Mod Rheumatol (2008) 18:86–90
DOI 10.1007/s10165-007-0010-3

CASE REPORT

Peripheral CD4+ T cells showing a Th2 phenotype in a patient with Mikulicz's disease associated with lymphadenopathy and pleural effusion

Katsuhisa Miyake · Masafumi Moriyama · Kumiko Aizawa · Shuji Nagano · Yasushi Inoue · Atsushi Sadanaga · Hitoshi Nakashima · Seiji Nakamura

Received: 20 July 2007 / Accepted: 11 September 2007 / Published online: 20 December 2007

Abstract Mikulicz's disease (MD) is a unique IgG4-related systemic disease indicated by enlargement of the lachrymal and salivary glands and which differs substantially from Sjögren's syndrome. A male patient with pleural effusion, swelling of the submandibular glands, and swelling of the paraaortic, mediastinal, and pararenal lymph nodes was diagnosed with MD. Analysis of peripheral CD4+ T cells from the patient revealed deviation of the Th1/Th2 balance to Th2. Prednisolone therapy ameliorated the disease and corrected the Th1/Th2 imbalance.

Keywords Mikulicz's disease · Sjögren's syndrome · Th1/Th2 balance

Case presentation

A 65-year-old man was referred to our institution with pleural effusion and swelling of the paraaortic, mediastinal, and pararenal lymph nodes in April of 2006. He had a history of bilateral painless cervical tumors, which were diagnosed as submandibular glandular swelling by echogram in May 2005. However, at that time he did not receive treatment and neglected the swelling. He had no history of allergic diseases such as bronchial asthma, atopic dermatitis, and allergic rhinitis. Physical examination showed no fever (body temperature 36.6°C), blood pressure of 134/80 mmHg, and a pulse rate of 68. The patient had no apparent symptoms of dry mouth or allergic reactions. Bilateral submandibular tumors were elastic hard (rt: 3 × 2.5 cm, lt: 3 × 3 cm). Swelling of the parotid salivary gland and preauricular lymph nodes was not detected. Chest sound was weak on the left side.

Laboratory findings showed an increased erythrocyte sedimentation rate (73 mm/h) and increased C-reactive protein (0.22 mg/dl). The hemoglobin concentration was 13.3 g/dl, the white blood cell count was 4,110/mm^3 (neutrophils 68.8%, lymphocytes 16.1%, monocytes 3.9%, eosinophils 10.2%, basophils 1.0%), and the platelet count was 27.1 × 10^4/mm^3. Serum chemistry data, including transaminase, lactate dehydrogenase (LDH) and creatinine, were all within normal limits. In immunological tests, antinuclear antibody (ANA), anti-SS-A antibody, anti-SS-B antibody and rheumatoid factor were all negative. The serum level of IgG was abnormally high, but IgA and IgM were within normal limits (3,142 ng/ml, 138 mg/dl, and 59 mg/dl, respectively). The serum IgE level was slightly elevated (735 mg/dl). The proportion of subclass IgG4 among the IgG molecules was 38%. Electrophoresis of serum protein showed polyclonal hypergammaglobulinemia. Serologic specimens were negative for cytomegalovirus, herpes simplex virus, Epstein-Barr virus, and mycoplasma, and the purified protein derivative in the tuberculin skin test was also negative. The Gum test and Saxon test

K. Miyake · K. Aizawa · S. Nagano · Y. Inoue · A. Sadanaga · H. Nakashima
Department of Medicine and Biosystemic Science, Graduate School of Medical Science, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

M. Moriyama · S. Nakamura
Department of Oral and Maxillofacial Surgery, Graduate School of Dental Science, Kyushu University, Fukuoka, Japan

Present Address:
H. Nakashima (✉)
Division of Nephrology and Rheumatology, Department of Internal Medicine, Fukuoka University School of Medicine, 7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan
e-mail: hnakashi@fukuoka-u.ac.jp

for saliva production were negative. Ophthalmologically, keratoconjunctivitis was not apparent and a Schirmer's test revealed normal lacrimal secretion.

A sonographic examination showed normal-sized parotid glands, but the submandibular glands were deformed: the right gland was spherical, the left gland was segmentally swelled, and both were hypoechoic. Swelling of multiple lymph nodes was also detected in both sides of the neck. A chest computed tomography (CT) scan revealed left pleural effusion, small patchy shadows in both lung fields, and enlargement of the mediastinal lymph nodes (Fig. 1a). In an abdominal CT scan, a renal hilar mass surrounding the pelvic ureter and swelling of paraaortic lymph nodes were apparent (Fig. 1b). A gallium-67 scintigram indicated abnormal uptake in the submandibular glands, lung hilus, and mediastinum (Fig. 1c), and a biopsy of the labial salivary glands (LSG) showed periductal lymphocytic infiltration with many plasmacytes and mild fibrosis (Fig. 1d).

Specimens were immunostained with anti-IgG4 monoclonal antibody (mAb) (1:500 dilution of mouse anti-human IgG4; The Binding Site), and this revealed abundant IgG4-bearing mononuclear cells infiltrating around acinar and ductal cells (Fig. 1e). Pleurocentesis performed on the left side showed turbid exudates, with a total cell count of 300/mm^3 in the pleural effusion. No malignant cells were detected, but numerous mononuclear cells (32% CD4+ T cells, 32% plasma cells) were present in the pleural effusion (Fig. 1f). Biochemical analysis of the effusion showed a total protein level of 6.8 g/dl, LDH activity of 137 IU/l, and a glucose level of 77 mg/dl. The concentration of IgG was 3,005 mg/dl, and that of IgG4 was 1,510 mg/dl. Bacterial culture and polymerase chain reaction (PCR) analysis for *Mycobacterium tuberculosis* DNA were negative. Only one of the three pairs of salivary glands was invaded, but bilateral submandibular glandular swelling had persisted for almost 1 year. Together with the histological findings in the labial salivary glands, polyclonal hypergammaglobulinemia with the hyper IgG4 subclass, and exclusion of diseases such as sarcoidosis, tuberculosis and malignant lymphoma, the data supported a diagnosis of Mikulicz's disease (MD). The patient was treated with 30 mg/day prednisolone (PSL) for 14 days, followed by tapering of PSL. Submandibular glandular swelling rapidly diminished, and there was a drastic reduction in pleural effusion and swelling of the paraaortic, mediastinal and pararenal lymph nodes.

Analysis of CD4+ T cells

PHA-stimulated IFN-γ production

Peripheral blood mononuclear cells (PBMNCs; 8×10^5) were incubated for 12, 24, 48, and 72 h at 37°C in 5% CO_2 in 0.2 ml RPMI 1640 (Nissui, Tokyo, Japan) containing 10% (v/v) fetal bovine serum with 5 μg/ml phytohemagglutinin (PHA). The culture supernatants were assayed for IFN-γ production by ELISA (Fig. 2a).

Intracellular cytokine detection

Flow cytometric determination of IFN-γ and IL-4 in the cytoplasm of peripheral CD4+ T cells was performed using

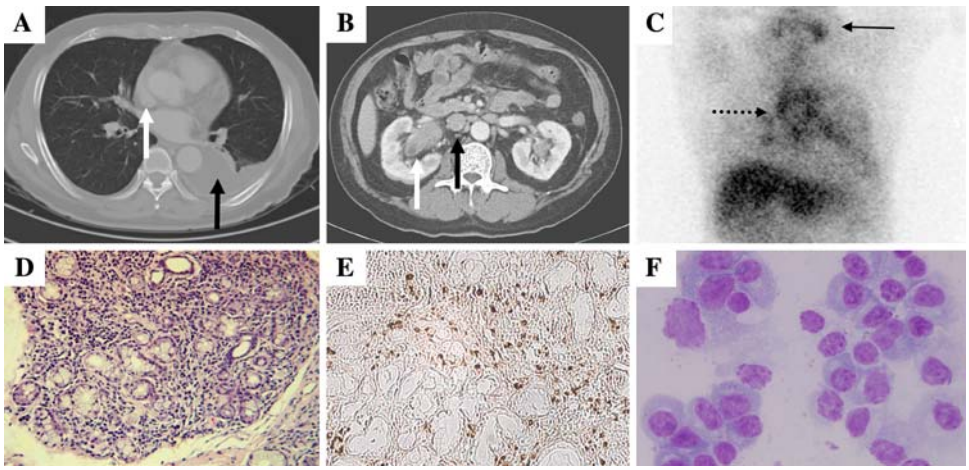

Fig. 1 Abnormal findings in examination of the patient. **a** Chest CT scan, indicating mediastinal lymph node swelling (*white arrow*) and pleural effusion (*black arrow*). **b** Abdominal CT scan, indicating a renal hilar mass surrounding the pelvic ureter (*white arrow*) and paraaortic lymph-node swelling (*black arrow*). **c** Gallium-67 scintigram, indicating abnormal uptake in the bilateral submandibular glands (*full arrow*) and uptake in the bilateral lung hilus and mediastinum (*dotted arrow*). **d** Histology of a biopsy of the labial salivary gland. **e** Plasma cells immunostained with anti-IgG4 monoclonal antibody in the biopsy specimen. **f** Plasma cells in the pleural effusion

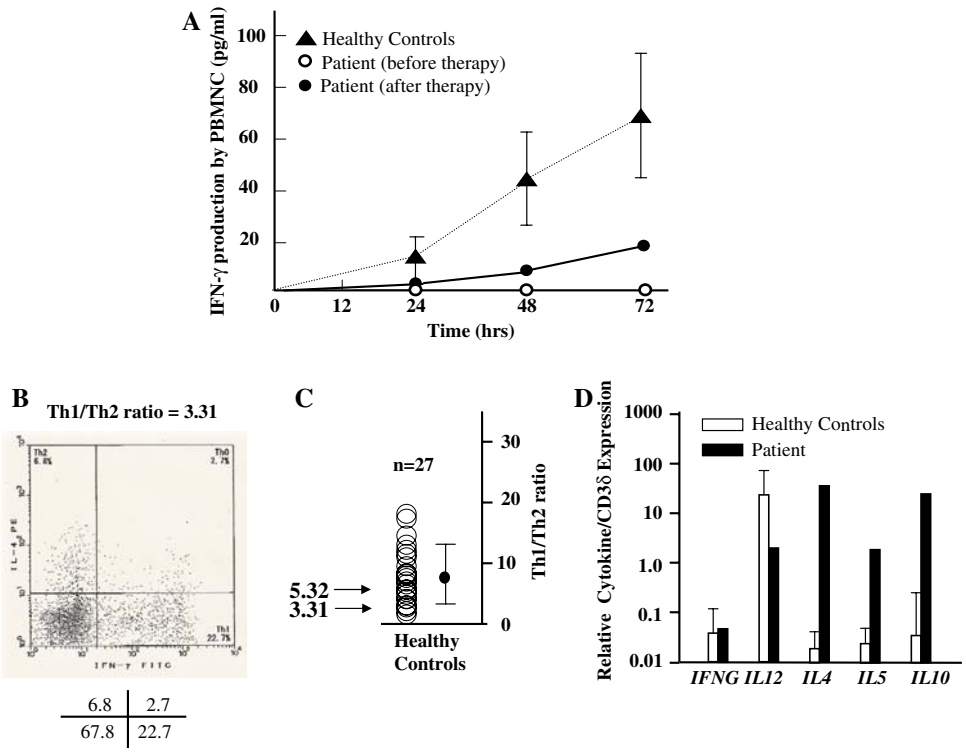

Fig. 2 Analysis of CD4+ T cells in the patient. **a** The amounts of IFN-γ produced by PBMNCs stimulated with PHA were determined periodically. **b** FACS results of intracellular IFN-γ and IL-4 cytokine assays of peripheral blood from the patient before therapy, showing that the percentages of Th1 cells [lower right region (LR)] and Th2 cells [upper left region (UL)] were 22.7 and 6.8%, respectively. **c** The Th1:Th2 balance was evaluated using the %LR to %UL ratio, and these ratios were 3.31 and 5.32 before and after therapy, respectively. **d** mRNA expression of IFN-γ, IL-12, IL-4, IL-5 and IL-10 in a biopsy specimen from the lip salivary gland

a published method [1–4]. Briefly, aliquots (500 µl) of heparinized whole blood were stimulated with a combination of 25 ng/ml phorbol myristate acetate (PMA) and 1 µg/ml ionomycin in the presence of 10 µg/ml brefeldin A (Sigma, St Louis, MO, USA), and then cultured for 4 h at 37°C in a humidified incubator containing 7% CO_2. Activated cultures were stained with 20 µl of peridinin chlorophyll protein-conjugated CD4-specific mAb (Becton Dickinson, San Jose, CA, USA) for 15 min at room temperature, and then treated with 2 ml of fluorescein-activated cell sorter (FACS) lysing solution (Becton Dickinson). After 5 min of incubation, the samples were centrifuged and combined with FACS permeabilization solution (Becton Dickinson) for 10 min at room temperature. The sample tubes were washed and incubated with fluorescein isothiocyanate (FITC)-conjugated IFN-γ specific mAb and phycoerythrin (PE)-conjugated IL-4-specific mAb (Becton Dickinson) for 30 min at room temperature. FITC-conjugated mouse IgG2a and PE-conjugated mouse IgG1 were used as controls. After washing again, the cells were resuspended in 1% paraformaldehyde and analyzed by flow cytometry. Data were obtained on a FACScan flow cytometer (Becton Dickinson) and results were analyzed using Cellquest software (Becton Dickinson). The percentages of IFN-γ- and IL-4-positive cells (% IFN-γ and% IL-4) were counted by FACS and the Th1:Th2 balance was evaluated from the ratio of % IFN-γ to % IL-4. The Th1:Th2 ratio for the patient was 3.3, whereas the mean ± SD for healthy controls was 7.77±4.24 (Fig. 2b, c).

Table 1 Laboratory data before and after PSL therapy

	Before PSL therapy	After PSL therapy
WBC (no/mm^3)	4,110	5,430
Eosinophils (%)	***10.2***	2.2
ESR (mm/h)	***100***	20
Total protein (g/dl)	***8.6***	7.2
Albumin (g/dl)	***3.5***	4.0
IgG (mg/dl)	***3,142***	***1,835***
IgG4 (% of IgG)	***38.0***	***34.4***
IgA (mg/dl)	125	120
IgM (mg/dl)	120	64
IgE (mg/dl)	***735***	33
Soluble IL-2 receptor (U/ml)	***2,345***	***984***

Bold and italicized numbers indicate abnormal values

Quantitative estimation of cytokine mRNA from salivary glands

Total RNA was prepared from LSG specimens by the acidified guanidinium–phenol–chloroform method [5–9] and quantitative cDNA amplification was performed according to the manufacturer's instructions. For controls, we used LSG specimens from 15 patients with mucocele who had no clinical or laboratory evidence of systemic autoimmune diseases. All samples were stored at −80°C before use. Informed consent was obtained from the study

participants. The cDNAs of the cytokines were analyzed by real-time PCR using LightCycler Fast Start DNA Master SYBR Green 1 (Roche Diagnostics, Mannheim, Germany) on a Light Cycler real-time PCR instrument (version 3.5; Roche Diagnostics). The cytokines examined were IFN-γ, IL-4, IL-5, IL-10 and IL-12 (Fig. 2d). The primer sequences and reaction conditions are shown in Table 2. To provide a meaningful comparison between different individuals or samples, we calculated the amount of cytokine PCR products relative to the amount of CD3δ (for standardization of T cell mRNA) in each sample.

Discussion

We have described a case of MD complicated by pleural effusion and swelling of several lymph nodes, in addition to symmetrical swelling of the submandibular glands. A number of plasma cells had infiltrated the pleura and pleural effusion. The histology of a biopsy specimen of the minor salivary glands was compatible with that of Sjögren's syndrome, but immunohistological analysis revealed infiltration of many IgG4-positive plasma cells around acinar and ductal cells. MD has often been considered as a subtype of Sjögren's syndrome [10], but MD actually represents a unique IgG4-related systemic disease with enlargement of the lachrymal and salivary glands, and differs substantially from Sjögren's syndrome [11, 12]. IgG4 is the rarest of the IgG subclasses, accounting for only 3–6% of total IgG in normal serum. It is a Th2-dependent isotype and has a low affinity for target antigen. Interleukin (IL)-4 directs naïve human B cells to switch to IgG4 and IgE production, [13] and therefore the high serum IgG4 and IgE levels in MD patients may be due to a Th2-predominant immune reaction.

The relationship of the Th1/Th2 imbalance to the pathogenesis of Sjögren's syndrome has been investigated widely, and a polarized Th1 balance has been associated with the immunopathology of the disease [14–17]. Numerous IFN-γ-positive CD4+ T cells are detected in the salivary glands of Sjögren's patients, and an intracellular cytokine assay demonstrated an increased frequency of IFN-γ-producing CD4+ T cells in the peripheral blood (PB) of patients compared with healthy controls [18]. These findings suggest that Th1 cells have a central role in the pathogenesis of Sjögren's syndrome.

In our patient, the serum showed polyclonal hypergammaglobulinemia and a high IgG4 level, accompanied with slightly elevated serum IgE, but anti-SS-A antibody, anti-SS-B antibody, and ANA were all negative. Therefore, we concluded that a Th2 immune reaction was predominant. The Th1 reaction was examined by addition of PHA to PBMNCs from the patient, and the Th1/Th2 balance in the PB was evaluated using an intracellular cytokine assay. No production of IFN-γ from PBMNCs was induced by PHA (Fig. 2a), and the Th1/Th2 ratio of 3.3 was much lower than the mean ratio in healthy controls (Fig. 2b, c). PSL therapy rapidly improved the patient's symptoms, with restoration of IFN-γ production from Th1 cells (Fig. 2a) and an increase in the Th1/Th2 ratio to 5.32 (Fig. 2b, c).

To evaluate the local immune reaction, we analyzed cytokine mRNA expression levels in salivary-gland specimens from the patient and compared the results with those from 15 healthy controls. The mRNA levels for the Th2 cytokines IL-4, IL-5 and IL-10 were 28.5, 1.82 and 19.3, respectively, for the patient, whereas the mean ($\pm$SD) levels for healthy controls were 0.013 ± 0.031, 0.0185 ± 0.0294, and 0.0464 ± 0.161, respectively; the levels of Th2 cytokines were significantly higher in the salivary glands

Table 2 Primers and conditions for real-time PCR amplification

Primer	Size (bp)		Sequence (5′ → 3′)	$MgCl_2$ (mM)	Annealing (°C)
CD3δ	184	Sense	GATGTCATTGCCACTCTGC	4	62
		Antisense	ACTTGTTCCGAGCCCAGTT		
IFN-γ	355	Sense	AGTTATATCTTGGCTTTTCA	4	58
		Antisense	ACCGAATAATTAGTCAGCTT		
IL-12	187	Sense	CCTGACCCACCCAAGAACTT	3	60
		Antisense	GTGGCTGAGGTCTTGTCCGT		
IL-4	203	Sense	CTGCCTCCAAGAACACAACT	3	62
		Antisense	CACAGGACAGGAATTCAAGC		
IL-5	104	Sense	ATGAGGATGCTTCTGCATTTG	3	63
		Antisense	TCAACTTTCTATTATCCACTCG		
IL-10	351	Sense	ATGCCCCAAGCTGAGAACCAA	3	63
		Antisense	TCTCAAGGGGCTGGGTCAGCTA		

from the patient. In contrast, the mRNA levels of the Th1 cytokines IFN-γ and IL-12 were 0.0416 and 2.37, respectively, in the patient, and 0.0377 ± 0.0050 and 19.4 ± 25.5, respectively, in healthy controls (Fig. 2d); therefore, significantly higher levels of Th1 cytokines were not seen in the salivary glands of the patient. These results indicate that the Th2 immune reaction predominated in the salivary glands of the patient and was responsible for the symptoms and signs. PSL therapy ameliorated these symptoms and signs, as well as correcting the Th1:Th2 imbalance.

The immune reactions in Sjögren's syndrome and MD appear to be deviations to Th1 and Th2, respectively, and the differences in symptoms and signs between these two diseases may be due to differences between Th1 and Th2 immune reactions. Yamamoto et al. [11] reported that keratoconjunctivitis sicca was present in only half of all patients with MD, and that sialography in patients with MD did not show the apple-tree sign. In addition, serological analyses showed that no case with MD displayed anti-SS-A or anti-SS-B antibodies [11]. Predominant infiltration of Th2 cells in the salivary glands of patients with MD may suppress production of IFN-γ from Th1 cells, which may result in protection of the acinar structure in the glands. Also, suppression of IFN-γ production may induce impaired synthesis of anti-SS-A and anti-SS-B antibodies from IgG1 [19–21].

The patient in our case had MD associated with lymphadenopathy and pleural effusion, and similar symptoms have not been described in other case reports of MD. His peripheral CD4+ T cells showed a Th2-predominant phenotype and Th2 cytokine mRNA expression levels were significantly elevated in his salivary glands. Further accumulation of patients with MD and careful clinical observation will improve the understanding of the pathogenesis of this disease.

Acknowledgments We are grateful to Dr. Hirotaka Higuchi and Dr. Katsuhiro Shimoda from Oita Tobu Hospital for introducing the patient to us and for providing detailed clinical information. There are no conflicts of interest associated with this article.

References

1. Brookes SM, Cohen SB, Price EJ, Webb LM, Feldmann M, Maini RN, et al. T cell clones from a Sjögren's syndrome salivary gland biopsy produce high levels of IL-10. Clin Exp Immunol. 1996;103:268–72.
2. Jung T, Schauer U, Heusser C, Neumann C, Rieger C. Detection of intracellular cytokines by flow cytometry. J Immunol Meth. 1993;159:197–207.
3. Maino VC, Picker LJ. Identification of functional subsets by flow cytometry: intracellular detection of cytokine expression. Cytometry. 1998;34:207–15.
4. Akahoshi M, Nakashima H, Tanaka Y, Kohsaka T, Nagano S, Ohgami E, et al. Th1/Th2 balance of peripheral T helper cells in systemic lupus erythematosus. Arthritis Rheum. 1999;42:1644–8.
5. Ohyama Y, Nakamura S, Matsuzaki G, Shinohara M, Hiroki A, Oka M, et al. T-cell receptor V alpha and V beta gene use by infiltrating T cells in labial glands of patients with Sjögren's syndrome. Oral Surg Oral Med Oral Path Oral Radiol Endod. 1995;79:730–7.
6. Mouri T, Nakamura S, Ohyama Y, Matsuzaki G, Shinohara M, Kishihara K, et al. T cell receptor V alpha and V beta gene usage by tumour-infiltrating lymphocytes in oral squamous cell carcinoma. Cancer Immunol Immunother. 1996;43:10–8.
7. Sasaki M, Nakamura S, Ohyama Y, Shinohara M, Ezaki I, Hara H, et al. Accumulation of common T cell clonotypes in the salivary glands of patients with human T lymphotropic virus type I-associated and idiopathic Sjögren's syndrome. J Immunol. 2000;164:2823–31.
8. Kumamaru W, Nakamura S, Kadena T, Yamada A, Kawamura E, Sasaki M, et al. T-cell receptor Vbeta gene usage by T cells reactive with the tumor-rejection antigen SART-1 in oral squamous cell carcinoma. Int J Cancer. 2004;108:686–95.
9. Kawamura E, Nakamura S, Sasaki M, Ohyama Y, Kadena T, Kumamaru W, et al. Accumulation of oligoclonal T cells in the infiltrating lymphocytes in oral lichen planus. J Oral Pathol Med. 2003;32:282–9.
10. Morgan WS, Castleman B. A clinicopathologic study of Mikulicz's disease. Am J Pathol. 1953;29:471–503.
11. Yamamoto M, Harada S, Ohara M, Suzuki C, Naishiro Y, Yamamoto H, et al. Clinical and pathological differences between Mikulicz's disease and Sjögren's syndrome. Rheumatology. 2005;44:227–34.
12. Yamamoto M, Ohara M, Suzuki C, Naishiro Y, Yamamoto H, Takahashi H, et al. Elevated IgG4 concentrations in serum of patients with Mikulicz's disease. Scand J Rheumatol. 2004;33:432–3.
13. Punnonen J, Aversa G, Cocks BG, McKenzie AN, Menon S, Zurawski G, et al. Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. Proc Natl Acad Sci USA. 1993;90:3730–4.
14. Fox RI, Kang HI, Ando D, Abrams J, Pisa E. Cytokine mRNA expression in salivary gland biopsies of Sjögren's syndrome. J Immunol. 1994;152:5532–9.
15. Ohyama Y, Nakamura S, Matsuzaki G, Shinohara M, Hiroki A, Fujimura T, et al. Cytokine messenger RNA expression in the labial salivary glands of patients with Sjögren's syndrome. Arthritis Rheum. 1996;39:1376–84.
16. Konttinen YT, Kemppinen P, Koski H, Li TF, Jumppanen M, Hietanen J, et al. T(H)1 cytokines are produced in labial salivary glands in Sjögren's syndrome, but also in healthy individuals. Scand J Rheumatol. 1999;28:106–12.
17. Price EJ, Venables PJ. The etiopathogenesis of Sjögren's syndrome. Semin Arthritis Rheum. 1995;25:117–33.
18. Ozaki Y, Amakawa R, Ito T, Iwai H, Tajima K, Uehira K, et al. Alteration of peripheral blood dendritic cells in patients with primary Sjögren's syndrome. Arthritis Rheum. 2001;44:419–31.
19. Bonfa E, Llovet R, Elkon K. Immunoblot analysis of IgG subclasses of multiple lupus autoantibodies. J Immunol. 1988;140:2231–6.
20. Jones CC, Hamilton RG, Jordon RE. Subclass distribution of human IgG autoantibodies in pemphigus. J Clin Immunol. 1988;8:43–9.
21. Lieu TS, Reimer CB, Sontheimer RD. Immunoglobulin class and subclass profile of the Ro/SS-A autoantibody response. J Invest Dermatol. 1988;90:158–64.