sophisticated validation study using randomly selected clinical case scenarios from various institutions and expert committee consensus diagnosis as the golden standard to test the three diagnostic systems for SS, to unify the criteria used for the diagnosis of SS, and ultimately to select the gold standard set of criteria for the diagnosis of SS in Japan.

Currently, the JPN diagnostic system is only used in Japan, because ACR and EULAR have never validated the JPN system. Therefore, we strongly hope that an ACR/EULAR collaborative initiative will validate JPN as well as the AECG and ACR systems.

In conclusion, although this study has a few limitations, the results obtained from it indicate the superiority of the JPN criteria, as it has higher sensitivity and specificity values for the diagnosis of SS in Japanese patients with SS than those of ACR and AECG.

Acknowledgments We thank Dr. F.G. Issa for critically reading the manuscript. This work was supported by Health and Labour Sciences Research Grants for research on intractable diseases (The Research Team for Autoimmune Diseases) from the Ministry of Health, Labour and Welfare of Japan.

Conflict of interest None.

References

1. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis. 2002;61:554–8.
2. Fujibayashi T, Sugai S, Miyasaka N, Hayashi Y, Tsubota K. Revised Japanese criteria for Sjögren's syndrome (1999): availability and validity. Mod Rheumatol. 2004;14:425–34.
3. Shiboski SC, Shiboski CH, Criswell L, Baer A, Challacombe S, Lanfranchi H, et al. American College of Rheumatology classification criteria for Sjögren's syndrome: a data-driven, expert consensus approach in the Sjögren's International Collaborative Clinical Alliance cohort. Arthritis Care Res. 2012;64:475–87.
4. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997;40:1725.
5. Petri M, Orbai AM, Alarcón GS, Gordon C, Merrill JT, Fortin PR, Bruce IN, Isenberg D, Wallace DJ, Nived O, Sturfelt G, Ramsey-Goldman R, Bae SC, Hanly JG, Sánchez-Guerrero J, Clarke A, Aranow C, Manzi S, Urowitz M, Gladman D, Kalunian K, Costner M, Werth VP, Zoma A, Bernatsky S, Ruiz-Irastorza G, Khamashta MA, Jacobsen S, Buyon JP, Maddison P, Dooley MA, van Vollenhoven RF, Ginzler E, Stoll T, Peschken C, Jorizzo JL, Callen JP, Lim SS, Fessler BJ, Inanc M, Kamen DL, Rahman A, Steinsson K, Franks AG Jr, Sigler L, Hameed S, Fang H, Pham N, Brey R, Weisman MH, McGwin G Jr, Magder LS. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum. 2012;64:2677–86.
6. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, Birnbaum NS, Burmester GR, Bykerk VP, Cohen MD, Combe B, Costenbader KH, Dougados M, Emery P, Ferraccioli G, Hazes JM, Hobbs K, Huizinga TW, Kavanaugh A, Kay J, Kvien TK, Laing T, Mease P, Ménard HA, Moreland LW, Naden RL, Pincus T, Smolen JS, Stanislawska-Biernat E, Symmons D, Tak PP, Upchurch KS, Vencovský J, Wolfe F, Hawker G. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2010;62:2569–81.

Japan College of Rheumatology
MODERN RHEUMATOLOGY

http://informahealthcare.com/mor
ISSN 1439-7595 (print), 1439-7609 (online)

Mod Rheumatol, 2013; Early Online: 1–7

DOI: 10.3109/14397595.2013.843765

informa
healthcare

ORIGINAL ARTICLE

Primary and secondary surveys on epidemiology of Sjögren's syndrome in Japan

Hiroto Tsuboi[1,14], Hiromitsu Asashima[1], Chinatsu Takai[1], Shinya Hagiwara[1], Chihiro Hagiya[1], Masahiro Yokosawa[1], Tomoya Hirota[1], Hisanori Umehara[2,14], Atsushi Kawakami[3,14], Hideki Nakamura[3,14], Hajime Sano[4,14], Kazuo Tsubota[5,14], Yoko Ogawa[5,14], Etsuko Takamura[6,14], Ichiro Saito[7,14], Hiroko Inoue[8,14], Seiji Nakamura[9,14], Masafumi Moriyama[9,14], Tsutomu Takeuchi[10,14], Yoshiya Tanaka[11,14], Shintaro Hirata[11,14], Tsuneyo Mimori[12,14], Hajime Yoshifuji[12], Akiko Ohta[13,14], Isao Matsumoto[1], and Takayuki Sumida[1,14]

[1]*Department of Internal Medicine, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan,* [2]*Department of Hematology and Immunology, Kanazawa Medical University, Kanazawa, Ishikawa, Japan,* [3]*Unit of Translational Medicine, Department of Immunology and Rheumatology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan,* [4]*Division of Rheumatology, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan,* [5]*Department of Ophthalmology, School of Medicine, Keio University, Tokyo, Japan,* [6]*Department of Ophthalmology, Tokyo Women's Medical University, School of Medicine, Tokyo, Japan,* [7]*Department of Pathology, Tsurumi University School of Dental Medicine, Tsurumi, Japan,* [8]*Department of Pharmacology, Nihon Pharmaceutical University, Saitama, Japan,* [9]*Section of Oral and Maxillofacial Oncology, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University, Fukuoka, Japan,* [10]*Division of Rheumatology, Department of Internal Medicine, School of Medicine, Keio University, Tokyo, Japan,* [11]*The First Department of Internal Medicine, School of Medicine, University of Occupational and Environmental Health, Japan, Kitakyushu, Japan,* [12]*Department of Rheumatology and Clinical Immunology, Kyoto University Graduate School of Medicine, Kyoto, Japan,* [13]*Department of Public Health, Saitama Medical University Faculty of Medicine, Saitama, Japan, and* [14]*The Research Team for Autoimmune Diseases, The Research Program for Intractable Disease of the Ministry of Health, Labor and Welfare (MHLW), Tokyo, Japan*

Abstract

Objective. To characterize the epidemiology of Sjögren's syndrome (SS), including prevalence, disease type, extra-glandular involvement, satisfaction of diagnostic criteria sets, and treatment used in Japan.

Methods. The Research Team for Autoimmune Diseases, the Research Program for Intractable Disease by the Ministry of Health, Labor and Welfare conducted primary and secondary surveys on epidemiology of SS in 2011. The primary survey covered 4,729 out of 14,095 Japan-wide Hospital Departments to investigate the prevalence of SS. The secondary survey encompassed 214 Hospital Departments that agreed to the survey, to characterize disease type, extra-glandular involvement, satisfaction of diagnostic criteria sets, and treatments.

Results. The number of patients with SS in Japan estimated by the primary survey was 68,483. The secondary survey involving data collected from 2,195 SS patients from 98 Hospital Departments showed that the mean age of patients was 60.8 ± 15.2 years, male/female ratio was 1/17.4, primary/secondary SS was about 60%/40% and glandular/extra-glandular form in primary SS was about 70%/25%. The satisfaction rate was 53.8% for the 1999 revised Japanese Ministry of Health criteria for the diagnosis of SS, 47.7% for the 2002 American–European Consensus Group classification criteria for SS and 49.6% for 2012 American College of Rheumatology classification criteria for SS. Corticosteroids were used by 752 of 2,195 patients (34%), immunosuppressants by 358 patients (16%), biologics by 68 patients (3%) and secretagogues by 695 patients (32%).

Conclusion. The surveys provided valuable information on the epidemiology of SS including prevalence, disease type, extra-glandular involvement, satisfaction of diagnostic criteria sets and treatments used today in Japan.

Keywords

Criteria, Epidemiology, Extra-glandular form, Glandular form, Sjögren's syndrome, Treatment

History

Received 30 May 2013
Accepted 18 June 2013
Published online 31 October 2013

Introduction

Sjögren's syndrome (SS) is an autoimmune disease that affects exocrine glands including salivary and lacrimal glands. It is characterized pathologically by lymphocytic infiltration into the exocrine glands, and clinically by dry mouth and dry eyes. SS is subcategorized into primary SS, which is not associated with any other well-defined connective tissue disease (CTD), and secondary SS, which is associated with other well-defined CTD [1]. Primary SS is further subdivided into the glandular form, with involvement of the exocrine glands only, and the extra-glandular form, with involvement of organs other than exocrine glands.

SS is a common autoimmune disease, and patients with SS present with a variety of clinical symptoms and signs other than dry mouth and dry eyes, such as general fatigue, arthralgia, myalgia, gastrointestinal symptoms, dysesthesia and depression. Accordingly, patients with SS might consult not only rheumatologists,

Correspondence to: Prof. Takayuki Sumida, Department of Internal Medicine, Faculty of Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba-city, Ibaraki 305-8575, Japan. Tel: +81-29-853-3221. Fax: +81-29-853-3222. E-mail: tsumida@md.tsukuba.ac.jp

dentists and ophthalmologists, but also general physicians, otolaryngologists, orthopedists, gastroenterologists, neurologists and psychiatrists. Several criteria sets have been proposed for the diagnosis of SS. Indeed, the revised criteria for the diagnosis of SS issued by the Japanese Ministry of Health (JPN) (1999) [2], as well as the American–European Consensus Group classification criteria for SS (AECG) (2002) [1], are usually used both in daily clinical practice and in clinical studies in Japan [3]. In addition to these two sets of diagnostic criteria, the American College of Rheumatology (ACR) recently published the ACR classification criteria for SS (2012), which were proposed by the Sjögren's International Collaborative Clinical Alliance (SICCA) [4].

The complex nature of SS makes it difficult to assess the precise epidemiology of SS [5]. Actually, the reported prevalence of SS varies widely among different studies, ranging from 0.1% to 4.8% [5]. A few studies have also estimated the population prevalence of SS in Japan. In 1993, The Research Team for Autoimmune Diseases and Epidemiology of the Japanese Ministry of Health estimates the number of patients with SS at 17000 [6]. In a more recent study conducted in 2008, the prevalence of SS defined by AECG criteria among Nagasaki atomic bomb survivors was 2.3% (23/1008) [7]. However, the exact incidence of SS throughout Japan is currently unknown.

To characterize the epidemiology of SS, including prevalence, disease type, extra-glandular involvement, satisfaction of criteria and treatment modalities used in Japan, The Research Team for Autoimmune Diseases, the Research Program for Intractable Disease by the Ministry of Health, Labor and Welfare (MHLW) conducted the primary and secondary survey on epidemiology of SS in 2011.

Methods

Primary survey

The Research Team for Autoimmune Diseases, the Research Program for Intractable Disease by MHLW conducted a primary survey on the epidemiology of SS in 2011. We identified the target departments of hospitals using the following protocol. We identified 7,999 Departments of Internal Medicine, 2,391 Departments of Ophthalmology, 2,011 Departments of Otolaryngology, 936 Departments of Rheumatology and 758 Departments of Oral Surgery (total 14,095 Hospital Departments) across Japan. These departments were divided into seven categories according to the number of beds in each hospital, including <100, 100–199, 200–299, 300–399, 400–499, and ≥ 500 beds, and university hospitals (Supplementary material, Table 1 available online at http://informahealthcare.com/doi/abs/10.3109/14397595.2013.843765). We selected the target departments at random by the following extractability, 5% for hospitals with <100 beds, 10% for hospitals with 100–199 beds, 20% for 200–299 beds,

Table 1. Reported patients in primary survey.

	Category	Target departments	Response (A)	Response rate (%)	Reported patients (B)	Total departments (C)	Total patients (D)
Internal Medicine	University hospital	147	40	27	1077	147	3958
	500 beds and over	384	69	18	1827	384	10168
	400–499 beds	256	43	17	132	321	985
	300–399 beds	265	46	17	195	662	2806
	200–299 beds	204	43	21	237	1023	5638
	100–199 beds	236	61	26	189	2367	7334
	Under 100 beds	158	36	23	26	3095	2235
	Total	1650	338	20	3683	7999	33125
Ophthalmology	University hospital	129	28	22	1544	129	7113
	500 beds and over	286	52	18	354	286	1947
	400–499 beds	169	37	22	143	211	815
	300–399 beds	153	39	25	62	387	615
	200–299 beds	78	9	12	12	391	521
	100–199 beds	59	5	8	4	582	466
	Under 100 beds	19	2	11	3	405	405
	Total	893	172	19	2122	2391	11883
Otolaryngology	University hospital	127	44	35	199	127	574
	500 beds and over	282	71	25	283	282	1124
	400–499 beds	160	39	24	30	204	157
	300–399 beds	148	30	20	70	369	123
	200–299 beds	71	18	25	19	355	375
	100–199 beds	44	17	39	0	446	0
	Under 100 beds	11	3	27	0	228	0
	Total	843	222	26	601	2011	2353
Rheumatology	University hospital	48	16	33	3045	48	9135
	500 beds and over	67	16	24	762	67	3191
	400–499 beds	48	11	23	100	48	436
	300–399 beds	67	12	18	220	67	1228
	200–299 beds	130	30	23	370	130	1603
	100–199 beds	270	54	20	102	270	510
	Under 100 beds	306	68	22	170	306	765
	Total	936	207	22	4769	936	16869
Oral surgery	University hospital	83	38	46	578	83	1262
	500 beds and over	167	53	32	504	167	1588
	400–499 beds	71	25	35	33	87	115
	300–399 beds	50	16	32	47	125	367
	200–299 beds	20	8	40	62	101	783
	100–199 beds	13	4	31	0	126	0
	Under 100 beds	3	1	33	2	69	138
	Total	407	145	36	1226	758	4253
Total		4729	1084	23	12401	14095	68483

Calculation of total patients (D); Sum of [Reported patients (B)/Response (A) X Total departments (C) in each category].

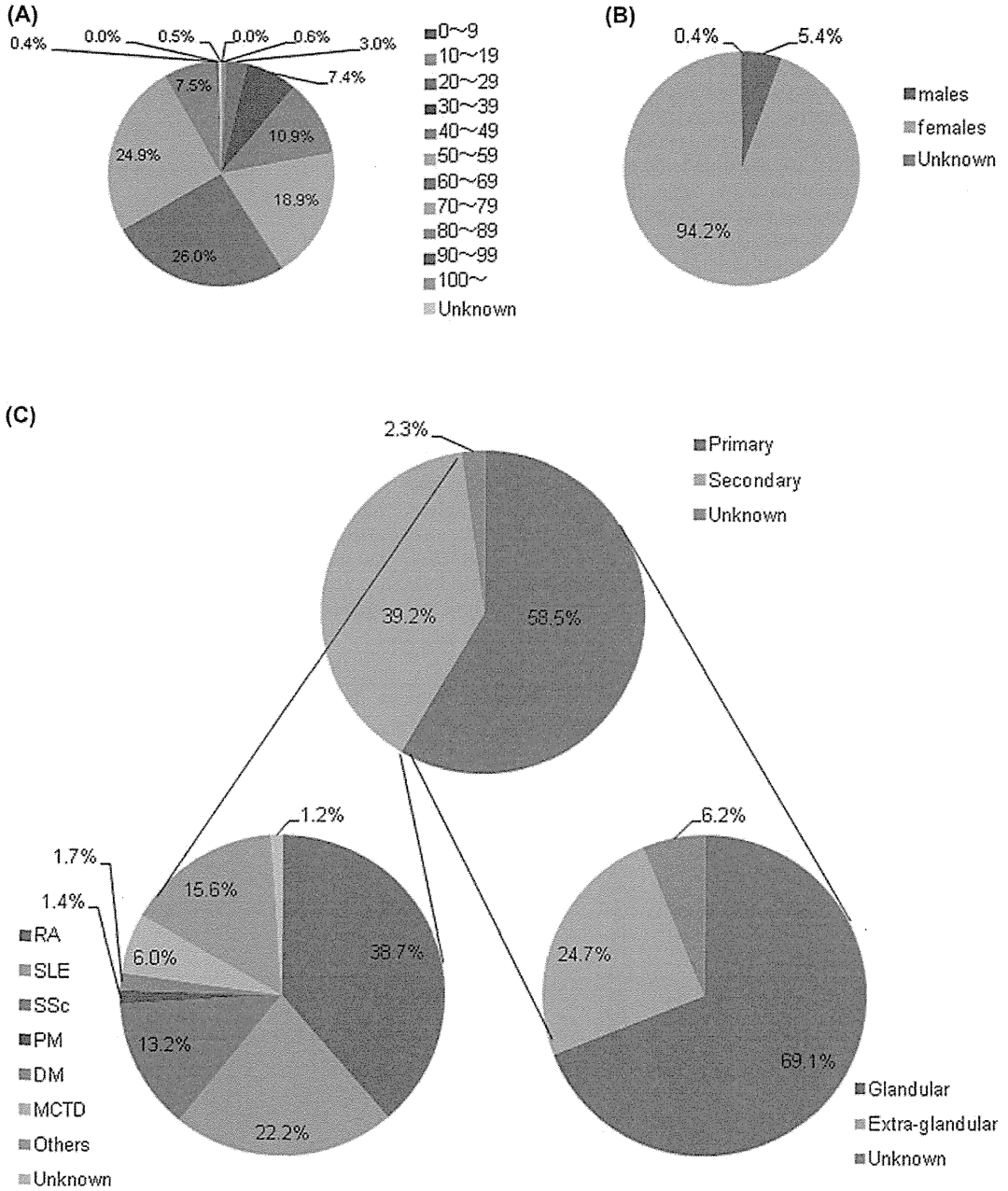

Figure 1. Characteristics of 2,195 patients with SS who participated in the secondary survey. (A). Age distribution; (B). Gender; (C). Disease type. *Top:* frequency of primary and secondary SS. *Bottom right:* frequency of glandular and extra-glandular forms among patients with primary SS. *Bottom left:* frequency of other CTDs in patients with secondary SS. RA: rheumatoid arthritis; SLE: systemic lupus erythematosus; SSc: systemic sclerosis; PM: polymyositis; DM: dermatomyositis; MCTD: mixed CTD.

40% for 300–399 beds, 80% for 400–499 beds and 100% for ≥500 beds, university hospitals, and Departments of Rheumatology (Supplementary material, Table 1 available online at http://informahealthcare.com/doi/abs/10.3109/14397595.2013.843765). Finally, we selected 1,650 Departments of Internal Medicine, 893 Departments of Ophthalmology, 843 Departments of Otolaryngology, 936 Departments of Rheumatology and 407 Departments of Oral Surgery (total 4,729 Hospital Departments). Thus, the primary survey was conducted in these 4,729 departments out of 14,095 Hospital Departments across Japan (Supplementary material, Table 1 available online at http://informahealthcare.com/doi/abs/10.3109/14397595.2013.843765). In the primary survey, we determined the number of patients with SS who consulted each department in 2010 (from 1 January to 31 December). Consent to participate in the secondary survey was obtained from each Hospital Department.

Secondary survey

The Research Team for Autoimmune Diseases also conducted a secondary survey on the epidemiology of SS in 2011. The secondary survey was performed in 214 Hospital Departments that agreed to participate in the survey. In the secondary survey, we investigated the effect of age, sex, disease type, extra-glandular

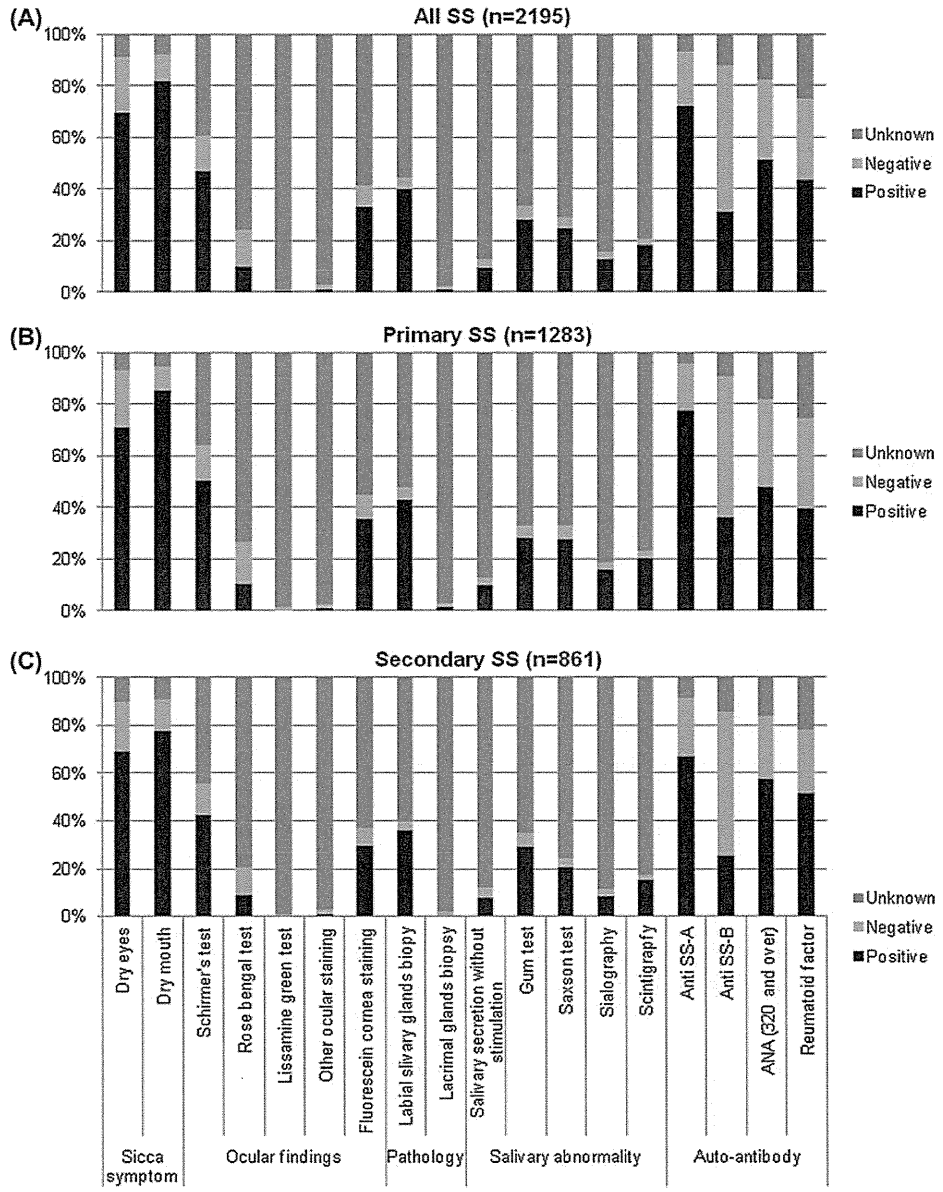

Figure 2. Positivity of various sicca symptoms and objective findings. The histogram shows proportion of patients with positive, negative and unknown (not performed) sicca symptoms and objective findings in (A) all patients with SS, (B) patients with primary SS and (C) patients with secondary SS.

involvements, satisfaction of criteria and treatment of patients with SS in Japan.

Results

Primary survey

Responses to the primary survey were received from 1,084 out of 4,729 Hospital Departments (response rate: 23%) (Table 1). The total number of patients with SS across Japan was calculated by the following formula: Sum of [Reported patients (B)/Return (A) X Total departments (C) in each category]. The estimated total number of patients with SS across Japan was 68,483 (Table 1). During the primary survey, 214 Hospital Departments consented to the secondary survey (Supplementary material, Table 2 available online at http://informahealthcare.com/doi/abs/10.3109/14397595.2013.843765).

Secondary survey

Responses to the secondary survey were received from 98 out of 214 Hospital Departments (response rate: 45.8%) (Supplementary material, Table 2 available online at http://informahealthcare.com/doi/abs/10.3109/14397595.2013.843765). Data were collected on 2,195 SS patients in the secondary survey. The mean age of the 2,195 SS patients was 60.8 ± 15.2 years, and the age of 69.7% of patients (1,530/2,195 patients) ranged from 50 to 79 years (Figure 1A). Furthermore, 94.2% of the patients were females, with a male/female ratio of 1/17.4 (Figure 1B). Primary SS was diagnosed in 1,283 out of 2,195 patients (58.5%), whereas secondary SS was diagnosed in 861 out of 2,195 patients (39.2%) (Figure 1C). With regard to primary SS, 886 out of 1,283 patients (69.1%) had the glandular form, and 317 out of 1,283 patients (24.7%) had the extra-glandular form (Figure 1C). In patients with secondary SS, rheumatoid arthritis (RA) was diagnosed in 38.7%

(333 out of 861 patients), and systemic lupus erythematosus (SLE) in 22.2% (191 out of 861 patients) (Figure 1C).

Figure 2 shows the proportion of patients with various sicca symptoms and the objective findings included by the diagnostic criteria set. The frequencies of dry eyes and mouth, and anti-SS-A antibody were high, while ocular staining and salivary examinations were not performed in many patients with SS (Figure 2A), primary SS (Figure 2B) and secondary SS (Figure 2C). Histopathological findings of labial salivary glands biopsies were positive in about 40% of patients, but the examination was performed in only about 40% of patients (Figure 2A–C). Interestingly, 597 out of 2,195 SS patients (27.2%) were seronegative SS (both anti-SS-A and SS-B antibodies were negative or unknown).

Figure 3 displays the Venn diagram showing comparison of satisfaction with JPN, AECG and ACR diagnostic criteria set in all SS, primary SS and secondary SS. The satisfaction rate was 53.8% (1,182/2,195) for JPN criteria, 47.7% (1,046/2,195) for AECG criteria, and 49.6% (1,089/2,195) for ACR criteria in all SS patients (Figure 3A). However, 798 out of 2,195 patients did not satisfy any criteria sets (Figure 3A). The satisfaction rate was 61.1% (784/1283) for JPN, 59.2% (760/1283) for AECG and 54.5% (699/1283) for ACR in primary SS patients (Figure 3B). The satisfaction rate was 44.9% (387/861) for JPN, 31.6% (272/861) for AECG and 44.1% (380/861) for ACR in secondary SS patients (Figure 3C). The agreement between the JPN and ACR criteria sets was good (kappa coefficient; 0.77–0.80), compared with moderate agreement between the AECG and the other two criteria sets (kappa coefficient; 0.50–0.56) in the diagnosis of all SS, primary SS and secondary SS patients (Table 2).

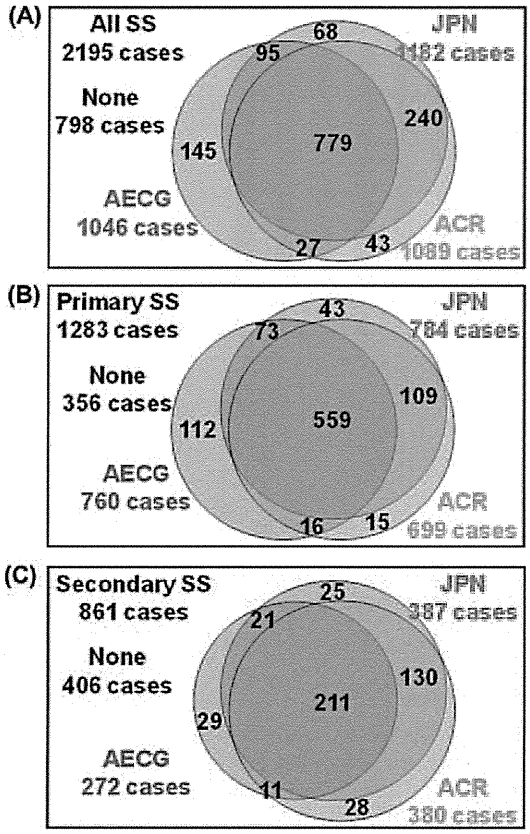

Figure 3. Venn diagrams showing comparison of satisfaction of the three tested criteria sets. (A) Comparison of satisfaction of the three tested criteria sets using data of all (n = 2,195) patients with SS. (B) Comparison of satisfaction of the three tested criteria sets using data of 1,283 patients with primary SS. (C) Comparison of satisfaction of the three tested criteria sets using data of 861 patients with secondary SS. Numbers: number of patients who satisfied each set of criteria, None: patients who did not satisfy the criteria of any of the three systems. JPN: The revised Japanese Ministry of Health criteria for the diagnosis of SS (1999); AECG: The American–European Consensus Group classification criteria for SS (2002); ACR: American College of Rheumatology classification criteria for SS (2012).

Table 2. Agreement between three criteria sets assessed by kappa coefficient.

	All SS 2195 cases	Primary SS 1283 cases	Secondary SS 861 cases
		kappa coefficient	
JPN vs. AECG	0.56	0.54	0.53
JPN vs. ACR	0.79	0.77	0.80
AECG vs. ACR	0.52	0.51	0.50

JPN: The revised Japanese Ministry of Health criteria for the diagnosis of SS (1999).
AECG: The American–European Consensus Group classification criteria for SS (2002).
ACR: American College of Rheumatology classification criteria for SS (2012).

Figures 4 and 5 summarize the types of treatment according to SS disease type. Corticosteroids were administrated in 752/2,195 SS patients (34.3%), in 270/1283 primary SS patients (21.0%) and in 475/861 secondary SS patients (55.2%) (Figure 4A). Among the corticosteroid-treated primary SS group, 126 patients (46.7%) had the glandular form, whereas 132 patients (48.9%) had the extra-glandular form. Immunosuppressants were used in 358/2,195 SS patients (16.3%), in 68/1,283 primary SS patients (5.3%) and in 287/861 secondary SS patients (33.3%) (Figure 4B). Among the immunosuppressants-treated primary SS group, 26 patients (38.2%) had the glandular form, whereas 38 patients (55.9%) had the extra-glandular form. Biologics were administrated in 68/2,195 SS patients (3.1%) (infliximab in 8, etanercept in 21, adalimumab in 10, tocilizumab in 13, abatacept in 10, rituximab in 1, and others and unknown in 5 patients), in 7/1,283 primary SS patients (0.5%) and in 59/861 secondary SS (6.9%) (Figure 5A). Among the biologics-treated secondary SS group, 49 patients (83.1%) had RA (Figure 5A). Secretagogues, such as pilocarpine and cevimeline, were administrated in 695/2,195 SS patients (31.7%), in 470/1,283 primary SS patients (36.6%) and in 212/861 secondary SS patients (24.6%) (Figure 5B).

Discussion

Although SS is a common autoimmune disease, the precise epidemiology of this disease remains poorly defined [8]. It is important to determine the epidemiology including prevalence, disease type, extra-glandular involvements, satisfaction of diagnostic criteria and treatment modalities for SS, because such information could help establish diagnostic and therapeutic guidelines, as well as validation of criteria sets. For this reason, the Research Team for Autoimmune Diseases, the Research Program for Intractable Disease of MHLW conducted primary and secondary surveys on the epidemiology of SS in Japan.

The study identified several important findings about SS. First, the estimated number of patients with SS in Japan was 68,483. Based on a total population of Japan at October 1, 2011 of 127,799,000, the prevalence of SS in Japan is 0.05%. This rate is lower than the minimum lowest prevalence of SS of 0.1 reported for other countries [5]. What is the reason for the low prevalence of SS in Japan? In many studies on the prevalence of SS performed in other countries, randomly selected population has been targeted, and SS criteria sets such as AECG criteria have been adopted for the diagnosis of SS [5]. On the other hand, in this survey, we targeted patients with SS who consulted their physicians, rather than

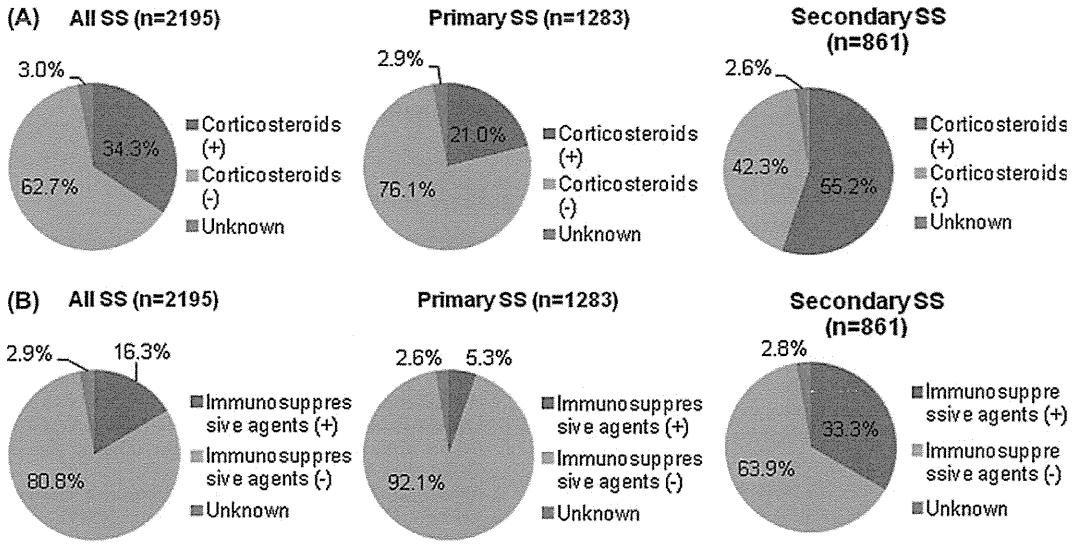

Figure 4. Treatments used in patients with SS and relationship between treatments and disease type (corticosteroids and immunosuppressive agents). (A) Corticosteroids. (B) Immunosuppressive agents. +: patients treated with drugs; -: patients treated without drugs.

the general public, and diagnosis of SS depended on the physician judgment. Thus, the present study underestimates the prevalence of SS in Japan, especially sub-clinical cases might be overlooked.

Second, the study characterized the distribution of age, gender and SS disease type in patients with SS in Japan. Previous reports indicated that SS affects mainly middle-aged females, with a female to male ratio of 9:1 [5]. We confirmed these features of SS in this survey, with the mean age of the group of 60.8 ± 15.2 years, and a female to male ratio of 17.4:1. Previous studies reported that almost half of SS patients develop extra-glandular disease, such as arthralgia/arthritis (>50%), interstitial nephritis (25%), interstitial lung diseases (30%) and peripheral polyneuropathy (20%) [5,9]. We demonstrated in the present study that the ratio of the primary to secondary SS was about 60% to 40%, with the glandular to extra-glandular form ratio of about 70% to 25% among patients with primary SS. We also confirmed the importance of screening for systemic organ involvement in SS patients.

Third, the satisfaction rate for three sets of criteria, including JPN, AECG and ACR, was only ~50% in this survey. This finding indicates that about half of the SS patients were not diagnosed by the diagnostic criteria but rather by the physician judgment. Importantly, pathological examination of labial salivary glands biopsy was performed only in about 40% of patients, but was not performed in about 60% of patients. This low frequency of labial

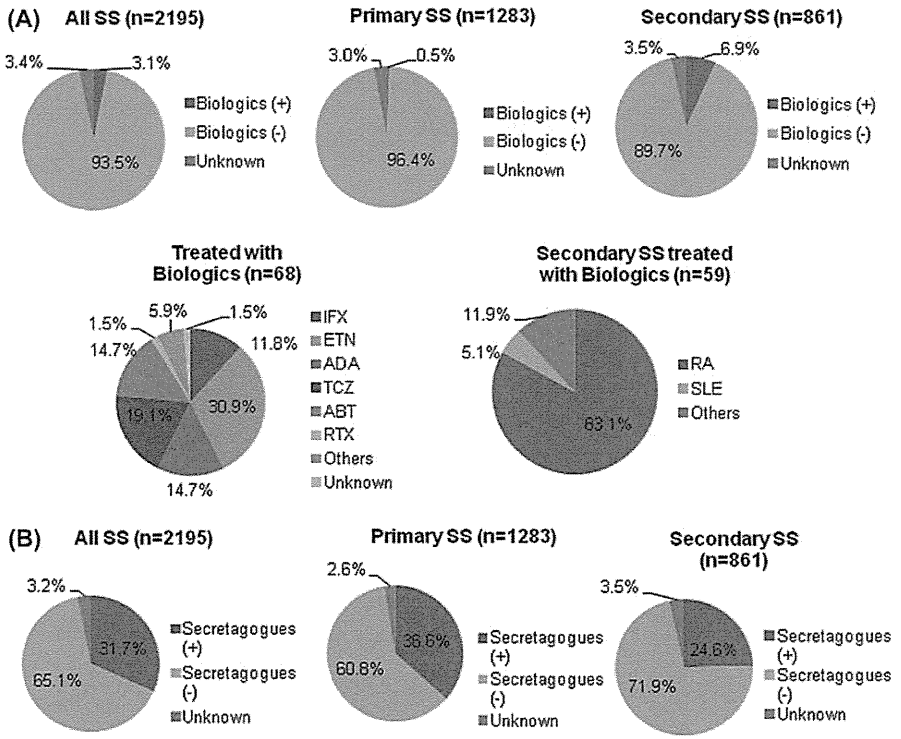

Figure 5. Treatments used in patients with SS and relationship between treatments and disease type (biologics and secretagogues). (A) Biologics. (B) Secretagogues. +, patients treated with drugs; -, patients treated without drugs; IFX, Infliximab; ETN, etanercept; ADA, adalimumab; TCZ, tocilizumab; ABT, abatacept; RTX, rituximab; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

salivary gland biopsy could be problematic, if the ACR criteria are to be adopted in Japan, because ACR criteria comprise only three items: autoantibodies, labial salivary glands biopsy and ocular staining [4]. Moreover, in this survey, the agreement between the JPN and ACR criteria sets was good, compared with moderate agreement between the AECG and the other two criteria sets in the diagnosis of SS. These results are consistent with the previous study performed by the Research Team for Autoimmune Diseases, the Research Program for Intractable Disease by MHLW using data of 694 patients with SS or suspected SS who had been checked for all four criteria of the JPN (pathology, oral, ocular and anti-SS-A/SS-B antibodies) [3].

Finally, this survey also characterized for the first time the treatment modalities applied for SS in Japan. Corticosteroids were used in 34% of patients, immunosuppressants in 16%. As expected, both corticosteroids and immunosuppressive agents were used mainly in patients with secondary SS. Interestingly, about half of primary SS patients treated with corticosteroids had the glandular form of SS, whereas only 38% of primary SS patients treated with immunosuppressants had the glandular form. Although the effectiveness of corticosteroids for glandular involvement of SS has not been established [10], in Japan, 10% of primary SS patients could be treated with corticosteroids for glandular involvement. On the other hand, immunosuppressive agents might be used in primary SS for mainly extra-glandular involvements. Biologics were administrated only in 3% of SS, and the main target of biologics was RA, which was associated with secondary SS. Secretagogues were used in 32% of patients with SS, and a larger proportion of patients with primary SS used these drugs than those with secondary SS. This finding suggests that dryness in primary SS is more severe than that in secondary SS.

Although this survey is cross-sectional, these important findings should be useful for the establishment of diagnostic and therapeutic guidelines for SS. Longitudinal surveys and prospective studies are needed to confirm these results.

In conclusion, the primary and secondary surveys employed in the present study provided valuable information on the epidemiology of SS, including prevalence, disease type, extra-glandular involvement, satisfaction of criteria and treatment used today in Japan.

Acknowledgements

We thank Dr. F. G. Issa for the critical reading of the manuscript. This work was supported by Health and Labour Sciences Research Grants for research on intractable diseases (The Research Team for Autoimmune Diseases) from the Ministry of Health, Labour and Welfare of Japan.

Supplementary material available online

Supplementary Table 1-2.

Conflict of interest

None

Authors' contributions

All authors contributed to the design of the study and data collection, and participated in the writing of the manuscript and all agree to accept equal responsibility for the accuracy of the contents of this paper.

References

1. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis. 2002;61(6):554–8.
2. Fujibayashi T, Sugai S, Miyasaka N, Hayashi Y, Tsubota K. Revised Japanese criteria for Sjögren's syndrome (1999): availability and validity. Mod Rheumatol. 2004;14(6):425–34.
3. Tsuboi H, Hagiwara S, Asashima H, Umehara H, Kawakami A, Nakamura H, et al. Validation of different sets of criteria for the diagnosis of Sjögren's syndrome in Japanese patients. Mod Rheumatol. 2013;23(2):219–25.
4. Shiboski SC, Shiboski CH, Criswell L, Baer A, Challacombe S, Lanfranchi H, et al. American College of Rheumatology classification criteria for Sjögren's syndrome: a data-driven, expert consensus approach in the Sjögren's International Collaborative Clinical Alliance cohort. Arthritis Care Res. 2012;64(4):475–87.
5. Mavragani CP, Moutsopoulos HM. The geoepidemiology of Sjögren's syndrome. Autoimmun Rev. 2010;9(5):A305–10.
6. The report from The Research Team for Autoimmune Diseases and Epidemiology of Japanese Ministry of Health, 1998 (in Japanese).
7. Hida A, Akahoshi M, Takagi Y, Ashizawa K, Imaizumi M, Soda M, et al. Prevalence of Sjogren syndrome among Nagasaki atomic bomb survivors. Ann Rheum Dis. 2008;67(5):689–95.
8. Binard A, Devauchelle-Pensec V, Fautrel B, Jousse S, Youinou P, Saraux A. Epidemiology of Sjögren's syndrome: where are we now? Clin Exp Rheumatol. 2007;25(1):1–4.
9. Vissink A, Bootsma H, Spijkervet FK, Hu S, Wong DT, Kallenberg CG. Current and future challenges in primary Sjögren's syndrome. Curr Pharm Biotechnol. 2012;13(10):2026–45.
10. Ramos-Casals M, Brito-Zerón P, Sisó-Almirall A, Bosch X, Tzioufas AG. Topical and systemic medications for the treatment of primary Sjögren's syndrome. Nat Rev Rheumatol. 2012;8(7):399–411.

Journal of Autoimmunity xxx (2013) 1–8

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

Review

T helper subsets in Sjögren's syndrome and IgG4-related dacryoadenitis and sialoadenitis: A critical review☆

Masafumi Moriyama [a], Akihiko Tanaka [a], Takashi Maehara [a], Sachiko Furukawa [a], Hitoshi Nakashima [b], Seiji Nakamura [a,*]

[a] Section of Oral and Maxillofacial Oncology, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
[b] Division of Nephrology and Rheumatology, Department of Internal Medicine, Faculty of Medicine, Fukuoka University, Fukuoka, Japan

ARTICLE INFO

Article history:
Received 10 July 2013
Accepted 15 July 2013

Keywords:
T helper subset
IgG4-related disease
IgG4-related dacryoadenitis and sialoadenitis
Sjögren's syndrome
Cytokine

ABSTRACT

IgG4-related disease (IgG4-RD) is a systemic disease characterized by the elevation of serum IgG4 and infiltration of IgG4-positive plasma cells in multiple target organs, including the pancreas, kidney, biliary tract and salivary glands. In contrast, Mikulicz's disease (MD) has been considered a subtype of Sjögren's syndrome (SS) based on histopathological similarities. However, it is now recognized that MD is an IgG4-RD distinguishable from SS and called as IgG4-related dacryoadenitis and sialoadenitis (IgG4-DS). Regarding immunological aspects, it is generally accepted that CD4+ T helper (Th) cells play a crucial role in the pathogenesis of SS. Since it is well known that IgG4 is induced by Th2 cytokines such as interleukin (IL)-4 and IL-13, IgG4-DS is speculated to be a unique inflammatory disorder characterized by Th2 immune reactions. However, the involvement of Th cells in the pathogenesis of IgG4-DS remains to be clarified. Exploring the role of Th cell subsets in IgG4-DS is a highly promising field of investigation. In this review, we focus on the selective localization and respective functions of Th cell subsets and discuss the differences between SS and IgG4-DS to clarify the pathogenic mechanisms of these diseases.

Sjögren's syndrome (SS) is an autoimmune disease characterized by lymphocytic infiltration into the salivary and lacrimal glands with concomitant autoantibody production and destruction of the glandular tissue. Patients typically experience symptoms of dry mouth (xerostomia) and dry eyes (keratoconjunctivitis sicca). Because of its characteristic lymphocytic infiltration and destruction of the salivary and lacrimal glands, SS is considered to be an ideal disease for studying patterns of cytokine production at the site of organ-specific autoimmune damage [1]. SS occurs alone as primary SS, or as secondary SS when underlying other connective tissue diseases [2]. Immunohistochemical studies demonstrated that the salivary glands are predominantly infiltrated by CD4+ T helper (Th) cells at an early stage of SS, and these cells are therefore thought to play a crucial role in the induction and/or maintenance of the disease [3]. In advanced stage, B cells predominate and these infiltration extends to occupy the acinar epithelium and further progress to hypergammaglobulinemia and B cell lymphoma [4]. Recent studies have suggested a central role of the epithelium in orchestrating the immune reaction by expressing HLA antigens, adhesion and costimulatory molecules, cytokines, and chemokines. Therefore, SS has been proposed as an etiological term "autoimmune epithelitis" [4–7], and it is of interest to examine the involvement of interaction between CD4+ Th cells and the epithelium in the initiation and progression of the disease process. Th cell populations comprise functionally distinct subsets characterized by specific patterns of cytokines and transcription factors. At least six Th subsets exist: Th0, Th1, Th2, Th17, regulatory T (Treg), and follicular helper T (Tfh) cells [8], which are suggested to be involved in the pathogenesis of SS [9–12].

On the other hands, Mikulicz's disease (MD) has been considered to be a subtype of SS based on histopathological similarities between the two diseases [13]. However, MD has a number of differences compared with typical SS including: 1) difference of gender distribution (MD occurs in both men and women, while SS occurs mainly in women); 2) persistent enlargement of lacrimal and salivary glands; 3) normal or mild salivary secretion dysfunction; 4) good responsiveness to corticosteroid treatment; 5) hypergammaglobulinemia and low frequency of anti SS-A and SS-B antibodies by serological analyses; and 6) multiple GC formation in

* Corresponding author. Tel.: +81 92 642 6447; fax: +81 92 642 6386.
E-mail address: seiji@dent.kyushu-u.ac.jp (S. Nakamura).

0896-8411/$ – see front matter © 2013 The Authors. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jaut.2013.07.007

Please cite this article in press as: Moriyama M, et al., T helper subsets in Sjögren's syndrome and IgG4-related dacryoadenitis and sialoadenitis: A critical review, Journal of Autoimmunity (2013), http://dx.doi.org/10.1016/j.jaut.2013.07.007

glandular tissue (Table 1). Previously, we reported that SS was characterized by periductal lymphocytic infiltration with atrophy or severe destruction of the acini, while MD showed non-periductal lymphocytic infiltration with hyperplastic GCs and mild destruction of the acini (Fig. 1) [14]. Fifteen of 66 patients with SS (23%) and 12 of 20 patients with MD (60%) showed ectopic GC formation in labial salivary glands (LSGs). Patients with MD showed a significantly higher frequency, higher number and larger size of GCs compared with SS patients [15]. In addition, Yamamoto et al. [16–18] reported that patients with MD had elevated levels of serum IgG4 and infiltrating IgG4-positive plasma cells in the gland tissues. Similar findings have been observed in autoimmune pancreatitis (AIP) [19], sclerosing cholangitis [20], tubulointerstitial nephritis [21], Ridel's thyroiditis [22] and Küttner's tumor [23]. These diseases are now referred to as IgG4-related disease (IgG4-RD) [24,25]. We recently described the concept of IgG4-RD and provided up-to-date information regarding this emerging disease entity [26]. Recent studies have referred to MD as IgG4-related dacryoadenitis and sialoadenitis (IgG4-DS) [15,27] (Table 2).

IgG4 molecules are symmetrical homobivalent antibodies that can exchange half-molecules (heavy and light chain) specific for two different antigens ("Fab-arm exchange"), which results in losing the ability to cross-link antigens and to form immune complexes [28]. In addition, IgG4 also can bind the Fc fragment of other IgG molecule, particularly other IgG4 molecules ("Fc–Fc interactions"). These IgG4 Fc–Fc interactions proceed to Fab-arm exchange reaction and may contribute to the anti-inflammatory activity, which includes a poor ability to induce complement and cell activation caused by low affinity for C1q (Fig. 2) [29]. Another characteristic is that IgG4 is a Th2-dependent immunoglobulin and has low affinity for its target antigen. interleukin (IL)-4 directs naive human B cell immunoglobulin isotype switching to IgG4 and IgE production [30]. We previously reported that peripheral CD4+ Th cells from patients with IgG4-DS revealed a deviation in the Th1/Th2 balance to Th2 and elevated expression of Th2-type cytokines [15,31,32]. Therefore, IgG4-DS is suggested to have a Th2-predominant phenotype. This review article will emphasize recent studies seeking to understand the role of Th cell subsets in primary SS and IgG4-DS.

1. Cytokine profiles of CD4+ Th cells

1.1. Th1/Th2 paradigm

Th1 cells support cell-mediated immunity and produce IL-2, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α, which induce inflammatory responses responsible for killing intracellular parasites and perpetuating autoimmune responses. However, excessive inflammatory responses can lead to uncontrolled tissue damage. Th2 cells produce IL-4, IL-5, and IL-13, which provide help for humoral immunity and promote IgE secretion and eosinophilic responses. Th2 responses can counteract Th1-mediated microbicidal action. Thus, the Th1/Th2 balance plays an important role in immunoregulation. In contrast, Th0 cells are characterized by the production of both Th1 and Th2 cytokines and are considered precursors of Th1 and Th2 cells. Several studies have revealed that autoimmune diseases are caused by disruption to the Th1/Th2 balance [33,34]. The relationship of Th1/Th2 imbalance to the pathogenesis of SS has been widely investigated. Polarized Th1 responses were associated with the immunopathology of SS [9]. High numbers of IFN-γ-positive CD4+ T cells were detected in the salivary glands of SS patients and intracellular cytokine analysis demonstrated the polarization of Th cells to a Th1 phenotype [35]. Furthermore, we reported that IL-2 and IFN-γ were consistently detected in all SS patients, while IL-4 and IL-5 were only detected in patients with high levels of B cell accumulation in the salivary glands [10,36]. Recently, Theander et al. [37] reported that the detection of GC-like structures (B cell accumulation) in LSG biopsy specimens from primary SS patients could be used as a highly predictive and easy-to-obtain marker for B cell lymphoma development. Taken together, these studies suggest that Th1 cytokines are essential for the induction and/or maintenance of SS, whereas Th2 cytokines may be involved in disease progression, especially local B cell activation. Our clinical data was demonstrated that Th1 and Th2 cytokine concentrations were significantly higher in saliva from SS patients than from controls, and the levels of Th2 cytokines were closely associated with increased lymphocytic accumulation in LSGs. Thus, the measurement of cytokines in saliva may be useful for diagnosis and to reveal disease status [12].

IgG4-DS patients frequently have a history of bronchial asthma and allergic rhinitis with severe eosinophilia and elevated serum IgE levels [38]. It is well known that allergic immune responses are induced by allergen-specific Th2 cytokines, such as IL-4 and IL-13, which promote the secretion of IgG4 and IgE by B cells [39]. Recent studies indicated that Th2 immune reactions contributed to IgG4-DS [15,32,40] and IgG4-related tubulointerstitial nephritis [31,41]. The expression profile of cytokines suggested that IgG4-DS was characterized by a deviation of the Th1/Th2 balance to a Th2 phenotype and elevated expression of Th2 cytokines. Contrary to our results, Ohta et al. [42] reported a strong predominance of Th1 and cytotoxic type 1 cells in the salivary glands from IgG4-DS patients. They concluded that disruption of the Th1/Th2 balance might be due to differences in the specimens examined or the severity of the disease.

Chemokines are important for leukocyte activation and chemotaxis. Interactions between chemokines and chemokine receptors promote the selective local infiltration of specific cells into inflamed areas. Furthermore, chemokines are intimately involved in maintenance of the Th1/Th2 balance and immune responses in cardiac allograft rejection [43], atopic keratoconjunctivitis [44], and cutaneous lupus erythematosus [45]. Chemokines also play a key role in lymphoid neogenesis in target organs [46]. Immunohistochemical staining in our studies indicated that Th2-type chemokines including macrophage-derived chemokine (MDC)/CCL22 and thymus and activation regulated chemokine (TARC)/CCL17, natural ligands for CCR4 on Th2 cells, were detectable in and around the ductal epithelial cells and GCs, while CCR4 was expressed on infiltrating lymphocytes in LSGs in both SS and IgG4-DS patients. Thus, interactions of CCR4 with MDC and TARC may play a critical role in the accumulation of Th2 cells and subsequently, the progression of SS and IgG4-DS [12,32]. In contrast, interferon gamma induced protein 10 (IP-10)/CXCL10, natural ligand for CXCR3 on Th1 cells, was detected in and around the ductal epithelial cells, while CXCR3 was only expressed on infiltrating lymphocytes in LSGs from SS patients [47].

Table 1
Clinical and laboratory findings of Sjögren's syndrome (SS) and IgG4-related dacryoadenitis and sialoadenitis (IgG4-DS). § IgG4 positive plasma cells/IgG positive plasma cells >50%.

	SS	IgG4-DS
Peak age of onset	40's and 50's	60's
Sex	Male ≪ Female	Male ≦ Female
Salivary secretion dysfunction	Moderate or severe	None or mild
Glandular swelling	Recurrent	Persistent
Sialography	Apple-tree sign	Parenchymal defect
$IgG4^+$ plasma cell infiltration§	Positive	Negative
Serum IgG	Often high	High
Serum IgG4	Normal	High
Serum complement	Normal	Often low
Anti SS-A/SS-B antibody (+)	High rate	Rare
Antinuclear antibody (+)	Often	Rare

Please cite this article in press as: Moriyama M, et al., T helper subsets in Sjögren's syndrome and IgG4-related dacryoadenitis and sialoadenitis: A critical review, Journal of Autoimmunity (2013), http://dx.doi.org/10.1016/j.jaut.2013.07.007

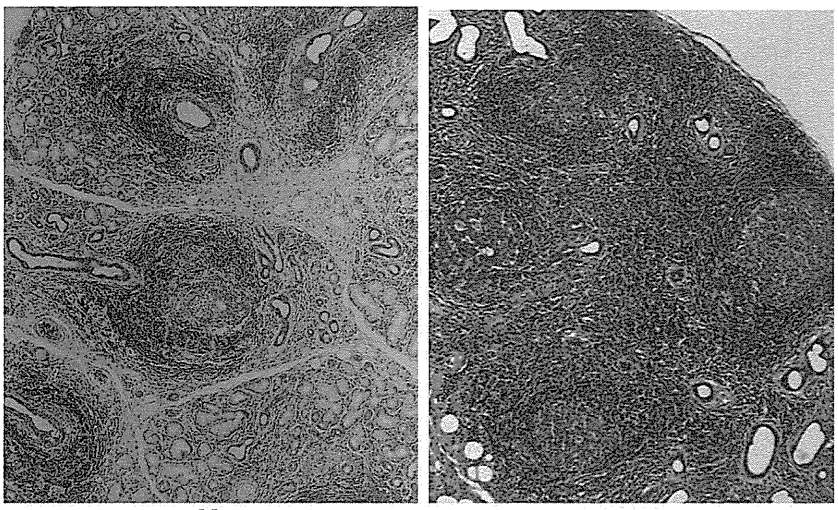

Fig. 1. Histopathological findings in salivary glands from patients with Sjögren's syndrome (SS) and IgG4-related dacryoadenitis and sialoadenitis (IgG4-DS). SS is characterized by periductal lymphocytic infiltration with atrophy or severe destruction of the acini, while IgG4-DS shows non-periductal lymphocytic infiltration with hyperplastic GCs and mild destruction of the acini. Abbreviations: GC, germinal center.

1.2. Th17 cells

The Th1/Th2 paradigm was recently expanded by the identification of Th17 cells, a subset of CD4+ Th cells characterized by their ability to produce IL-17. Several studies have reported that IL-17 was detected in epithelial and infiltrating mononuclear cells in LSGs from patients with SS. In addition, Th17 cells are "tissue seeking" and intimately involved in the initiation of SS [48]. Youinou et al. [49] reported that Th17 cells orchestrate autoreactive GCs. However, Our previous data in selectively extracted lesions from LSGs by laser capture microdissection showed that the expressions of Th17-related molecules in infiltrating lymphocytes outside ectopic GCs were higher than inside ectopic GCs [36]. Interestingly, a subset of Th17/Th1 cells identified in the gut of Crohn's disease patients may co-express IFN-γ and IL-17 [50]. Both Th1 and Th17 cells were involved in the pathogenesis of SS [51], and the early induction of a CD4+ Th1/Th17 pathway caused the systemic release of IL-17 in mice [52]. Our previous data suggest that both Th1 and Th17 cells present around the ductal epithelial cells might be of critical importance in the initiation of SS. Furthermore, the destruction of epithelial by Th1 and Th17 cells are thought to play an important pathogenetic role by the occurrence of infiltrating lesions in various epithelial tissues as well as the increased epithelial expression of various immunoactive molecules. Thus, SS has been described as "autoimmune epithelitis" [6]. In contrast, Th17-related molecules were rarely expressed in patients with IgG4-DS [32,36]. As mentioned above, IgG4-DS showed non-periductal lymphocytic infiltration and mild destruction of the epithelial cells. These findings were speculated that IgG4-DS might be a "non- autoimmune epithelitis".

Table 2
Role of Th subsets in IgG4-related disease (IgG4-RD). Abbreviations: Th, T helper; MD, Mikulicz's disease; AID, activation-induced cytidine deaminase; LSG, labial salivary gland; Tc1, T cytotoxic type 1; Tfh, follicular helper T; NLR, nucleotide-binding oligomerization domain-like receptor; TLR, Toll-like receptor; AIP, autoimmune pancreatitis; BAFF, B-cell activating factor belonging to the tumor necrosis factor family; APRIL, a proliferation-inducing ligand; Treg, regulatory T; TGF-β, transforming growth factor β.

Principal findings	Reference
Overexpression of IL-21 by Th2 cells play a key role in germinal center formation and IgG4 production in IgG4-DS.	[15]
Peripheral CD4+ T cells from the patient with MD reveal the deviation of the Th1/Th2 balance to Th2.	[31]
Th2 and regulatory immune reactions play a key role of IgG4 production in MD.	[32]
The production of IgG4 antibodies appears to be driven in part by Th2 cytokines that mediate allergic responses and IgE production.	[38]
Th2 cells are involved in the pathogenesis of IgG4-related lacrimal gland enlargement.	[39]
Overexpressions of IL-10, TGF-β, and AID in LSGs play important roles in the pathogenesis of IgG4-RD, such as IgG4-specific class-switch recombination and fibrosis.	[81]
IgG4-related tubulointerstitial nephritis shows amplification of IL-10 and TGF-β.	[41]
Th1 and Tc1 cell populations and IL-17 expression are involved in the mechanism of pathogenesis of IgG4-related sclerosing sialadenitis.	[42]
IgG4-related interstitial nephritis shows Tfh cells in enhancing a skewed B-cell terminal maturation and of CD20 + B cells in disease progression.	[66]
Activation of NLR and TLR in monocytes from AIP patients induces IgG4 production by B cells.	[76]
BAFF and APRIL are useful markers for predicting disease activity in IgG4-RD.	[78]
The progression and induction of AIP was supported by increased memory Treg and Th2 immune responses.	[80]

1.3. Regulatory T cells

Treg cells, identified by the expression of Foxp3, are essential for the maintenance of immunological self-tolerance and immune homeostasis to prevent the development of various inflammatory diseases. It achieves this either by direct contact with effector immune cells and/or by secreting anti-inflammatory cytokines such as IL-10 and transforming growth factor (TGF)-β. Treg cells exert their effects through the modulation of both T and B cell responses. Two subsets of Treg cells, CD4+ CD25+ Foxp3+ Treg cells [53] and IL-10–producing Tr1 cells [54] are crucial for regulating effector T cell functions. CD4+ CD25+ Foxp3+ Treg cells can prevent

Please cite this article in press as: Moriyama M, et al., T helper subsets in Sjögren's syndrome and IgG4-related dacryoadenitis and sialoadenitis: A critical review, Journal of Autoimmunity (2013), http://dx.doi.org/10.1016/j.jaut.2013.07.007

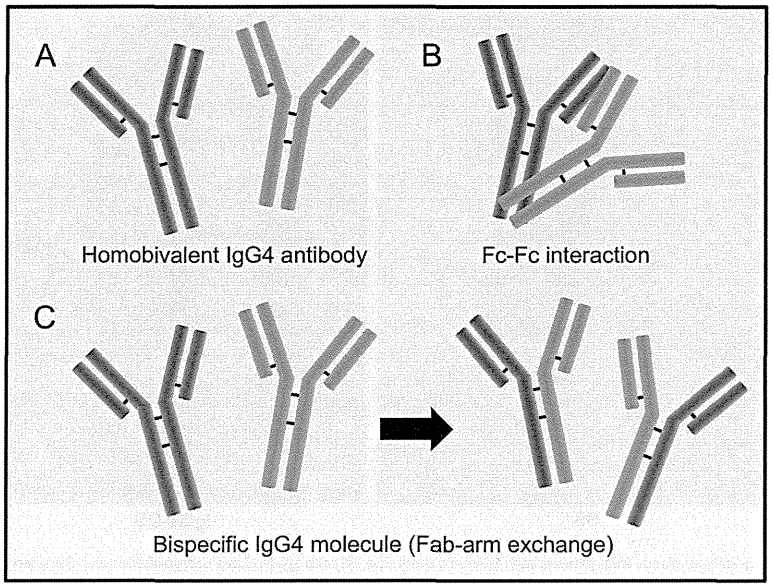

Fig. 2. Unique structure of IgG4 antibody. A, IgG4 antibody consists of two heavy chains and two light chains. B, Fc fragment of IgG4 can interact with the Fc fragment of another IgG4 molecule. C, Exchange of half-molecules (Fab-arm exchange) results in IgG4 combining two different specificities in a single molecule (bispecific antibody).

autoimmune hepatitis and primary biliary cirrhosis [55]. Mice with defects in Treg cell generation often develop T cell-mediated systemic autoimmune responses that affect multiple organs. Kolkowski et al. [56] demonstrated that salivary glands in SS constitutively expressed IL-10 and TGF-β. Other studies reported a significant reduction of Tregs in LSGs and peripheral blood from SS patients that might be involved in the pathogenesis of salivary gland destruction [57,58]. In contrast, Gottenberg et al. [59] reported increased Treg cell numbers in the peripheral blood of SS patients. Therefore, it is unclear whether Tregs are involved in the pathogenesis of SS. According to recent data, Foxp3+ T-regulatory cell frequency in the salivary glands of SS patients correlates with inflammation grade and certain risk factors for lymphoma development [60]. While in early and moderate infiltrations a compensatory control of Tregs in response to Th17 expansion seems to occur, in advanced SS lesions Tregs may fail to control the immune mediated tissue injury [7,61]. Increased levels of Treg cells in salivary glands from SS patients might suggest negative feedback is more active than in healthy subjects. Therefore, Treg cells might be not involved in the initiation of disease.

Zen et al. [62] reported that significant numbers of CD4+ CD25+ Foxp3+ Tregs infiltrated the affected tissues in cases of autoimmune pancreato-cholangitis (AIPC), which is one of IgG4-RD. Furthermore, another study demonstrated that IL-10 decreased IL-4-induced IgE switching but increased IL-4-induced IgG4 production [63]. We found that IL-4, IL-10, and Foxp3 were positively correlated with the IgG4/IgG ratio in the salivary glands from patients with IgG4-DS [32]. These results suggest that Th2 and regulatory immune reactions might play key roles in IgG4 production.

2. Role of IL-21 in SS and IgG4-DS

2.1. Follicular helper T cells

Tfh cells were recently identified as a unique Th phenotype, expressing high levels of CXCR5, a chemokine receptor [64]. Several studies reported that Tfh cells control the functional activity of effector Th cells and promote ectopic GC formation by IL-21, which contributed to impaired B cell differentiation [65,66]. Once GCs are formed, Tfh cells are required for their maintenance and the regulation of B cell differentiation into plasma cells and memory B cells. Several studies in SS patients demonstrated that IL-21 was increased in serum and high levels of IL-21 receptor were present on the surface of most B cells [67]. Furthermore, IL-4 and IL-21 receptors knockout mice have greatly reduced IgG responses, indicating that IL-21 co-operates with IL-4 to regulate humoral immune responses [68]. We previously observed that Tfh-related molecules, CXCR5 and B-cell lymphoma 6 protein (Bcl-6), were highly expressed on infiltrating lymphocytes in ectopic GCs of LSG lesions from both SS and IgG4-DS patients [15,36]. These results provide strong support for Tfh cells in the progression of disease as a lymphoproliferative disorder, particularly in the growth and activation of ectopic GC formation (Fig. 3).

IL-21 was mainly produced by Th2 and Th17 cells in addition to Tfh cells [68,69]. Interestingly, high IL-21 expression was only detected outside ectopic GCs in patients with IgG4-DS in our immunohistological analyses. The expression patterns of Th2-related molecules (IL-4, CCR4 and c-Maf) in LSGs were similar to that of IL-21 in patients with IgG4-DS. In contrast, Th17-related molecules were rarely expressed in patients with IgG4-DS. Furthermore, IL-21 positively correlated with the number of GCs formed in LSGs from patients with IgG4-DS [15]. Taken together, these findings suggest that excessive IL-21 production by Th2 cells in salivary glands from IgG4-DS patients might induce Bcl-6 expression in B cells resulting in multiple GC formation. Furthermore, IL-21 directly inhibited IL-4-induced IgE production [70], and IgG4 class switching was induced by co-stimulation with IL-4 and IL-21 in humans and mice [71]. In addition, IL-21 induced IL-10 production by mitogen-stimulated peripheral blood mononuclear cells in humans [72]. Therefore, we speculate that IL-21 correlates with IL-4 and IL-10 for IgG4 class switching. In the current study, we found that IL-21 positively correlated with the IgG4/IgG ratio in immunohistochemically positive cells

Please cite this article in press as: Moriyama M, et al., T helper subsets in Sjögren's syndrome and IgG4-related dacryoadenitis and sialoadenitis: A critical review, Journal of Autoimmunity (2013), http://dx.doi.org/10.1016/j.jaut.2013.07.007

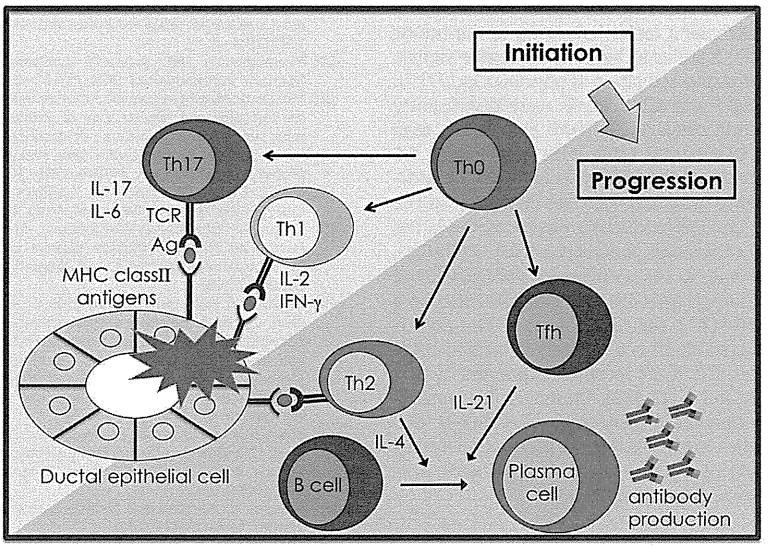

Fig. 3. Schematic model of Th cell network in SS. Th1 and Th17 cells are involved in early stages of disease, while Th2 and Tfh cells are associated with GC formation in the late stage. Abbreviations: Th, T helper; Tfh, follicular helper T.

[15] suggesting that IL-21 might also be involved in the class switching of IgG4 in IgG4-DS [73].

2.2. Innate immunity in IgG4-DS

Macrophages act as cells in the immune response to foreign invaders of the body, by presenting pathogenic antigens to antigen-specific Th cells. Historically, they have been classified into two distinct macrophage phenotypes, "classically activated" proinflammatory (M1) and "alternatively activated" anti-inflammatory (M2) macrophages [74]. M2 macrophages are activated by IL-4, produce high levels of IL-10 and are important for debris scavenging, wound healing and fibrosis. These polarized macrophage populations can also contribute to systemic diseases [75]. Watanabe et al. [76] demonstrated that abnormal innate immune responses induced via Toll-like receptor signaling in macrophages might enhance Th2 immune responses and the immunopathogenesis of IgG4-RD. Our current studies observed that IgG4-DS patients showed predominant infiltration by M2 macrophages that secreted IL-10 and IL-13 in salivary glands.

Dendritic cells (DCs) are professional antigen presenting cells that bridge innate and adaptive immunity. Expression of

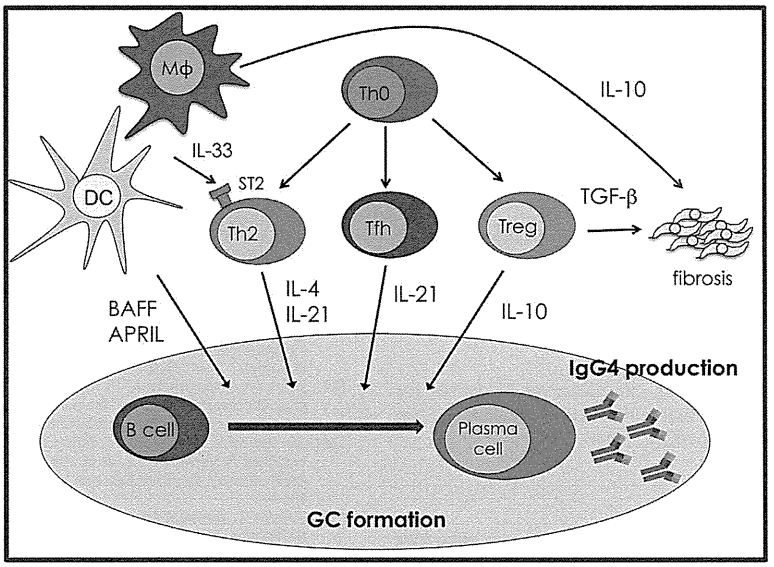

Fig. 4. Schematic model of Th cell and innate immune network in IgG4-DS. Th2, Treg, and Tfh cells play key roles in GC formation and IgG4 production. Dendritic cells and macrophages promote Th2 immune reaction by IL-33 as well as BAFF and APRIL. Abbreviations: Treg, regulatory T; BAFF, B cell activating factor belonging to the tumor necrosis factor family; APRIL, a proliferation-inducing ligand.

Please cite this article in press as: Moriyama M, et al., T helper subsets in Sjögren's syndrome and IgG4-related dacryoadenitis and sialoadenitis: A critical review, Journal of Autoimmunity (2013), http://dx.doi.org/10.1016/j.jaut.2013.07.007

DC-derived TNF-family ligands such as a proliferation-inducing ligand (APRIL) and B cell activating factor belonging to the tumor necrosis factor family (BAFF) is induced by innate immune signals to promote the differentiation and activation of plasma cells [77]. In IgG4-RD patients, serum BAFF and APRIL levels were significantly higher than in healthy individuals [78]. BAFF and APRIL may contribute to progressive plasmacyte infiltration and ectopic GC formation in the target organs of patients with IgG4-RD. In addition, BAFF and APRIL enhance IgG4 and IgE class switching in the presence of IL-4 [79]. Th2 cytokine production was increased in the tissues of patients with autoimmune pancreatitis [80]. Therefore, BAFF and APRIL may contribute to the pathogenesis of IgG4-RD in concert with Th2 cells. Although IgG4-RD was considered to be a Th2-dependent disease [40,41,81], the mechanism of Th2 polarization has yet to be elucidated. IL-33 is a recently identified cytokine that directly stimulates ST2, IL-33 receptor, expressed by Th2 cells to produce IL-4, IL-5, and IL-13 [82]. Moreover, the genetic polymorphism of IL-33 in humans is associated with allergic diseases [83]. Our current studies suggest that IL-33 production by DCs and M2 macrophages might play a key role in Th2 cytokine production and the pathogenesis of IgG4-DS (Fig. 4).

3. Conclusions

Research accumulated in recent years makes it increasingly clear that the immunological backgrounds are entirely different between SS and IgG4-DS. However, additional research is required to elucidate further the pathogenesis of IgG4-DS, especially the development of a mouse model of IgG4-DS. Although Glucocorticoids are the standard treatment for IgG4-RD, Yamamoto et al. [84] reported that the relapse rate of IgG4-DS during steroid therapy is 26.8%. A more thorough understanding of the complex mechanisms of IgG4-DS, especially the role of Th subset-related cytokines, could lead to the development of novel pharmacological strategies aimed at disrupting the cytokine network and inhibiting the initiation and/or progression of IgG4-DS. Finally, it should be noted that while this thesis focuses primarily on T cells, that there have recently been other extensive reviews and hypotheses published on Sjogren's syndrome, reflecting its increased interest not only to basic immunologists, but also to rheumatologists [4,85–116].

Competing interests

The authors declare no competing interests.

Author contributions

All authors provided substantial contributions to discussions of content, and to reviewing and editing the manuscript before submission. M Moriyama researched the data and wrote the article.

Acknowledgments

This work was supported by the "Research on Measures for Intractable Diseases" Project, a matching fund subsidy from the Ministry of Health Labour and Welfare, Japan.

References

[1] Youinou P. Sjogren's syndrome: a quintessential B cell-induced autoimmune disease. Jt Bone Spine 2008;75:1–2.
[2] Gershwin ME. The mosaic of autoimmunity. Autoimmun Rev 2008;7:161–3.
[3] Adamson 3rd TC, Fox RI, Frisman DM, Howell FV. Immunohistologic analysis of lymphoid infiltrates in primary Sjogren's syndrome using monoclonal antibodies. J Immunology (Baltimore, Md 1950) 1983;130:203–8.
[4] Tzioufas AG, Kapsogeorgou EK, Moutsopoulos HM. Pathogenesis of Sjogren's syndrome: what we know and what we should learn. J Autoimmun 2012;39:4–8.
[5] Moutsopoulos HM. Sjogren's syndrome: autoimmune epithelitis. Clin Immunol Immunopathol 1994;72:162–5.
[6] Mitsias DI, Kapsogeorgou EK, Moutsopoulos HM. The role of epithelial cells in the initiation and perpetuation of autoimmune lesions: lessons from Sjogren's syndrome (autoimmune epithelitis). Lupus 2006;15:255–61.
[7] Mavragani CP, Moutsopoulos HM. The geoepidemiology of Sjogren's syndrome. Autoimmun Rev 2010;9:A305–10.
[8] King C, Tangye SG, Mackay CR. T follicular helper (TFH) cells in normal and dysregulated immune responses. Annu Rev Immunol 2008;26:741–66.
[9] Mitsias DI, Tzioufas AG, Veiopoulou C, Zintzaras E, Tassios IK, Kogopoulou O, et al. The Th1/Th2 cytokine balance changes with the progress of the immunopathological lesion of Sjogren's syndrome. Clin Exp Immunol 2002;128:562–8.
[10] 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 Sjogren's syndrome. Arthritis Rheum 1996;39:1376–84.
[11] van Woerkom JM, Kruize AA, Wenting-van Wijk MJ, Knol E, Bihari IC, Jacobs JW, et al. Salivary gland and peripheral blood T helper 1 and 2 cell activity in Sjogren's syndrome compared with non-Sjogren's sicca syndrome. Ann Rheum Dis 2005;64:1474–9.
[12] Moriyama M, Hayashida JN, Toyoshima T, Ohyama Y, Shinozaki S, Tanaka A, et al. Cytokine/chemokine profiles contribute to understanding the pathogenesis and diagnosis of primary Sjogren's syndrome. Clin Exp Immunol 2012;169:17–26.
[13] Morgan WS, Castleman B. A clinicopathologic study of Mikulicz's disease. Am J Pathol 1953;29:471–503.
[14] M.Moriyama, A.Tanaka, T.Maehara, Y.Ohyama, M.Shimizu, H.Nakashima, et al. Clinical characteristics of Mikulicz's disease as an IgG4-related disease. *Clin Oral Investig.* in press.
[15] Maehara T, Moriyama M, Nakashima H, Miyake K, Hayashida JN, Tanaka A, et al. Interleukin-21 contributes to germinal centre formation and immunoglobulin G4 production in IgG4-related dacryoadenitis and sialoadenitis, so-called Mikulicz's disease. Ann Rheum Dis 2012;71:2011–20.
[16] Yamamoto M, Takahashi H, Sugai S, Imai K. Clinical and pathological characteristics of Mikulicz's disease (IgG4-related plasmacytic exocrinopathy). Autoimmun Rev 2005;4:195–200.
[17] Yamamoto M, Takahashi H, Naishiro Y, Isshiki H, Ohara M, Suzuki C, et al. Mikulicz's disease and systemic IgG4-related plasmacytic syndrome (SIPS). Nihon Rinsho Meneki Gakkai Kaishi 2008;31:1–8.
[18] Yamamoto M, Takahashi H, Ohara M, Suzuki C, Naishiro Y, Yamamoto H, et al. A new conceptualization for Mikulicz's disease as an IgG4-related plasmacytic disease. Mod Rheumatol 2006;16:335–40.
[19] Hamano H, Kawa S, Horiuchi A, Unno H, Furuya N, Akamatsu T, et al. High serum IgG4 concentrations in patients with sclerosing pancreatitis. N Engl J Med 2001;344:732–8.
[20] Zen Y, Harada K, Sasaki M, Sato Y, Tsuneyama K, Haratake J, et al. IgG4-related sclerosing cholangitis with and without hepatic inflammatory pseudotumor, and sclerosing pancreatitis-associated sclerosing cholangitis: do they belong to a spectrum of sclerosing pancreatitis? Am J Surg Pathol 2004;28:1193–203.
[21] Takeda S, Haratake J, Kasai T, Takaeda C, Takazakura E. IgG4-associated idiopathic tubulointerstitial nephritis complicating autoimmune pancreatitis. Nephrol Dial Transplant 2004;19:474–6.
[22] Hamed G, Tsushima K, Yasuo M, Kubo K, Yamazaki S, Kawa S, et al. Inflammatory lesions of the lung, submandibular gland, bile duct and prostate in a patient with IgG4-associated multifocal systemic fibrosclerosis. Respirology 2007;12:455–7.
[23] Kitagawa S, Zen Y, Harada K, Sasaki M, Sato Y, Minato H, et al. Abundant IgG4-positive plasma cell infiltration characterizes chronic sclerosing sialadenitis (Kuttner's tumor). Am J Surg Pathol 2005;29:783–91.
[24] Umehara H, Okazaki K, Masaki Y, Kawano M, Yamamoto M, Saeki T, et al. A novel clinical entity, IgG4-related disease (IgG4RD): general concept and details. Mod Rheumatol 2012;22:1–14.
[25] Stone JH, Zen Y, Deshpande V. IgG4-related disease. N Engl J Med 2012;366:539–51.
[26] Umehara H, Okazaki K, Masaki Y, Kawano M, Yamamoto M, Saeki T, et al. Comprehensive diagnostic criteria for IgG4-related disease (IgG4-RD). Mod Rheumatol 2011;2012(22):21–30.
[27] Stone JH, Khosroshahi A, Deshpande V, Chan JK, Heathcote JG, Aalberse R, et al. Recommendations for the nomenclature of IgG4-related disease and its individual organ system manifestations. Arthritis Rheum 2012;64:3061–7.
[28] van der Neut Kolfschoten M, Schuurman J, Losen M, Bleeker WK, Martinez-Martinez P, Vermeulen E, et al. Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. Sci (New York, NY) 2007;317:1554–7.
[29] Rispens T, Ooievaar-De Heer P, Vermeulen E, Schuurman J, van der Neut Kolfschoten M, Aalberse RC. Human IgG4 binds to IgG4 and conformationally altered IgG1 via Fc-Fc interactions. J Immunology (Baltimore, Md 1950) 2009;182:4275–81.
[30] 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 U S A 1993;90:3730–4.

Please cite this article in press as: Moriyama M, et al., T helper subsets in Sjögren's syndrome and IgG4-related dacryoadenitis and sialoadenitis: A critical review, Journal of Autoimmunity (2013), http://dx.doi.org/10.1016/j.jaut.2013.07.007

[31] Miyake K, Moriyama M, Aizawa K, Nagano S, Inoue Y, Sadanaga A, et al. Peripheral CD4+ T cells showing a Th2 phenotype in a patient with Mikulicz's disease associated with lymphadenopathy and pleural effusion. Mod Rheumatol 2008;18:86–90.
[32] Tanaka A, Moriyama M, Nakashima H, Miyake K, Hayashida JN, Maehara T, et al. Th2 and regulatory immune reactions contribute to IgG4 production and the initiation of Mikulicz disease. Arthritis Rheum 2012;64:254–63.
[33] Kennedy MK, Torrance DS, Picha KS, Mohler KM. Analysis of cytokine mRNA expression in the central nervous system of mice with experimental autoimmune encephalomyelitis reveals that IL-10 mRNA expression correlates with recovery. J Immunology (Baltimore, Md 1950) 1992;149:2496–505.
[34] Rapoport MJ, Jaramillo A, Zipris D, Lazarus AH, Serreze DV, Leiter EH, et al. Interleukin 4 reverses T cell proliferative unresponsiveness and prevents the onset of diabetes in nonobese diabetic mice. J Exp Med 1993;178:87–99.
[35] Ozaki Y, Amakawa R, Ito T, Iwai H, Tajima K, Uehira K, et al. Alteration of peripheral blood dendritic cells in patients with primary Sjogren's syndrome. Arthritis Rheum 2001;44:419–31.
[36] Maehara T, Moriyama M, Hayashida JN, Tanaka A, Shinozaki S, Kubo Y, et al. Selective localization of T helper subsets in labial salivary glands from primary Sjogren's syndrome patients. Clin Exp Immunol 2012;169:89–99.
[37] Theander E, Vasaitis L, Baecklund E, Nordmark G, Warfvinge G, Liedholm R, et al. Lymphoid organisation in labial salivary gland biopsies is a possible predictor for the development of malignant lymphoma in primary Sjogren's syndrome. Ann Rheum Dis 2011;70:1363–8.
[38] Nirula A, Glaser SM, Kalled SL, Taylor FR. What is IgG4? A review of the biology of a unique immunoglobulin subtype. Curr Opin Rheumatol 2011;23:119–24.
[39] Finkelman FD, Boyce JA, Vercelli D, Rothenberg ME. Key advances in mechanisms of asthma, allergy, and immunology in 2009. J Allergy Clin Immunol 2010;125:312–8.
[40] Tsuboi H, Matsuo N, Iizuka M, Tsuzuki S, Kondo Y, Tanaka A, et al. Analysis of IgG4 class switch-related molecules in IgG4-related disease. Arthritis Res Ther 2012;14:R171.
[41] Nakashima H, Miyake K, Moriyama M, Tanaka A, Watanabe M, Abe Y, et al. An amplification of IL-10 and TGF-beta in patients with IgG4-related tubulointerstitial nephritis. Clin Nephrol 2010;73:385–91.
[42] Ohta N, Makihara S, Okano M, Kurakami K, Ishida A, Furukawa T, et al. Roles of IL-17, Th1, and Tc1 cells in patients with IgG4-related sclerosing sialadenitis. Laryngoscope 2012;122:2169–74.
[43] Oberbarnscheidt MH, Walch JM, Li Q, Williams AL, Walters JT, Hoffman RA, et al. Memory T cells migrate to and reject vascularized cardiac allografts independent of the chemokine receptor CXCR3. Transplantation 2011;91:827–32.
[44] Yamagami S, Ebihara N, Amano SY. Chemokine receptor gene expression in giant papillae of atopic keratoconjunctivitis. Mol Vis 2005;11:192–200.
[45] Gambichler T, Genc Z, Skrygan M, Scola N, Tigges C, Terras S, et al. Cytokine and chemokine ligand expression in cutaneous lupus erythematosus. Eur J Dermatol 2012;22:319–23.
[46] Amft N, Curnow SJ, Scheel-Toellner D, Devadas A, Oates J, Crocker J, et al. Ectopic expression of the B cell-attracting chemokine BCA-1 (CXCL13) on endothelial cells and within lymphoid follicles contributes to the establishment of germinal center-like structures in Sjogren's syndrome. Arthritis Rheum 2001;44:2633–41.
[47] Ogawa N, Ping L, Zhenjun L, Takada Y, Sugai S. Involvement of the interferon-gamma-induced T cell-attracting chemokines, interferon-gamma-inducible 10-kd protein (CXCL10) and monokine induced by interferon-gamma (CXCL9), in the salivary gland lesions of patients with Sjogren's syndrome. Arthritis Rheum 2002;46:2730–41.
[48] Nguyen CQ, Yin H, Lee BH, Carcamo WC, Chiorini JA, Peck AB. Pathogenic effect of interleukin-17A in induction of Sjogren's syndrome-like disease using adenovirus-mediated gene transfer. Arthritis Res Ther 2010;12:R220.
[49] Youinou P, Pers JO. Disturbance of cytokine networks in Sjogren's syndrome. Arthritis Res Ther 2011;13:227.
[50] McGeachy MJ, Bak-Jensen KS, Chen Y, Tato CM, Blumenschein W, McClanahan T, et al. TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. Nat Immunol 2007;8:1390–7.
[51] Sakai A, Sugawara Y, Kuroishi T, Sasano T, Sugawara S. Identification of IL-18 and Th17 cells in salivary glands of patients with Sjogren's syndrome, and amplification of IL-17-mediated secretion of inflammatory cytokines from salivary gland cells by IL-18. J Immunology (Baltimore, Md 1950) 2008;181:2898–906.
[52] Nguyen CQ, Hu MH, Li Y, Stewart C, Peck AB. Salivary gland tissue expression of interleukin-23 and interleukin-17 in Sjogren's syndrome: findings in humans and mice. Arthritis Rheum 2008;58:734–43.
[53] Shevach EM, DiPaolo RA, Andersson J, Zhao DM, Stephens GL, Thornton AM. The lifestyle of naturally occurring CD4+ CD25+ Foxp3+ regulatory T cells. Immunol Rev 2006;212:60–73.
[54] Pot C, Apetoh L, Kuchroo VK. Type 1 regulatory T cells (Tr1) in autoimmunity. Semin Immunol 2011;23:202–8.
[55] Lan RY, Cheng C, Lian ZX, Tsuneyama K, Yang GX, Moritoki Y, et al. Liver-targeted and peripheral blood alterations of regulatory T cells in primary biliary cirrhosis. Hepatology 2006;43:729–37.
[56] Kolkowski EC, Reth P, Pelusa F, Bosch J, Pujol-Borrell R, Coll J, et al. Th1 predominance and perforin expression in minor salivary glands from patients with primary Sjogren's syndrome. J Autoimmun 1999;13:155–62.
[57] Li X, Li X, Qian L, Wang G, Zhang H, Wang X, et al. T regulatory cells are markedly diminished in diseased salivary glands of patients with primary Sjogren's syndrome. J Rheumatol 2007;34:2438–45.
[58] Roca V, Calafat M, Larocca L, Ramhorst R, Farina M, Franchi AM, et al. Potential immunomodulatory role of VIP in the implantation sites of prediabetic nonobese diabetic mice. Reproduction 2009;138:733–42.
[59] Gottenberg JE, Lavie F, Abbed K, Gasnault J, Le Nevot E, Delfraissy JF, et al. CD4 CD25high regulatory T cells are not impaired in patients with primary Sjogren's syndrome. J Autoimmun 2005;24:235–42.
[60] Christodoulou MI, Kapsogeorgou EK, Moutsopoulos NM, Moutsopoulos HM. Foxp3+ T-regulatory cells in Sjogren's syndrome: correlation with the grade of the autoimmune lesion and certain adverse prognostic factors. Am J Pathol 2008;173:1389–96.
[61] Christodoulou MI, Kapsogeorgou EK, Moutsopoulos HM. Characteristics of the minor salivary gland infiltrates in Sjogren's syndrome. J Autoimmun 2010;34:400–7.
[62] Zen Y, Fujii T, Harada K, Kawano M, Yamada K, Takahira M, et al. Th2 and regulatory immune reactions are increased in immunoglobin G4-related sclerosing pancreatitis and cholangitis. Hepatology 2007;45:1538–46.
[63] Meiler F, Klunker S, Zimmermann M, Akdis CA, Akdis M. Distinct regulation of IgE, IgG4 and IgA by T regulatory cells and toll-like receptors. Allergy 2008;63:1455–63.
[64] Morita R, Schmitt N, Bentebibel SE, Ranganathan R, Bourdery L, Zurawski G, et al. Human blood CXCR5(+) CD4(+) T cells are counterparts of T follicular cells and contain specific subsets that differentially support antibody secretion. Immunity 2011;34:108–21.
[65] Vinuesa CG, Linterman MA, Goodnow CC, Randall KL. T cells and follicular dendritic cells in germinal center B-cell formation and selection. Immunol Rev 2010;237:72–89.
[66] Zaidan M, Cervera-Pierot P, de Seigneux S, Dahan K, Fabiani B, Callard P, et al. Evidence of follicular T-cell implication in a case of IgG4-related systemic disease with interstitial nephritis. Nephrol Dial Transplant 2011;26:2047–50.
[67] Yuan SL, Jiang L, Zhang XL, Li SF, Duan HM, Wang XF. Serum IL-21 level in patients with primary Sjogren's syndrome and clinical significance of IL-21. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 2007;23:124–6.
[68] Ozaki K, Spolski R, Feng CG, Qi CF, Cheng J, Sher A, et al. A critical role for IL-21 in regulating immunoglobulin production. Sci (New York, NY) 2002;298:1630–4.
[69] Wurster AL, Rodgers VL, Satoskar AR, Whitters MJ, Young DA, Collins M, et al. Interleukin 21 is a T helper (Th) cell 2 cytokine that specifically inhibits the differentiation of naive Th cells into interferon gamma-producing Th1 cells. J Exp Med 2002;196:969–77.
[70] Suto A, Nakajima H, Hirose K, Suzuki K, Kagami S, Seto Y, et al. Interleukin 21 prevents antigen-induced IgE production by inhibiting germ line C(epsilon) transcription of IL-4-stimulated B cells. Blood 2002;100:4565–73.
[71] Kitayama D, Sakamoto A, Arima M, Hatano M, Miyazaki M, Tokuhisa T. A role for Bcl6 in sequential class switch recombination to IgE in B cells stimulated with IL-4 and IL-21. Mol Immunol 2008;45:1337–45.
[72] Wood N, Bourque K, Donaldson DD, Collins M, Vercelli D, Goldman SJ, et al. IL-21 effects on human IgE production in response to IL-4 or IL-13. Cell Immunol 2004;231:133–45.
[73] Fox RI. The distinct pathogenesis of IgG4 Mickulicz's disease and Sjogren's syndrome in Japan; the role of IL-21 and IL-6. Ann Rheum Dis 2012;71:1919–20.
[74] Kigerl KA, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG. Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. J Neurosci 2009;29:13435–44.
[75] Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. J Immunology (Baltimore, Md 1950) 2006;177:7303–11.
[76] Watanabe T, Yamashita K, Fujikawa S, Sakurai T, Kudo M, Shiokawa M, et al. Involvement of activation of toll-like receptors and nucleotide-binding oligomerization domain-like receptors in enhanced IgG4 responses in autoimmune pancreatitis. Arthritis Rheum 2012;64:914–24.
[77] Mackay F, Schneider P, Rennert P, Browning J. BAFF and APRIL: a tutorial on B cell survival. Annu Rev Immunol 2003;21:231–64.
[78] Kiyama K, Kawabata D, Hosono Y, Kitagori K, Yukawa N, Yoshifuji H, et al. Serum BAFF and APRIL levels in patients with IgG4-related disease and their clinical significance. Arthritis Res Ther 2012;14:R86.
[79] Litinskiy MB, Nardelli B, Hilbert DM, He B, Schaffer A, Casali P, et al. DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. Nat Immunol 2002;3:822–9.
[80] Okazaki K, Uchida K, Fukui T. Recent advances in autoimmune pancreatitis: concept, diagnosis, and pathogenesis. J Gastroenterol 2008;43:409–18.
[81] Kanari H, Kagami S, Kashiwakuma D, Oya Y, Furuta S, Ikeda K, et al. Role of Th2 cells in IgG4-related lacrimal gland enlargement. Int Arch Allergy Immunol 2010;152(Suppl. 1):47–53.
[82] Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity 2005;23:479–90.
[83] Sakashita M, Yoshimoto T, Hirota T, Harada M, Okubo K, Osawa Y, et al. Association of serum interleukin-33 level and the interleukin-33 genetic variant with Japanese cedar pollinosis. Clin Exp Allergy 2008;38:1875–81.

Please cite this article in press as: Moriyama M, et al., T helper subsets in Sjögren's syndrome and IgG4-related dacryoadenitis and sialoadenitis: A critical review, Journal of Autoimmunity (2013), http://dx.doi.org/10.1016/j.jaut.2013.07.007

[84] Yamamoto M, Takahashi H, Ishigami K, Yajima H, Shimizu Y, Tabeya T, et al. Evaluation and clinical validity of a new questionnaire for Mikulicz's disease. Int J Rheumatol 2012;2012:283459.
[85] Vissink A, Bootsma H, Spijkervet FK, Hu S, Wong DT, Kallenberg CG. Current and future challenges in primary Sjogren's syndrome. Curr Pharm Biotechnol 2012;13:2026–45.
[86] Barrera MJ, Bahamondes V, Sepulveda D, Quest AF, Castro I, Cortes J, et al. Sjogren's syndrome and the epithelial target: a comprehensive review. J Autoimmun 2013;42:7–18.
[87] Furness S, Worthington HV, Bryan G, Birchenough S, McMillan R. Interventions for the management of dry mouth: topical therapies. Cochrane Database Syst Rev 2011;7:CD008934.
[88] Thabet Y, Le Dantec C, Ghedira I, Devauchelle V, Cornec D, Pers JO, et al. Epigenetic dysregulation in salivary glands from patients with primary Sjogren's syndrome may be ascribed to infiltrating B cells. J Autoimmun 2013;41:175–81.
[89] Lu Q, The critical importance of epigenetics in autoimmunity. J Autoimmun 2013;41:1–5.
[90] Yao Q, Altman RD, Wang X. Systemic lupus erythematosus with Sjogren syndrome compared to systemic lupus erythematosus alone: a meta-analysis. J Clin Rheumatol 2012;18:28–32.
[91] Gliozzi M, Greenwell-Wild T, Jin W, Moutsopoulos NM, Kapsogeorgou E, Moutsopoulos HM, et al. A link between interferon and augmented plasmin generation in exocrine gland damage in Sjogren's syndrome. J Autoimmun 2013;40:122–33.
[92] Ebert EC. Gastrointestinal and hepatic manifestations of Sjogren syndrome. J Clin Gastroenterol 2012;46:25–30.
[93] Mavragani CP, Fragoulis GE, Moutsopoulos HM. Endocrine alterations in primary Sjogren's syndrome: an overview. J Autoimmun 2012;39:354–8.
[94] Singh N, Cohen PL. The T cell in Sjogren's syndrome: force majeure, not spectateur. J Autoimmun 2012;39:229–33.
[95] Amador-Patarroyo MJ, Arbelaez JG, Mantilla RD, Rodriguez-Rodriguez A, Cardenas-Roldan J, Pineda-Tamayo R, et al. Sjogren's syndrome at the crossroad of polyautoimmunity. J Autoimmun 2012;39:199–205.
[96] Scofield RH. Vasculitis in Sjogren's syndrome. Curr Rheumatol Rep 2011;13:482–8.
[97] Arentz G, Thurgood LA, Lindop R, Chataway TK, Gordon tP. Secreted human Ro52 autoantibody proteomes express a restricted set of public clonotypes. J Autoimmun 2012;39:466–70.
[98] Agmon-Levin N, Kivity S, Tzioufas AG, Lopez Hoyos M, Rozman B, Efes I, et al. Low levels of vitamin-D are associated with neuropathy and lymphoma among patients with Sjogren's syndrome. J Autoimmun 2012;39:234–9.
[99] Porola P, Mackiewicz Z, Laine M, Baretto G, Stegaev V, Takakubo Y, et al. Laminin isoform profiles in salivary glands in Sjogren's syndrome. Adv Clin Chem 2011;55:35–59.
[100] Cornec D, Devauchelle-Pensec V, Tobon GJ, Pers JO, Jousse-Joulin S, Saraux A. B cells in Sjogren's syndrome: from pathophysiology to diagnosis and treatment. J Autoimmun 2012;39:161–7.
[101] Bournia VK, Vlachoyiannopoulos PG. Subgroups of Sjogren syndrome patients according to serological profiles. J Autoimmun 2012;39:15–26.
[102] Sim S, Wolin SL. Emerging roles for the Ro 60-kDa autoantigen in noncoding RNA metabolism. Wiley Interdiscip Rev RNA 2011;2:686–99.
[103] Seror R, Bootsma H, Bowman SJ, Dorner T, Gottenberg JE, Mariette X, et al. J Autoimmun 2012;39:97–102.
[104] Fischer M, Horn IS, Bertolini J, Dietzsch S, Fuchs M, Dietz A. Laryngeal MALT lymphoma with known Sjogren syndrome 2011;59:111–4.
[105] Tzioufas AG, Vlachoyiannopoulos PG. Sjogren's syndrome: an update on clinical, basic and diagnostic therapeutic aspects. J Autoimmun 2012;39:1–3.
[106] Guerrier T, Le Pottier L, Devauchelle V, Pers JO, Jamin C, Youinou P. Role of toll-like receptors in primary Sjogren's syndrome with a special emphasis on B-cell maturation within exocrine tissues. J Autoimmun 2012;39:69–76.
[107] Abdulahad WH, Kroese FG, Vissink A, Bootsma H. Immune regulation and B-cell depletion therapy in patients with primary Sjogren's syndrome. J Autoimmun 2012;39:103–11.
[108] Takahashi H, Yamamoto M, Tabeya T, Suzuki C, Naishiro Y, Shinomura Y, et al. The immunobiology and clinical characteristics of IgG4 related diseases. J Autoimmun 2012;39:93–6.
[109] Dorner T, Radbruch A, Burmester GR. B-cell-directed therapies for autoimmune disease. Nat Rev Rheumatol 2009;5:433–41.
[110] Sarkar PK, Patel N, Furie RA, Talwar A. Pulmonary manifestations of primary Sjogren's syndrome. Indian J Chest Dis Allied Sci 2009;51:93–101.
[111] Pavlakis PP, Alexopoulos H, Kosmidis ML, Mamali I, Moutsopoulos HM, Tzioufas AG, et al. Peripheral neuropathies in Sjogren's syndrome: a critical update on clinical features and pathogenetic mechanisms. J Autoimmun 2012;39:27–33.
[112] Manoussakis MN, Tsinti M, Kapsogeorgou EK, Moutsopoulos HM. The salivary gland epithelial cells of patients with primary Sjogren's syndrome manifest significantly reduced responsiveness to 17β-estradiol. J Autoimmun 2012;39:64–8.
[113] Konttinen YT, Fuellen G, Bing Y, Porola P, Stegaev V, Trokovic N, et al. Sex steroids in Sjogren's syndrome. J Autoimmun 2012;39:49–56.
[114] Ramirez M, Ramos-Casals M, Graus F. Central nervous system involvement in primary Sjogren syndrome. Med Clin (Barc) 2009;133:349–59.
[115] Brito-Zeron P, Retamozo S, Gandia M, Akasbi M, Perez-De-Lis M, Diaz-Lagares C, et al. Monoclonal gammopathy related to Sjogren syndrome: a key marker of disease prognosis and outcomes. J Autoimmun 2012;39:43–8.
[116] Ice JA, Li H, Adrianto I, Lin PC, Kelly JA, Montgomery CG, et al. Genetics of Sjogren's syndrome in the genome-wide association era. J Autoimmun 2012;39:57–63.

Please cite this article in press as: Moriyama M, et al., T helper subsets in Sjögren's syndrome and IgG4-related dacryoadenitis and sialoadenitis: A critical review, Journal of Autoimmunity (2013), http://dx.doi.org/10.1016/j.jaut.2013.07.007

別紙４

研究成果の刊行に関する一覧表

研究代表者　京都大学ウイルス研究所　教授　松岡雅雄
研究分担者　熊本大学　教授　谷原秀信

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Inoue T, Kawaji T, **Tanihara H.**	Elevated levels of multiple biomarkers of Alzheimer's disease in the aqueous humor of eyes with open-angle glaucoma.	Invest Ophthalmol Vis Sci.	54(9)	5353-8	2013

Elevated Levels of Multiple Biomarkers of Alzheimer's Disease in the Aqueous Humor of Eyes With Open-Angle Glaucoma

Toshihiro Inoue, Takahiro Kawaji, and Hidenobu Tanihara

Department of Ophthalmology, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan

Correspondence: Toshihiro Inoue, Department of Ophthalmology, Faculty of Life Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan; noel@da2.so-net.ne.jp.

Submitted: April 17, 2013
Accepted: July 6, 2013

Citation: Inoue T, Kawaji T, Tanihara H. Elevated levels of multiple biomarkers of Alzheimer's disease in the aqueous humor of eyes with open-angle glaucoma. *Invest Ophthalmol Vis Sci.* 2013;54:5353–5358. DOI: 10.1167/iovs.13-12245

Purpose. To investigate levels of biomarkers of Alzheimer's disease in the aqueous humor in patients with open-angle glaucoma (OAG).

Methods. Aqueous humor samples were collected from 38 patients with cataracts, 20 patients with POAG, and 32 patients with exfoliation glaucoma. Aqueous levels of apolipoprotein (Apo) AI, ApoCIII, ApoE, transthyretin (TTR), complement factor H, complement C3, and α2-macroglobulin (α2M) were determined by multiplex bead immunoassay. Clinical data were obtained from medical charts.

Results. Age and sex did not differ significantly among groups. POAG patients had significantly higher aqueous levels of Apo AI, ApoCIII, ApoE, TTR, and α2M compared with cataract patients ($P < 0.001$, $P = 0.036$, $P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively). Corresponding values for exfoliation glaucoma patients were also significantly higher compared with those for cataract patients ($P = 0.003$, $P = 0.009$, $P < 0.001$, $P < 0.001$, and $P < 0.001$), and those levels correlated positively with each other in eyes with OAG ($P < 0.001$ for all combinations). Complement factor H and complement C3 levels did not differ among groups. Mean deviation values for the Humphrey visual field test correlated positively with levels of Apo AI, ApoE, TTR, and complement factor H in OAG patients ($P = 0.026$, 0.012, 0.008, and 0.027, respectively), but age and IOP values were not correlated with protein levels.

Conclusions. OAG patients had elevated levels of multiple biomarkers of Alzheimer's disease in the aqueous humor.

Keywords: Alzheimer's disease, glaucoma, aqueous humor, biomarker, apolipoprotein

Glaucoma is an ocular neurodegenerative disease and is the leading cause of blindness throughout the world.[1] Various factors accelerate the progression of glaucomatous optic neuropathy, and a poor response to treatments aimed at reducing IOP is one of the most important risk factors for blindness in patients with glaucoma. IOP is determined by the balance between the inflow and outflow of the aqueous humor, which includes numerous biologically active factors. We previously reported that the aqueous humor of eyes with open-angle glaucoma (OAG) contained higher levels of multiple cytokines and growth factors compared with nonglaucomatous eyes, as assessed by using the multiplex bead immunoassay.[2] Data suggest that the aqueous humor may be a useful sample for detecting biomarkers and that these factors may be involved in the development and/or progression of glaucomatous optic neuropathy.

Alzheimer's disease (AD) is one of the most common causes of dementia in elderly patients, who may have progressive neurodegenerative disorders. Given the limitations of AD diagnosis and therapy, potential fluid biomarkers of AD, especially in the cerebrospinal fluid (CSF), have been sought, and some of the biomarkers that have been identified were expected to be useful as aids in the diagnosis and treatment of AD.[3] Some of those biomarkers are related to the pathogenesis of AD. For instance, the genotype of apolipoprotein (Apo) E is the well-known risk factor for progression of AD, and Apo E is involved in the aggregation and clearance of amyloid-β, the major component of amyloid plaques which initiate a pathogenic cascade leading to AD.[4] Transthyretin (TTR), an another biomarker of AD, directly interacts with amyloid-β and inhibits its ability to aggregate.[5,6]

Because both AD and glaucoma are age-related progressive neurodegenerative diseases, possible pathologic characteristics that the two diseases may share have been investigated. For instance, some reports found that patients with AD had an increased occurrence of glaucoma.[7–9] Another report indicated that the retinal ganglion cell layer was thinner and that cupping of the optic nerve head was larger in patients with AD compared with age-matched controls.[10] Thus, available evidence suggests a link between AD and glaucoma. Also, aqueous levels of some AD-related biomarkers, such as apolipoproteins and TTR, were reportedly high in eyes with POAG.[11–14] Previous reports thus suggested shared characteristics between AD and glaucoma with regard to fluid biomarkers. However, multiple quantitative analyses of AD-related biomarkers in POAG eyes have not been completed, and such correlations remain to be confirmed. In addition, fewer reports about aqueous levels of these biomarkers in eyes with exfoliation glaucoma (ExG) were published compared with reports about eyes with POAG.

www.iovs.org | ISSN: 1552-5783

TABLE. Characteristics of the Study Patients

Characteristic	Cataract (Nonglaucomaus) Patients	POAG Patients	ExG Patients
Number of patients	38	20	32
Male/female	20/18	15/5	19/13
Age, y			
Mean ± SD	75.5 ± 5.7	71.6 ± 7.3	73.9 ± 7.1
Range	61–83	57–83	56–83
Preoperative IOP, mm Hg			
Mean ± SD	12.4 ± 2.7	26.8 ± 7.3*	26.9 ± 7.4*
Range	7.7–21.0	14.0–45.0	15.0–43.7
MD in Humphrey visual field analysis, dB			
Mean ± SD	Untested	−16.8 ± 9.4	−18.7 ± 8.5
Range	Untested	−29.1 to −2.0	−31.0 to −2.8
Number of glaucoma eye drops			
Mean ± SD	0	2.7 ± 0.7	2.9 ± 0.6
Range	0	1–4	1–4
Duration of glaucoma therapy, mo			
Mean ± SD	0	98.8 ± 83.9	53.1 ± 48.4†
Range	0	13.8–367.1	2.5–185.4

* $P < 0.01$ compared with control (cataract) patients.
† $P < 0.05$ compared with POAG patients.

Here, we report simultaneous increases in the levels of AD-related biomarkers in the aqueous humor samples obtained from eyes with OAG (POAG and ExG). The levels of the analytes correlated strongly with each other and correlated partly with the severity of visual field defects in OAG eyes.

MATERIALS AND METHODS

Patients

This cross-sectional study was approved by the institutional review board of Kumamoto University, Kumamoto, Japan. All procedures conformed to the Declaration of Helsinki, and each patient gave informed consent for participation in the study. Included in this study were glaucomatous patients with ExG or POAG who underwent cataract surgery and/or trabeculectomy. The control group was comprised of cataractous patients without glaucoma who underwent phacoemulsification. Exclusion criteria were as follows: (1) eyes with ocular diseases other than cataract and/or glaucoma and (2) eyes with a history of intraocular surgery including laser treatment. When both eyes of a patient met the inclusion criteria, we included in our analysis only the eye that was treated first.

IOP values were measured by using a Goldmann tonometer during 1 PM and 4 PM, and the mean of three continuous measurements before surgery was said to be the preoperative IOP. In patients with OAG, visual fields were measured with the Humphrey Visual Field Analyzer within 3 months of the objective surgery except for one patient, whose data were unreliable because of severe visual loss.

Collection of the Aqueous Humor Samples

Aqueous humor samples were collected at the beginning of surgery, before any incisional procedures, from 38 cataractous (nonglaucomatous) eyes (patients) and 52 eyes (patients) with phakic OAG (20 patients with POAG and 32 patients with ExG). The Table gives the characteristics of the patients. Of those, 47, 48, and 46 patients received eye drops of β-blockers, prostaglandin analogues, and carbonic anhydrase inhibitors at the time of surgery, respectively. Aqueous humor (70–100 μL) was withdrawn by using a limbal paracentesis with a 30-gauge needle and a tuberculin syringe. Contact with intraocular tissues was carefully avoided, and contamination of samples with blood was prevented. Samples were immediately frozen on dry ice and were stored in a freezer at −80°C until measurements were made by means of a multiplex bead-based immunoassay.

Multiple Immunoassay Analyses

Analyses utilized a multiplex bead-based immunoassay, xMAP, and Human Neurodegenerative Disease Panel 1 (Luminex, Austin, TX), and levels of seven neurodegenerative disease-related proteins in the aqueous humor, Apo AI, ApoCIII, ApoE, TTR, complement factor H, complement component C3, and α2-macroglobulin (α2M), were determined as described previously.[2] Briefly, a 25-μL aliquot of the aqueous humor sample was transferred to a plate, and part of each aliquot was placed into one of the capture microsphere multiplexes. After incubation at 4°C for 18 hours, multiplexed cocktails of biotinylated reporter antibodies were mixed and then incubated at room temperature for 1 hour. Multiplexes were developed by using an excess of streptavidin-phycoerythrin solution. The solution was mixed with each multiplex, after which incubation at room temperature proceeded for 30 minutes. Vacuum filtration was used to reduce the volumes of the multiplexed reactions, and then the volumes were increased by dilution with a matrix buffer. A Luminex 200 instrument (Luminex) was used for analysis, and data were interpreted via proprietary data analysis software (DNASIS Plex version 2.5; Hitachi Software Engineering, Tokyo, Japan).

Data Analysis

Data were analyzed by using the JMP Version 8 statistical package program (SAS Institute, Cary, NC). The unpaired two-tailed *t*-test and χ^2 test were used for comparison of characteristics of patients in the groups. The Tukey-Kramer honestly significant difference test was utilized to compare the levels of aqueous proteins in the groups. Correlations among analytes were assessed by calculating Spearman correlation coefficients. A P value of less than 0.05 was considered statistically significant.

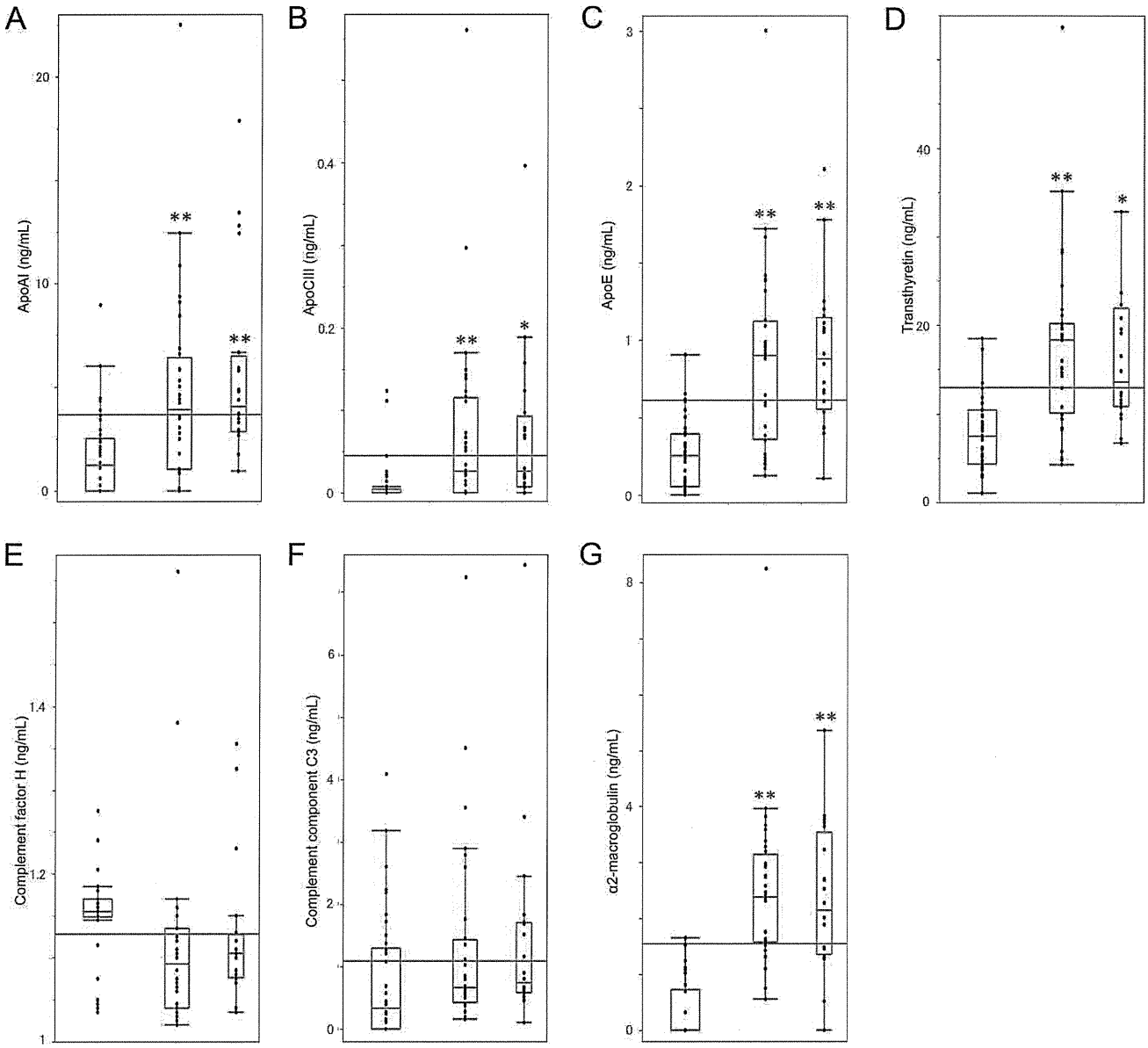

FIGURE 1. Comparison of levels of analytes in eyes of control patients (cataractous; *left columns*), and in eyes with ExG (*middle columns*), or POAG (*right columns*). Distributions of the concentrations of Apo AI (**A**), ApoCIII (**B**), ApoE (**C**), TTR (**D**), complement factor H (**E**), complement component C3 (**F**), and α2-macroglobulin (α2M) (**G**) are presented with the *quantile box plots*. The mean of the total sample is indicated by a *horizontal line* across each plot. The median is indicated by a *horizontal line* in the body of a box. The 75th and 25th quantiles are represented in the *top* and *bottom* of a box. The box covers the interquantile range of the data. The 10th and 90th quantiles are the *lines above* and *below* each box. $^{**}P < 0.01$, $^{*}P < 0.05$.

RESULTS

Increased Multiple AD-Related Protein Levels in the Aqueous Humor Obtained From OAG Eyes

Figure 1 presents the results of AD-related protein measurements. In control (nonglaucomatous) samples, mean values ± SD of Apo AI, ApoCIII, ApoE, TTR, complement factor H, complement component C3, and α2M were 1.65 ± 2.03, 0.01 ± 0.03, 0.25 ± 0.22, 7.67 ± 3.97, 1.15 ± 0.06, 0.76 ± 1.02, and 0.33 ± 0.57 ng/mL, respectively. Compared with the control cases, ExG cases had significantly higher levels of Apo AI (2.86-fold, $P = 0.0028$), ApoCIII (6.51-fold, $P = 0.0088$), ApoE (3.37-fold, $P < 0.0001$), TTR (2.27-fold, $P < 0.0001$), and α2M (7.51-fold, $P < 0.0001$). Corresponding values in POAG cases were also significantly higher than those in control cases (3.55-fold, $P = 0.0003$; 6.26-fold, $P = 0.0360$; 3.58-fold, $P < 0.0001$; 2.07-fold, $P = 0.0002$; and 6.99-fold, $P < 0.0001$, respectively). In contrast, levels of complement factor H and complement component C3 were not different among the groups. Levels of any measured protein in the POAG and ExG groups did not differ significantly.

Multiple Correlations Among AD-Related Protein Levels in the Aqueous Humor of OAG Eyes

Figure 2 shows multiple correlations among proteins measured in the aqueous humor of 52 OAG eyes. Statistical analysis