254.0 ± 50.3 mg/dl (P < 0.05) after glucocorticoid treatment. Histopathologically, only MD was associated with prominent infiltration of IgG4-positive plasmacytes into lachrymal and salivary glands.

Conclusion. Mikulicz’s disease is quite different from SS clinically and histopathologically. MD is suggested to be an IgG4-related systemic disease.

Key words: Immunoglobulin γ4, Interferon γ, Interleukin 4, Mikulicz’s disease, Sjögren’s syndrome.

In 1888, Johann von Mikulicz-Radecki reported a case with bilateral, painless and symmetrical swelling of the lachrymal, parotid and submandibular glands [1]. Schaffer linked the case with obvious diseases, such as sarcoidosis and lymphoma, presenting these symptoms as Mikulicz’s syndrome and idiopathic cases as Mikulicz’s disease (MD) in 1927 [2]. In 1933, Sjögren summarized 19 cases with keratoconjunctivitis sicca, two of which displayed swelling of major salivary glands [3]. The concept of Sjögren’s syndrome (SS) was established after this report. In 1953, Morgan and Castleman examined specimens from 18 cases diagnosed with MD. Finding that both MD and SS were histologically similar, they announced that most cases reported as MD represented MD. We have previously described elevated IgG4 levels in the serum of these patients [8]. We therefore undertook further clinical and histopathological analysis of the differences between MD and SS.

Materials and methods

We first summarized the clinical characteristics of our MD patients and measured serum IgG subclasses and cytokines of patients with MD, SS with glandular swelling (SSw) and without glandular swelling (SSo), and patients with only a dry mouth who were not diagnosed as having SS (DY). Next, we examined the changes in IgG subclasses in the MD group according to steroid therapy. Finally, we analysed gland specimens immunohistochemically.

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Patients and materials

Analyses were performed in seven patients with MD (two men, five women) who consulted doctors at Sapporo Medical University and its related facilities between April 1997 and October 2003. MD was diagnosed according to the following criteria: (i) persistent (longer than 3 months) symmetrical swelling of more than two lachrymal and major salivary glands; (ii) prominent mononuclear infiltration of lachrymal and salivary glands; and (iii) exclusion of other diseases presenting glandular swelling, such as sarcoidosis and lymphoproliferative disease. Another 14 patients with primary SS were diagnosed in accordance with the revised European criteria [9]. The five patients in the SS group presented lateral lachrymal or salivary gland swelling (SSw), and anti-SS-A antibodies were detected serologically. Serum samples from SSw patients were collected when the patients presented glandular swelling. The 12 patients with DY were also used as controls. Serum samples were obtained before and after therapy, and stored at –80°C. Formalin-fixed paraffin-embedded blocks of minor salivary gland tissue from the above groups were analysed. Blocks of the lachrymal and submandibular glands, cervical lymph nodes and bone marrow from patients with MD were also examined. Written consent for use of the information from these cases was obtained from the patients in accordance with the Declaration of Helsinki.

Nephelometry

Pretherapy serum levels of IgG subclasses from the patients were measured with a Behring nephelometer (Dade Behring, Deerfield, IL, USA) using IgG subclasses (BS-NIA IgG1–4; The Binding Site, Birmingham, UK) as antibodies for 0.4 ml serum samples. Diluted samples (N-dilution liquid; Oriental Yeast, Japan, in 1:20–100) of standard and control sera were introduced into the reaction tubes of the nephelometer. Appropriate anti-IgG subclass reagents and reaction buffer (N-responsive buffer liquid; Oriental Yeast, Tokyo, Japan) were added. Dispersion strength, according to irradiation from a light-emitting diode, was measured at a wavelength of 840 nm and contrasted with dispersion by available light after 10 s and 6 min. IgG subclass concentrations in test samples were calculated relative to calibration curves, obtained using nephelometric IgG subclass standard sera. A control serum was assayed to confirm the validity of calibration curves and the accuracy of IgG subclass determinations. Next, the post-therapy samples from three patients with MD were used to measure IgG subclass levels. We examined the changes in IgG subclasses in the MD patients according to steroid therapy.

Enzyme-linked immunosorbent assay

Interferon (IFN) γ was determined using sera from five patients with MD, five patients with SSw, five patients with SSo and 12 patients with DY. IFN-γ was measured using a Human IFN-γ ELISA kit (BioSource, Sunnyvale, CA, USA). Standards, controls and samples (50 μl) were introduced into the appropriate microplate wells and 50 μl of biotin conjugate was added, and the wells were incubated for 1.5 h. Each reacted well was washed to remove unbound IFN-γ. We added 100 μl of streptavidin-horseradish peroxidase working solution to each well, and incubated for 45 min. Each well was washed. Stabilized chromogen (100 μl) was added, and the wells were incubated for 30 min. Then 100 μl of stop solution was introduced into the wells and coloured reaction product was measured photometrically at 450 nm. The concentrations of IFN-γ in test samples were calculated relative to calibration curve values.

Interleukin (IL)-4 was measured in the serum of five patients with MD, five patients with SSw, nine patients with SSo and 12 patients with DY using the methods described by Kricka [10]. Chemiluminescent enzyme immunoassay was performed in microplate wells. Wells were coated with unlabelled monoclonal anti-IL-4 antibodies (mouse anti-human IL-4 antibody; BD Biosciences, Pharmingen, San Diego, CA, USA) and washed. Test samples and standard (recombinant IL-4 protein; R & D Systems, Minneapolis, MN, USA) and control sera were introduced into the appropriate wells and incubated. Reacted wells were washed to remove unbound IL-4. Secondary antibodies (biotinylated anti-human IL-4 antibody; BD Pharmingen) were added to each well and unbound conjugate was removed by washing. Plates were incubated with substrate solution and luminosity was measured. The concentrations of IL-4 in test samples were calculated relative to calibration curve values and the IFN-γ/IL-4 ratio was then calculated.

Immunohistochemistry

For all immunostainings, each monoclonal antibody was reacted for 24 h at 4°C with steam after endogenous peroxidase activity had been stopped in each section. Primary antibodies comprised anti-IgG4 antibodies (mouse anti-human IgG4; The Binding Site) diluted 1:500. Secondary antibodies (biotinylated anti-mouse IgG [H+L]-; Vector Laboratories, Burlingame, CA, USA) were diluted 1:500. Nuclear staining was performed using haematoxylin after indirect peroxidase staining in labial salivary gland specimens from the patients with MD, SSw, SSo and DY. Lachrymal and submandibular glands, cervical lymph nodes and bone marrow from the patients with MD were also examined. Next, gland specimens from patients with MD were stained using anti-CD138 antibodies (CD138; Biogenesis, Poole, UK) diluted 1:250 to identify IgG4-producing cells.

Statistical analysis

The Mann-Whitney U test was used for comparisons of data between subject groups, and P values of <0.05 were considered statistically significant. Statistical processing was performed using StatView version 5.0 software (SAS Institute, Cary, NC, USA).

Results

Patient profiles

Background characteristics of the patients with MD are shown in Table 1. Mean age was 66.71 ± 5.31 years. Their symptoms were not consistent with the revised European criteria for SS [9], although specimens of swollen lachrymal and minor salivary glands displayed severe mononuclear infiltration. Salivary function was only slightly decreased (Saxon’s test, 1.96 ± 2.14 g/2 min), and was improved (post-therapy improvement, 535.60 ± 485.70%) following administration of glucocorticoids. (The mean dose of prednisolone started at 40 mg/day and decreased to 5–10 mg/day. The steroid treatment was continued because of recurrent glandular swelling.) Sialography yielded normal results, and the apple-tree sign, which is typical of SS, was not seen. Half of the MD patients did not have keratoconjunctivitis sicca. Although cases 2, 4 and 7 displayed the presence of anti-nuclear antibody, and cases 2 and 4 also displayed hypocomplementaemia and they did not satisfy the criteria for systemic lupus erythematosus [11]. Serologically, mean total IgG level was 3835.9 ± 2702.7 mg/dl in MD. No case of MD displayed anti-SS-A or anti-SS-B antibodies. Cases 1 and 4 were complicated with retroperitoneal fibrosis, and cases 2 and 5 exhibited tubulointerstitial nephritis.
Differences between Mikulicz’s disease and Sjögren’s syndrome

**Table 1. Background data for patients with MD**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yr), sex</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>KCS</th>
<th>IgG (mg/dl)</th>
<th>IgG4 (mg/dl)</th>
<th>ANA</th>
<th>SS-A</th>
<th>SS-B</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>70, F</td>
<td>n.d.</td>
<td>n.d.</td>
<td>(+)</td>
<td>2011</td>
<td>228</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>Retroperitoneal fibrosis</td>
</tr>
<tr>
<td>2.</td>
<td>72, M</td>
<td>1.23</td>
<td>4.63</td>
<td>(+)</td>
<td>9470</td>
<td>2940</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>Tubulointerstitial nephritis</td>
</tr>
<tr>
<td>3.</td>
<td>69, F</td>
<td>0.24</td>
<td>2.98</td>
<td>(−)</td>
<td>1860</td>
<td>409</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>Tubulointerstitial nephritis</td>
</tr>
<tr>
<td>4.</td>
<td>68, F</td>
<td>5.46</td>
<td>4.15</td>
<td>(+)</td>
<td>2630</td>
<td>1160</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>Retroperitoneal fibrosis</td>
</tr>
<tr>
<td>5.</td>
<td>64, M</td>
<td>2.43</td>
<td>4.18</td>
<td>(−)</td>
<td>5100</td>
<td>1360</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>Tubulointerstitial nephritis</td>
</tr>
<tr>
<td>6.</td>
<td>68, F</td>
<td>0.42</td>
<td>3.41</td>
<td>(+)</td>
<td>2960</td>
<td>1280</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>Tubulointerstitial nephritis</td>
</tr>
<tr>
<td>7.</td>
<td>56, F</td>
<td>n.d.</td>
<td>n.d.</td>
<td>(−)</td>
<td>2820</td>
<td>811</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>Tubulointerstitial nephritis</td>
</tr>
</tbody>
</table>

Saxon-t, Saxon’s test; KCS, keratoconjunctivitis sicca; ANA, antinuclear antibody; SS-A, anti-SS-A antibody; SS-B, anti-SS-B antibody; n.d., not done.

**Fig. 1.** Serum IgG subclasses in MD and SS. Serum IgG4 levels were significantly higher and serum IgG1 levels were significantly lower in MD than in SS. MD; Mikulicz’s disease, SSw; Sjögren’s syndrome with glandular swelling, SSo; Sjögren’s syndrome without glandular swelling. Bars represent standard deviations.

**IgG subclasses**

Serum IgG subclasses of these patients, as measured by nephelometry, are shown in Fig. 1. The IgG4 level was 1169.7 ± 892.2 mg/dl in MD, 24.4 ± 7.0 mg/dl in SSw (P < 0.005), 82.6 ± 189.7 mg/dl in SSo (P < 0.005) and 41.1 ± 34.4 mg/dl in DY (P < 0.0005). IgG4 as a percentage of total IgG was 29.99 ± 9.68% in MD, 1.28 ± 8.2% in SSw (P < 0.005), 2.85% in SSo (P < 0.005) and 1.63 ± 8.2% in DY (P < 0.0005).

**Cytokines**

Before therapy, IFN-γ concentration was 0.78 ± 0.23 IU/ml in MD, 0.20 ± 0.07 IU/ml in SSw (P < 0.01), 0.58 ± 0.86 IU/ml in SSo (P = 0.12) and 0.092 ± 0.090 IU/ml in DY (P < 0.005) (normal value < 0.11 IU/ml). The IL-4 level was 2.14 ± 0.31 pg/ml in MD, 57.02 ± 14.05 pg/ml in SSw (P < 0.05), 116.24 ± 207.65 pg/ml in SSo (P < 0.005) and 3.00 ± 1.31 pg/ml in DY (P = 0.10) (normal value < 4.0 pg/ml). The IFN-γ/IL-4 ratio of 0.392 ± 0.083 in MD, 0.004 ± 0.002 in SSw (P < 0.05), 0.012 ± 0.009 in SSo (P < 0.05) and 0.040 ± 0.039 in DY (P < 0.005).

**Changes in IgG subclass levels following glucocorticoid therapy**

Figure 2 shows the changes in IgG subclasses following steroid therapy in MD. The IgG4 level decreased from 1820.0 ± 975.1 mg/dl to 254.0 ± 50.3 mg/dl (P < 0.05) after treatment, and IgG4/total IgG percentage declined from 37.20 ± 2.78 to 19.39 ± 8.29% (P < 0.05). The IgG1 level decreased from 1706.7 ± 854.9 to 176.7 ± 313.3 mg/dl (P < 0.05) after therapy, but the ratio increased from 36.23 ± 0.70% to 45.86 ± 5.44% (P < 0.05).

**Immunohistochemistry**

Anti-IgG4 antibody staining showed that the specimens from the minor salivary glands in MD revealed numerous IgG4-producing cells infiltrating around acinar and ductal cells (Fig. 3a). Specimens from patients with SSw (Fig. 3b), SSo and DY showed no IgG4-producing cells. In MD patients, numerous infiltrating cells with IgG4 were recognized in the lachrymal and submandibular glands (Fig. 4a, b). Large numbers of cells with IgG4 were also apparent in lymphoid tissue, such as lymph nodes and bone marrow (Fig. 4c, d). IgG4-producing cells in the glands were identified as plasma cells on anti-CD138 antibody staining.

**Discussion**

It is usually the gland of SS that is repeatedly swelling. MD presents with bilateral and persistent swelling of the lachrymal and salivary glands, but since the findings of Morgan and Castleman were published in 1953 it has been considered as being included under primary SS or as a subtype of primary SS. Our cases of MD patients displayed a gender ratio of 1:2.5 (M:F). In SS the ratio is about 1:20 [12]. In the present study, keratoconjunctivitis sicca was present in only half of all patients with MD, and the symptoms were very mild. The sialography in patients with MD did not show the apple-tree sign in any. This apple-tree sign represents contrast...
medium filling out from salivary acini and ducts of severely degenerated or destroyed glands in SS [13]. The present results seem to indicate a lack of glandular destruction in MD. This suggestion would explain two other clinical and histopathological observations. First, salivary function in MD improved significantly after steroid therapy, whereas SS is considered unable to be improved using corticosteroids [5]. Second, gland cells in MD display less apoptosis, as noted by Tsubota [6, 7], while SS is associated with apoptosis due to autoimmune disease. These findings strongly suggest that MD represents an entity separate from clinical and histopathological SS.

Serological analyses revealed that no case with MD displayed anti-SS-A or -SS-B antibodies, instead displaying elevated IgG4 concentrations, which is one of serological characteristics of MD. Our analysis revealed that IgG4 in the patients with MD constituted about 30% of total IgG, which was quite an unusual finding. We could not find this phenomenon in other any connective tissue disease [8], including SSw. In healthy adults, relative serum concentrations of the human IgG subclasses are as follows: IgG1 > IgG2 > IgG3 = IgG4 [14, 15]. In Japanese populations, mean levels for each IgG subclass are as follows: IgG1, 65%; IgG2, 25%; IgG3, 6%; IgG4, only 4% [16]. The Japanese population shows almost no differences from other populations in the percentages of Ig subclasses to total IgG. IgG4 is not generally associated with differences in sex and age, and both the IgG4 level and the IgG4/total IgG ratio are basically constant [17]. The physiological role of IgG4 remains unknown, except for actions as a blocking antibody in allergic reactions, as seen in the present study. IgG4 usually responds to allergens, especially to polysaccharides [18]. However, we are still looking for the antigen responsible for the increased IgG4 in MD. It may only be increased in some responses.

In the regulation of IgG4 production, Jeannin found that isotype switching to IgE or IgG4 in B cells needs two signals, namely Th2-type cytokines such as IL-4 and IL-13, and interactions between CD40 on B cells and CD40 ligand on T cells [19]. Production of IgG4 for mononuclear cells in vitro increased with elevated IFN-γ/IL-4 ratio and decreased with a low IFN-γ/IL-4 ratio [20, 21]. Our measurements showed that the IFN-γ/IL-4 ratio
FIG. 4. Other glands and lymphoid tissues in MD. Anti-IgG4 antibody stain. (a) Numerous mononuclear cells with IgG4 are apparent in the lacrimal glands. Magnification ×200. (b) Abundant plasma cells with IgG4 are present around the lymphoid follicles in the submandibular glands. Magnification ×200. (c) IgG4-bearing cells are apparent in the cervical lymph nodes. Magnification ×400. (d) Cells with IgG4 are present in bone marrow. IgG4-producing cells are thus present in both central and peripheral lymphoid tissues. Magnification ×400. This figure may be viewed in colour as supplementary data at Rheumatology Online.
FIG. 4. Continued.
was very high in MD, and raised IgG4 production was considered dependent on the high IFN-γ/IL-4 ratio.

Infiltrating cells in the lachrymal and salivary glands of SS are known to predominantly comprise IgG- and IgA-bearing plasma-cytes [22]. We revealed that IgG4-bearing plasmacytes were abundant in the lachrymal and salivary glands in MD, histologically. Moreover, in the present study we found this phenomenon in the central and peripheral lymphoid tissues of the patients with MD. However, our analysis could not detect any plasma cells with IgG4 in specimens from patients with SSw, SSo or DY. This seems to confirm that MD represents a quite different entity to SS. This finding suggests that MD is not a disease that occurs only in lachrymal and salivary glands, and it is possible that MD is a systemic disease.

In the present study, we showed that the pathogenesis MD involves IgG4. Elevated concentrations of IgG4 are known to be present in pemphigus vulgaris [23], pemphigus foliaceus [24] and some types of sclerosing pancreatitis [25]. Oliveira found that IgG4-related immune complexes was involved in the pathogenesis of some membranous nephropathies [26, 27]. Recent analyses of antigens for IgG4 have revealed that desmoglein 3 represents the antigen in pemphigus vulgaris, while desmoglein 1 is the antigen in pemphigus foliaceus [23, 24]. These antigens are adhesion molecules that help to maintain skin structures. IgG4 can act as a pathogenic antibody [23, 24]. The amount of IgG4 may be reflected in the severity of pemphigus vulgaris [28], and also in MD activity. Autoimmune pancreatitis has recently been the focus of interest in pancreatology. Abdominal computed tomography shows a diffuse pancreas or limited swelling of the pancreas, and endoscopic retrograde cholangio-pancreatography discloses a sclerosing pancreatic duct. The condition has good responsiveness to glucocorticoids, unlike the usual chronic pancreatitis. Serologically, there is also hypergammaglobulinaemia, especially elevated IgG4 [25].

It is known that autoimmune pancreatitis with elevated IgG4 is sometimes complicated by retroperitoneal fibrosis [29], sclerosing cholangitis [30], and so on. Kamisawa et al. proposed a new concept of 'IgG4-related autoimmune disease' around autoimmune pancreatitis, placing the emphasis on the importance of IgG4 in the pathogenesis [31]; also included here are Riedel’s thyroiditis [31], tubulointerstitial nephritis [32] and seronegative SS (SS without anti-SS-A or -SS-B antibodies) [31].

We consider that there are some problems in the concept of 'IgG4-related autoimmune disease around autoimmune pancreatitis'. The cases of seronegative SS reported by Kamisawa et al. may be equivalent to what we have described as MD. Some of our MD cases displayed complications of retroperitoneal fibrosis and tubulointerstitial nephritis, but autoimmune pancreatitis was not seen.

In future, we intend to examine the role of the elevated IgG4 in the pathogenesis of MD, and the relationships between MD and the IgG4-related autoimmune diseases discussed above.

Our research disclosed that MD represents an IgG4-related systemic disease that differs substantially from SS. The lachrymal and salivary secretion in MD can be expected to improve following adequate treatments. We consider that the administration of MD must be considered separately from that of SS.

### Key messages

- Mikulicz’s disease is quite different from Sjögren’s syndrome both clinically and histopathologically.
- Mikulicz’s disease is suggested to be an IgG4-related systemic disease.
The authors declare that they have no conflicts of interest with regard to the publication of this study.

References