

A case of Mikulicz's disease with Th2-biased cytokine profile: possible feature discriminable from Sjögren's syndrome

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Abstract This article concerns a male patient with Mikulicz's disease (MD) accompanied with marked elevation of serum immunoglobulin (Ig)G4 and IgE levels. His peripheral blood mononuclear cells (PBMC) showed markedly enhanced in vitro production of interleukin (IL)-4, IL-5, IL-13, but not interferon gamma (IFN- γ) compared with patients with Sjögren's syndrome (SS) and healthy donors, suggesting distinct Th2 bias in this MD patient. Besides the prominent infiltration of IgG4-producing plasma cells, the enhanced expression of both CD40 and CD40 ligand (CD40L) were observed in the

swollen salivary gland of the MD patient, suggesting enhanced signaling pathways for the induction of IgG4 and IgE switching. Possible differences between MD and SS in light of their underlying pathogenesis are discussed.

Keywords Mikulicz's disease · Immunoglobulin G4 (IgG4) · CD40 ligation · Th2 · Sjögren's syndrome

Introduction

Mikulicz's disease (MD) is a unique condition in which idiopathic, bilateral, painless, and symmetrical swelling of the lacrimal, parotid, and submandibular glands. Because MD and Sjögren's syndrome (SS) are histologically similar, MD has been considered as a variant of SS [1]. However, there are some clinical differences between MD and typical SS. In MD, the enlargement of lacrimal and salivary glands is persistent, and secretory dysfunction is either not detectable or moderate. Further, MD shows good responsiveness to corticosteroid treatment. Serologically, MD patients exhibit normal or hyper gammaglobulinemia and normo- or hypocomplementemia but lack anti-SS-A and anti-SS-B antibodies. Recently, it has also been reported that MD patients show marked serum immunoglobulin G4 (IgG4) elevation and infiltration of plasmacytes expressing IgG4 in the enlarged lacrimal and salivary glands. However, the specimens from SS patients show no IgG4-producing plasmacytes, and serum IgG4 elevation is currently thought to be one of the prominent characteristics of MD [2–4]. In addition, systemic IgG4-related plasmacytic syndrome, such as autoimmune pancreatitis and sclerosing cholangitis, occur frequently in MD, and its association has been

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attractive in the field of rheumatology and clinical immunology [5, 6].

Case report

In October 2006, a 61-year-old man presented with malaise, mild dry mouth, morning finger stiffness, and bilateral swelling around the eyelids and submandibular regions. He was suspected of having SS or malignant lymphoma and was admitted to our hospital for further examination in November 2006. He had past histories of bronchial asthma and allergic rhinitis. On physical examination, the bilateral lacrimal glands, parotid, and submandibular glands were markedly swollen, but there was no pain. He had no skin lesions. The cardiovascular and respiratory systems were both normal. Hepatosplenomegaly, lymphadenopathy, and neurological abnormalities were not observed. In the blood test results, a normal complete blood cell count was observed except for severe eosinophilia (2,782/ μ l). Laboratory tests revealed hypocomplementemia [C3, 51 mg/dl (normal, 86–160); C4, 9 mg/dl (17–45); CH50, 22 U/ml (30–40)] and elevated C1q immune complex [4.1 IU/ml (<3.0)]. C-reactive protein was positive (0.39 mg/dl). Antibodies, including rheumatoid factor, anticyclic citrullinated peptide (anti-CCP) antibody, myeloperoxidase antineutrophil cytoplasmic antibody (MPO-ANCA), antinuclear antibody (ANA), anti-SS-A, and anti-SS-B, were negative. Serum total IgG and IgE were markedly elevated at 7,319 mg/dl and 1,629 IU/ml, respectively. Characteristically, IgG4 increased polyclonally (3,073 mg/dl, 42% of total IgG). Hepatobiliary and pancreatic enzymes were normal, except for impaired glucose tolerance by 75 g oral glucose tolerance test (75 g OGTT) [blood glucose level at 120 min of 75 g OGTT, 210 mg/ml; hemoglobin (Hb) A1c, 6.1%]. Urinary concentration test and renal function was normal, and he had no renal tubular dysfunction. Neither lacrimal nor salivary function was disturbed (gum test 15 ml/10 min; Schirmer's test, right eye 12 mm/5 min, left eye 25 mm/5 min). Ophthalmologic examination was negative for keratoconjunctivitis sicca (KCS). Computed tomography (CT) scans revealed remarkable diffuse enlargement with wedge-shaped contour of the bilateral parotid, submandibular, and lacrimal glands. Abdominal ultrasonography and CT scans revealed a diffuse swelling of the pancreas. Endoscopic retrograde cholangiopancreatography (ERCP) showed segmental irregular narrowing of the main pancreatic duct and cholangiectasis, suggesting the presence of autoimmune pancreatitis and its related sclerosing cholangitis. Gallium-67 scintigram showed abnormal accumulation in bilateral lacrimal and submandibular glands and the center of the epigastrium. One of the swollen submandibular glands was biopsied, and

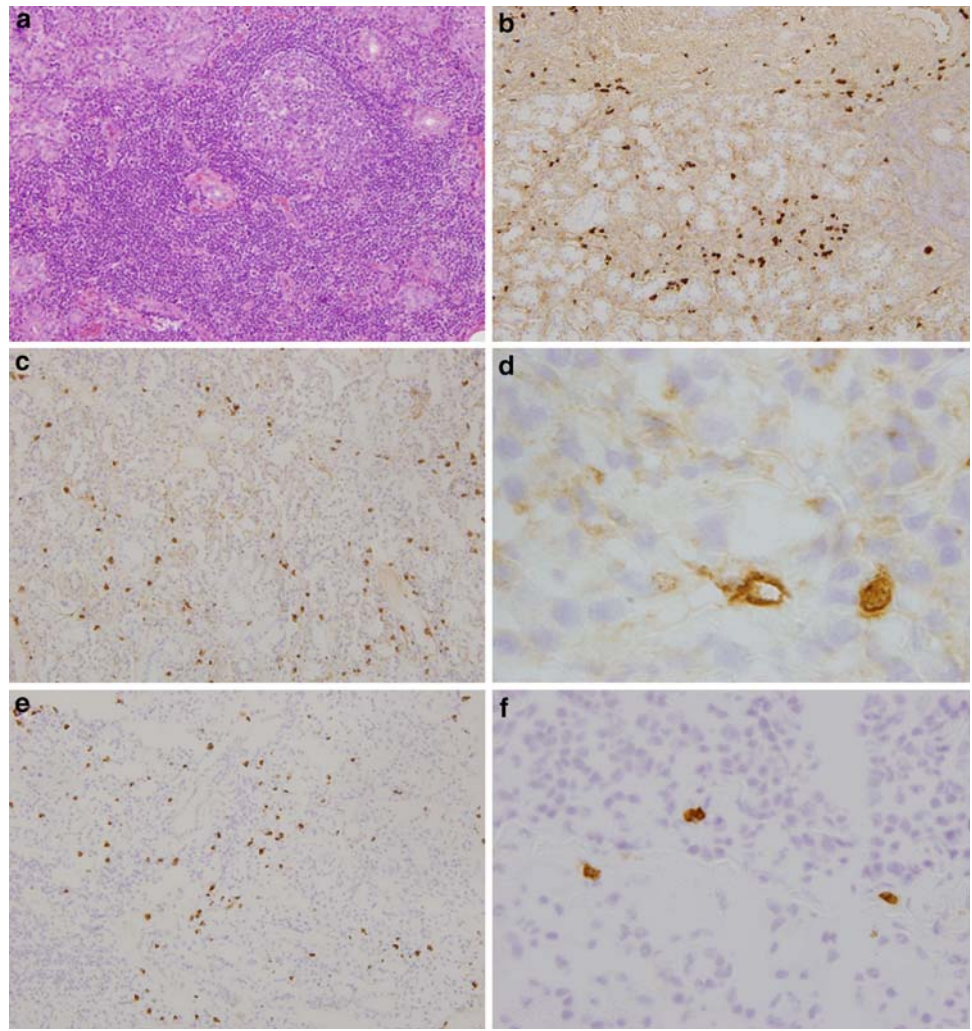
subsequent histopathological examination revealed diffuse infiltration of predominantly mononuclear cells and plasmacytes expressing IgG4 (anti-IgG4 antibody; clone HY6025, Zymed Laboratories, South San Francisco, CA, USA) (Fig. 1a, b). We diagnosed the patient as having MD on the basis of the serological and histopathological features. High-dose oral corticosteroid administration (prednisolone 50 mg/day) was initiated. After this treatment, the enlargement of the lacrimal and salivary glands rapidly regressed and severe eosinophilia diminished. Elevated serum IgG, IgG4, and IgE were remarkably decreased (IgG, 1,534 mg/dl; IgG4, 788 mg/dl, 51% of total IgG; IgE 314 IU/ml) within 4 weeks. Pancreatitis and sclerosing cholangitis evaluated by ERCP had substantially improved 2 months later.

To further examine the pathophysiology underlying the clinical symptoms in MD, fresh peripheral blood mononuclear cells (PBMC) were collected from this MD patient, two primary SS patients diagnosed by the criteria of SS [7] before treatment, and three normal healthy donors. Levels of interleukin (IL)-4, IL-5, IL-13, and interferon gamma (INF- γ) production from PBMCs with or without T-cell stimulation, antihuman CD3 antibody or phorbol-12-myristate-13-acetate (PMA) plus calcium ionophore A23187, were compared. Culture supernatants were collected at 6 and 48 h, and cytokines (IL-4, IL-5, IL-13, INF- γ) were measured by enzyme-linked immunosorbent assay (ELISA). As shown in Table 1, levels of IL-4 and IL-5 production of the MD patient were remarkably high compared with those of the SS patients and healthy donors. The levels of IL-13 in the MD patient were also significantly higher than in the healthy donors. In contrast, levels of INF- γ were not significantly altered among the group. In addition to IgG4, the expressions of CD40 and CD40L were also evaluated on sections from fresh-frozen blocks of a submandibular gland from the patient. Immunohistochemistry using anti-CD40 (clone EA-5, Ancell, Bayport, MN, USA) and anti-CD40 L antibodies (24-31, Ancell) showed a significant number of positive strong staining cells, and these looked like lymphocytes or plasmacytes morphologically (Fig. 1c–f).

Discussion

Since Morgan and Castleman concluded on the basis of histopathological similarity that MD and SS were the same entity in 1953 [1], MD has been thought to be a variant of SS. However, recent reports have pointed out differences in the clinical and histopathologic findings. In SS, the disturbed salivary function rarely recovers with corticosteroid treatment, whereas in MD, it may recover well. In addition, the infiltrating cells in affected glands are mainly CD4⁺ T

Fig. 1 Specimens of submandibular glands in a Mikulicz's disease (MD) patient. **a** H&E staining, $\times 1:400$. There is massive lymphocytic and plasmacytic infiltration forming lymphoid follicle into the interacinar region of submandibular glands. **b** Anti-IgG4 monoclonal antibody staining, $\times 1:200$. It exhibits abundant infiltration of plasmacytes with IgG4 between two acinar cells or around lymphofollicles. **c** Anti-CD40 antibody staining, $\times 1:200$, **d** $\times 1:1000$. It exhibits infiltration of lymphocytes or plasmacytes stained with CD40 between acinar cells. **e** Anti-CD40 ligand (L) antibody staining, $\times 1:200$, **f** $\times 1:400$. It exhibits infiltration of lymphocytes or plasmacytes stained with CD40L between acinar cells



cells in SS, whereas plasma cells are normally present and glandular damage is less severe. It has also been reported that glandular cell apoptosis is less often observed in MD [8]. In addition, elevated serum IgG4 levels and prominent infiltration of IgG4-producing plasma cells have been considered to be a characteristic of MD [2–4], leading to a high incidence of systemic IgG4-related plasmacytic syndrome, such as autoimmune pancreatitis and sclerosing cholangitis and allergic disease in this kind of disease. In fact, the case presented here had a history of bronchial asthma and allergic rhinitis, showed severe eosinophilia and elevated serum IgE, and high levels of IL-5 in PBMC. IL-5 is important for eosinophilic infiltration and activation [9]. In this report, we first demonstrated the CD40 ligation on submandibular glands and high levels of IL-13 in PBMC of the MD patient. Levels of IL-4 production of the MD patient were also remarkably high compared with those of the SS patients and healthy donors. IL-4 or IL-13 and CD40L have been suggested to use common signaling pathways for the induction of IgG4 and IgE switching

[10–12]: the first, furnished by IL-4 or IL-13, induces the expression of the sterile and transcript; the second, provided by the B/T-cell interaction and mimicked in vitro by CD40 triggering, induces expression of the mature and transcript [13]. In submandibular glands of our MD patient, expression of CD40 or CD40L was identified with lymphocytes and also plasmacytes. The result showed clear contrast to the previous study by Dimitriou et al. demonstrating the presence of CD40 or CD40L identified with B cell or CD4⁺ T cells in salivary gland of the SS patient [14]. In our MD patient, plasmacytes also participated in CD40 ligation that different from SS. Recently, Azuma et al. reported a case of SS with a Th2-biased property, such as elevation of serum IgE, IL-5, and eosinophil counts; the patient was later rediagnosed as having MD [15]. Miyake et al. reported peripheral CD4⁺ T cells from an MD patient who showed a Th2-predominant phenotype and elevated Th2 cytokine (IL-4, IL-5, and IL-10) but INF- γ as Th1 cytokine similarly to our results, in his salivary glands compared with healthy donors. The patient also

Table 1 In vitro cytokine production from peripheral blood mononuclear cells (PBMCs) with or without T-cell stimulation

Cytokine	Object	Incubation 6 h			Incubation 48 h		
		Stimulation (–)	Anti-CD3	PMA + A23187	Stimulation (–)	Anti-CD3	PMA + A23187
IL-4 (pg/ml)	M	1.43	31.00	610.00	0.35	63.50	3,113.00
	S1	n.d.	9.14	16.16	n.d.	0.99	17.70
	S2	<0.39	<0.39	33.40	<0.39	6.11	61.80
	H1	<0.39	3.09	105.60	<0.39	3.08	173.90
	H2	2.74	2.43	106.90	<0.39	3.08	46.20
	H3	<0.39	3.05	102.40	<0.39	4.31	115.40
IL-5 (pg/ml)	M	<11.7	16.45	427.10	<11.7	1369.00	5,444.00
	S1	n.d.	<11.7	<11.7	n.d.	<11.7	<11.7
	S2	<11.7	<11.7	<11.7	<11.7	<11.7	<11.7
	H1	<11.7	<11.7	<11.7	<11.7	183.20	130.30
	H2	<11.7	<11.7	29.04	<11.7	381.80	437.50
	H3	<11.7	<11.7	20.90	<11.7	245.00	140.90
IL-13 (pg/ml)	M	<1.56	10.74	107.1	<1.56	343.20	817.00
	S1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	S2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	H1	<1.56	5.32	29.53	<1.56	209.10	95.12
	H2	<1.56	5.45	34.36	<1.56	198.80	108.10
	H3	<1.56	3.74	24.88	11.82	154.90	74.01
IFN- γ (pg/ml)	M	76.76	731.25	4,377.96	<15.6	28,478.10	53,559.10
	S1	n.d.	4,097.40	2,570.20	n.d.	5,281.90	32,804.00
	S2	<15.6	143.30	587.10	<15.6	18,203.00	21,498.00
	H1	<15.6	343.38	2,665.10	27.6	77,834.40	22,419.10
	H2	<15.6	865.74	1,771.17	22.4	38,402.90	12,030.80
	H3	<15.6	686.26	5,525.40	5,525.5	215,180.60	39,086.30

PMA phorbol-12-myristate-13-acetate, A23187 calcium ionophore, IL interleukins, IFN interferon, M the present MD patient; S1, S2 SS patients; H1–3 healthy donors, n.d. not done

had elevated eosinophils and IgE levels in his serum [16]. Moreover, Zen et al. showed increased Th2 cytokines (IL-4, IL-5, IL-13) in situ using fresh and formalin-fixed specimens of IgG4-related sclerosing pancreatitis and cholangitis [autoimmune pancreatocholangitis (AIPC)] compared with controls, such as primary sclerosing cholangitis and primary biliary cirrhosis [17]. These results are in line with our results in which Th2-biased cytokine was shown not only in MD but also in AIPC. It is possible that IgG4-positive multiorgan lymphoproliferative syndrome (IgG4⁺ MOLPS) by Masaki [6] has common pathogenesis related to Th2 bias.

Here we found a distinct Th2 bias in the MD patient, which has not been detected in SS or healthy donors, whereas a couple of past reports indicated the Th1 bias in definite SS [18–20]. The bulk of this literature, as well as analysis of our own case, may suggest that the pathogenesis and resultant clinical manifestation of MD would be distinguishable from those of SS, and therefore it should be proposed that MD and SS are similar but definitely distinct disease entities.

Conflict of interest statement None.

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