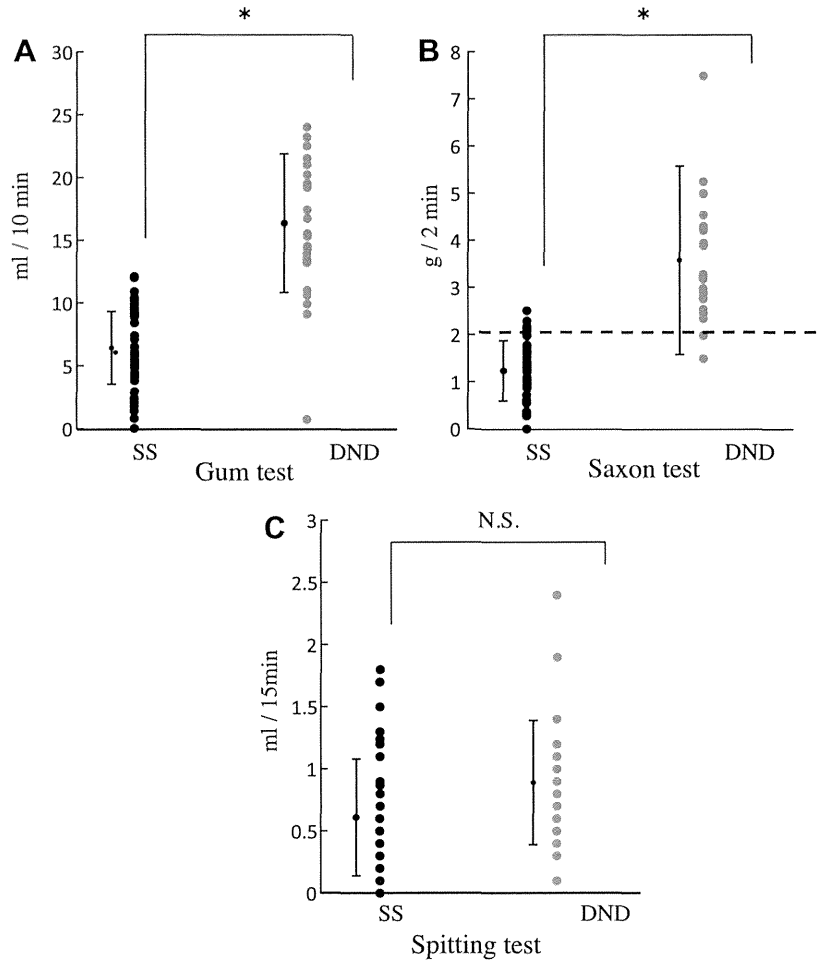

Fig. 2. (A–C) Stimulated and unstimulated salivary flow rates of patients with dry mouth. Results of the stimulated salivary flow rate (SSFR) and unstimulated salivary flow rate (USFR) of SS patients and DND patients. The decrease in SSFR of SS patients was only slightly more than that of DND patients (Mann–Whitney *U*-test, $^{*}p < 0.01$).

Table 2
The frequency of classifications as "decreased" in SSFR and USFR.

	SSFR		USFR
	Gum test	Saxon test	Spitting test
SS	44/50 (88.0%)*	43/50 (86.0%)*	47/50 (94.0%)
DND	3/26 (11.5%)	2/26 (7.7%)	24/26 (92.3%)

χ^2 test.
The definitions of "decreased" by each test is indicated in Section 2. SSFR, stimulated salivary flow rate; USFR, unstimulated salivary flow rate.
* $p < 0.01$.

with that of DND patients (mean: gum test, 16.4 mL/10 min; Saxon test, 3.58 g/2 min) (Mann–Whitney *U*-test, $p < 0.05$, Fig. 2). There was no significant difference in the USFR between SS patients (mean: 0.61 mL/15 min) and DND patients (mean: 0.90 mL/15 min). According to the frequency of the classifications of "decreased" in SS patients and DND patients, the SSFR of SS patients (gum test, 88.0%; Saxon test, 86.0%) was significantly higher than that of DND patients (gum test, 11.5%; Saxon test, 7.7%), but there was no significant difference in the USFR (Tables 2 and 3).

3.3. Correlations among the measurement methods of salivary flow rate

The correlations among the measurement methods of the gum test, Saxon test, and spitting test for patients with dry mouth were investigated. In SS patients, there was a positive correlation between the gum test and Saxon test (Pearson's product–moment correlation coefficient, $p < 0.01$, Fig. 3); there were also positive correlations between the spitting test and gum test, as well as between the spitting test and Saxon test (Pearson's product–moment correlation coefficient, $p < 0.05$, Fig. 3). In DND patients, there was a positive correlation between the gum test and Saxon test (Pearson's product–moment correlation coefficient, $p < 0.05$, Fig. 4). However, a clear correlation was not found between the spitting test and

Table 3
Classification of xerostomia (dry mouth).

Dry mouth caused by salivary gland dysfunction
(1) Sjögren's syndrome
(2) Radiation-induced dry mouth
(3) Dry mouth associated with aging
(4) Graft-versus-host disease (GVHD)
(5) Sarcoidosis
(6) Acquired immunodeficiency syndrome (AIDS)
(7) Malignant lymphoma
(8) Idiopathic xerostomia
Dry mouth associated with neurogenic or neuropsychiatric disorders and drugs
(1) Dry mouth associated with neurogenic or neuropsychiatric disorders
(2) Drug-induced dry mouth
Dry mouth associated with systemic diseases or metabolic disorders
(1) Dry mouth associated with systemic and metabolic diseases
(2) Dry mouth induced by excessive oral vaporization

J. Jpn. Soc. Oral Mucous Membr. 2008;14:86–8.

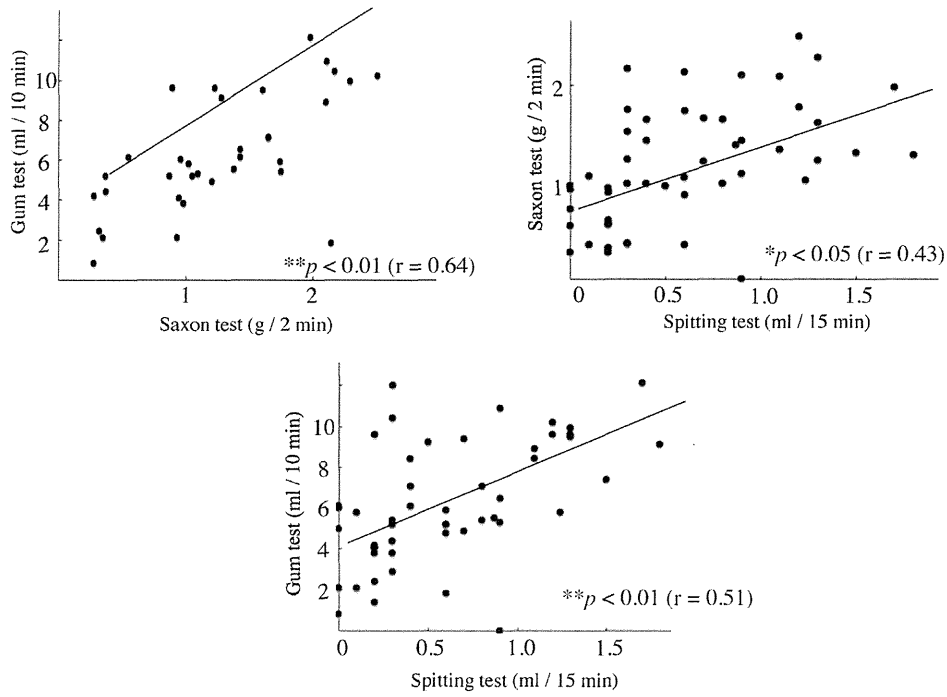

Fig. 3. Correlations among the measurement methods of the salivary flow rate in SS patients. Correlations among the gum test, Saxon test and spitting test in SS patients. Positive correlations were shown between the gum test and Saxon test (Pearson's product–moment correlation coefficient $r = 0.64$, $^{**}p < 0.01$), between the gum test and spitting test ($r = 0.43$, $^{*}p < 0.05$) and between the Saxon test and spitting test ($r = 0.51$, $^{**}p < 0.01$).

gum test, or between the spitting test and Saxon test (Pearson's product–moment correlation coefficient, N.S., Fig. 4).

4. Discussion

Recently, the number of patients with dry mouth has increased. Additionally, it has been reported that increases in social stress and drug use, an aging society, and changes in chewing habits can cause dry mouth [1–10,15–17]. SS is caused by salivary-gland dysfunction, but radiotherapy of the head and neck as well as atrophy due to aging is also important [5,6]. DND is caused mainly by depression, stress and drugs (e.g., antianxiety, antidepressant, antihypertensive). This mechanism of action is by suppression of the salivary secretory nerve system such as the central nervous system (CNS)

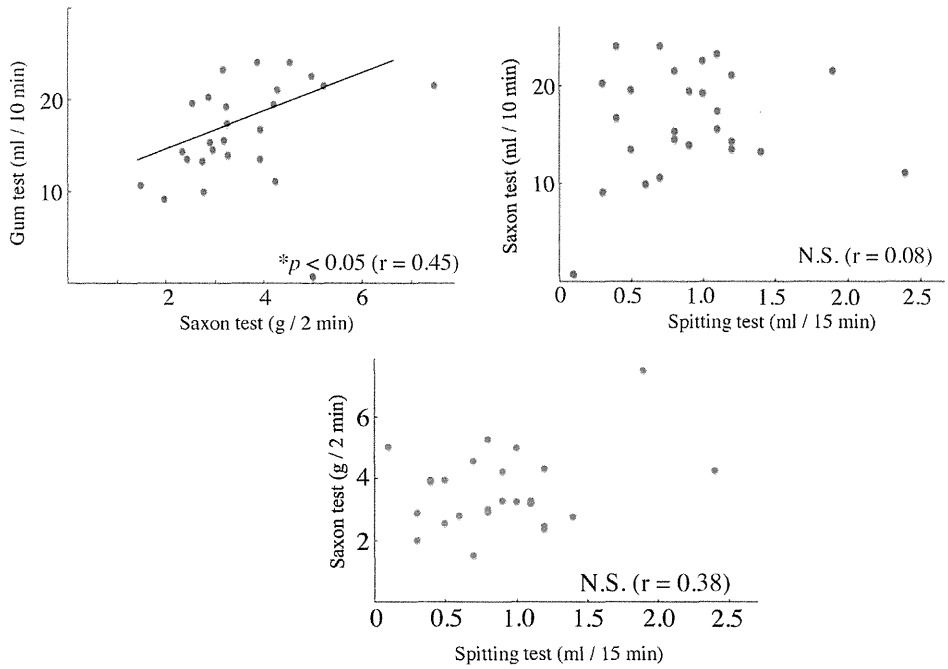

Fig. 4. Correlations among measurement methods of the salivary flow rate in DND patients. Correlations among the gum test, Saxon test and spitting test in DND patients. A positive correlation was shown between the gum test and Saxon test (Pearson's product–moment correlation coefficient $r = 0.45$, $^{*}p < 0.05$), but a correlation was not recognized between the gum test and spitting test ($r = 0.08$, no significant difference) or between the Saxon test and spitting test ($r = 0.38$, no significant difference).

or the salivary nucleus of the facial nervous system [7–9,18,19]. In addition, evaporation of saliva associated with breathing through the mouth, hyperventilation, opening the mouth, and swallowing disorders can also result in dry mouth [20]. These patients are considered to present with xerostomia in the absence of hyposalivation, because the salivary flow rate is less than the rate of fluid loss from the mouth by evaporation and by the absorption of water through the oral mucosa [1]. Therefore, in the present study, we focused on patients with SS and DND who composed a majority of the population with dry mouth.

Another problem is that a definitive test for the diagnosis of dry mouth is lacking. Although it is possible to distinguish SS from DND by all the examinations in the criteria of SS, these examinations can be painful and complicated for patients. For the increasing number of patients with dry mouth, simple methods were needed to understand clinical conditions and help a rapid and more precise diagnosis.

With respect to major complaints, "dryness of eyes" was observed more frequently in SS patients, and "feeling oral pain" was observed more frequently in DND patients. This difference could be used to distinguish DND patients from SS patients, but it is difficult to distinguish them using this difference alone because all patients with dry mouth complained of "xerostomia". In the comparison of the VAS between SS patients and DND patients, three items associated with the diet of DND patients were milder than those of SS patients. These results suggested that the saliva of DND patients was secreted normally at meal times. Thus, a VAS seemed to be useful also in distinguishing DND patients from SS patients.

According to salivary flow rates, the mean volume of the SSFR in SS patients was reduced significantly compared with that of DND patients. Moreover, the comparison between SS patients and DND patients showed that the prevalence of the classification of "decreased" in the SSFR in SS patients was higher than in DND patients, also there was no significant difference in the USFR. Positive correlations among the gum test, Saxon test, and spitting test were found in SS patients, as well as the gum test and Saxon test in DND patients. These results suggest that the decreases in the SSFR and USFR in SS patients were caused by salivary-gland dysfunctions, and that decrease in the USFR in DND patients was by suppression of the CNS and the nervous system in the salivary glands [7–9]. Upon consideration of the normal SSFR in DND patients, these suppressions in DND patients might be broken down by stimulation such as food intake. However, it is still necessary to elucidate the mechanisms underlying drug-induced hyposalivation because different results might be derived depending on the inhibition strength or period of CNS or administration period of the drugs.

Upon perusal of the results of the VAS, SSFR, and USFR, it appears that the decrease in the USFR caused chronic symptoms such as xerostomia and hyposalivation, and that the decrease in the SSFR caused all the complaints except oral pain itemized in the VAS. Oral pain was not correlated with the decrease in the SSFR and USFR, because DND patients might have increased sensitivity for the pain. These findings suggest that the VAS, SSFR, and USFR are available for distinction between DND and SS despite simple methods that can be undertaken readily by general dentists. However, measurements of salivary flow rates often vary depending upon the measurement conditions, and often lack accuracy. Moreover, the gum test is sometimes difficult for patients wearing dentures, and the Saxon test often causes nausea. Considering the positive correlation between the gum test and Saxon test observed in the present study, it is preferable to carry out only one test or the other. Newer test methods include measurement of the USFR by the cotton roll method, measurement of water content in the oral mucosa using a moisture-checking device, and a saliva wetness test using test papers [21]. Previously, we confirmed the correlation and consistency in measurement of the moisture of the tongue surface [21,22] using an oral-moisture checking device (Moisture Checker for Mucus®), of which the accuracy has been established. The test of water content in the oral mucosa using an oral moisture-checking device is simple and could be used in the criteria for the diagnosis of SS. According to our studies of SS [5,23–30], soluble substances such as cytokines and chemokines are produced in the salivary glands and can be detected in saliva. Hence, these substances could be used to diagnose SS in the future.

In order to distinguish DND from SS, the SSFR, USFR, and VAS are useful and simple methods for taking into account both criteria for SS. These tests can be carried out even in general dental clinics. This is very important as the first step to distinguish DND from SS. Subsequently, if a patient is suspected of having SS attends a general dental clinic, he/she can be referred to special facilities to undergo lip biopsy, scintigraphy of the salivary glands or sialography.

Conflict of interest

None declared.

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None.

Author contributions

All authors were involved in drafting the article or revising it, and all authors approved the final version. Dr. Hayashida and Dr. Minami had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analyses. Conception and design of the study was done by Hayashida and Nakamura. Hayashida, Minami and Moriyama took care of data acquisition. Minami, Hayashida and Toyoshima did the analysis and interpretation of the data.

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Review

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

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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

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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

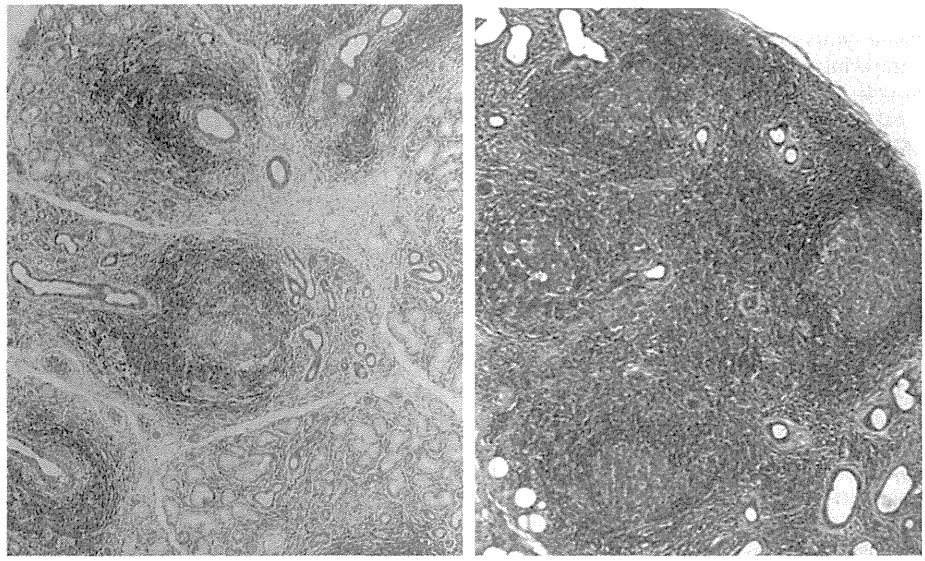

Fig. 1. Histopathological findings in salivary glands from patients with Sjögren's syndrome (SS) and IgG4-related dacryoadenitis and sialoadenitis (IgG4-DS). SS is characterized by periductal lymphocytic infiltration with atrophy or severe destruction of the acini, while IgG4-DS shows non-periductal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

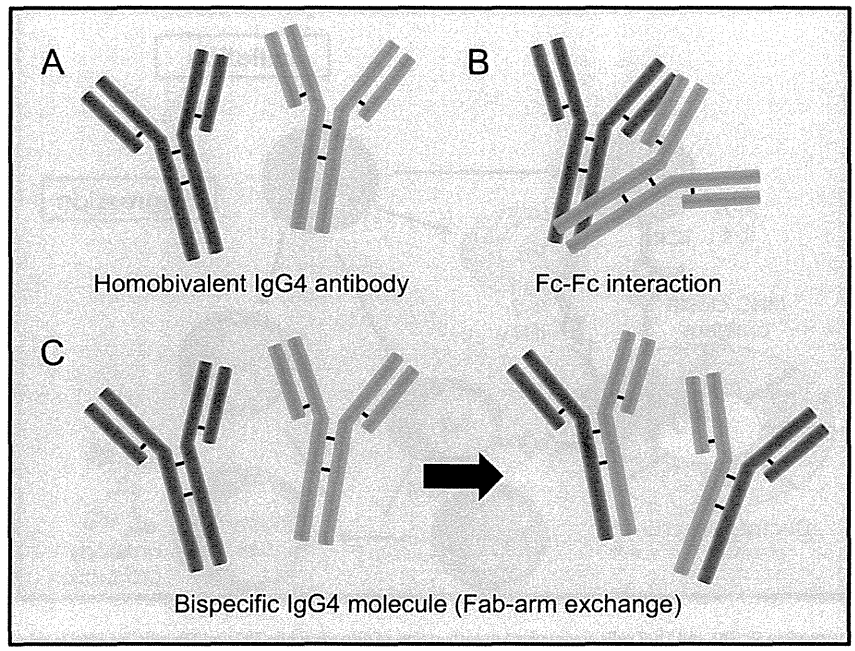

Fig. 2. Unique structure of IgG4 antibody. A, IgG4 antibody consists of two heavy chains and two light chains. B, Fc fragment of IgG4 can interact with the Fc fragment of another IgG4 molecule. C, Exchange of half-molecules (Fab-arm exchange) results in IgG4 combining two different specificities in a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

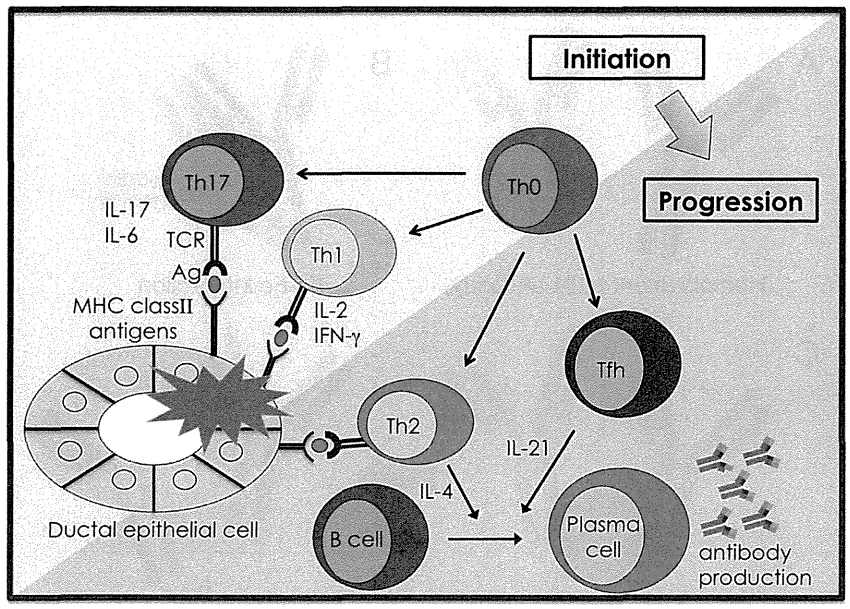

Fig. 3. Schematic model of Th cell network in SS. Th1 and Th17 cells are involved in early stages of disease, while Th2 and Tfh cells are associated with GC formation in the late stage. Abbreviations: Th, T helper; Tfh, follicular helper T.

[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

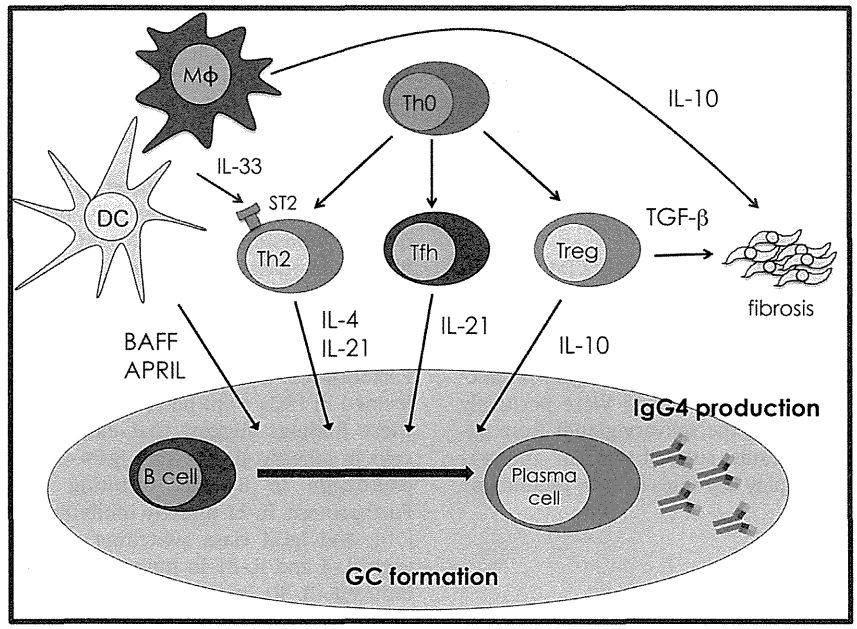

Fig. 4. Schematic model of Th cell and innate immune network in IgG4-DS. Th2, Treg, and Tfh cells play key roles in GC formation and IgG4 production. Dendritic cells and macrophages promote Th2 immune reaction by IL-33 as well as BAFF and APRIL. Abbreviations: Treg, regulatory T; BAFF, B cell activating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

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.

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ORIGINAL ARTICLE

Nationwide epidemiological survey of 169 patients with adult Still's disease in Japan

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Abstract

Objectives. A nationwide survey was conducted to assess the number of patients, clinical aspects, treatment, and prognosis of adult Still's disease (ASD) in Japan.
Methods. A primary questionnaire was sent to randomly selected medical institutions in order to estimate the number of patients. We sent a secondary questionnaire to the same institutions to characterize the clinical manifestations and treatment of ASD.
Results. The estimated prevalence of ASD was 3.9 per 100,000. Analysis of 169 patients showed a mean age at onset of 46 years. The main clinical symptoms were fever, arthritis, and typical rash in agreement with previous surveys. Oral glucocorticoids were used to treat 96% of the patients, while methotrexate was used in 41% and biological agents were used in 16%. Lymphadenopathy and macrophage activation syndrome were significantly associated with increased risk of relapse ($P < 0.05$, each). Patients who achieved remission after tocilizumab therapy had significantly longer disease duration (6.2 years) than patients who did not (1.9 years) ($p < 0.05$).
Conclusions. The 2010–2011 nationwide survey of ASD identified important changes in treatment and improvement of prognosis compared with previous surveys.

Keywords

Adult Still's disease, Ferritin, Methotrexate, Multicenter study, Tocilizumab

History

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Introduction

Adult Still's disease (ASD) was first reported by Bywaters in 1971 [1] as an idiopathic systemic inflammatory disease with three main symptoms: quotidian fevers, arthritis, and evanescent rash. ASD is difficult to diagnose due to the lack of specific clinical manifestations and serum biomarkers. The ASD classification proposed by Yamaguchi et al. [2] in 1992 is used worldwide based on its high sensitivity and specificity. Two major epidemiological surveys were conducted in Japan by the research group of the Ministry of Health and Welfare of Japan in 1988 and 1994 [3,4]. Since the last survey, hyperferritinemia has been added to the Yamaguchi's criteria for reference and this has allowed easier and earlier diagnosis of ASD, and probably changed its clinical manifestations and prognosis. In addition, while ASD is generally treated with glucocorticoids, glucocorticoid-resistant ASD has recently been treated with methotrexate (MTX) or biological agents that are effective against rheumatoid arthritis (RA). Such drugs seem to have improved the course and prognosis of ASD over the past two decades. Indeed, some studies have shown the efficacy of immunosuppressive agents like cyclosporine A (CyA) and biologic agents like tumor necrosis factor (TNF) inhibitors or anti-interleukin (IL)-6 receptor antibody in the treatment of small cohort of patients with ASD [5–7]. Therefore, the research group for autoimmune diseases of the Ministry of Health, Labour and Welfare of Japan conducted another nationwide survey of ASD between 2010 and 2011 to estimate the number of ASD patients in Japan and to assess the clinical manifestations, treatment, course, and prognosis of this disease.

Patients and methods

The survey was performed in two parts: the primary survey was designed to estimate the number of ASD patients treated at medical institutions, while the secondary survey assessed the clinical manifestations of ASD. In the primary survey, we randomly selected medical institutions that were stratified according to the number of beds and posted a questionnaire to the Department of Internal Medicine or Rheumatology about the number of ASD patients treated between January 1 and December 31, 2010.

The diagnosis of ASD depended on physician's judgment. We subsequently sent another questionnaire to the same hospitals in 2011 to obtain detailed information about the patients. To comply with the Personal Information Protection Law in Japan, all information that could identify an individual were made anonymous.

Subjects of the survey included ASD patients aged 16 years or older, who met Yamaguchi's criteria, and attended and/or were admitted to the hospital between January 1 and December 31, 2010. In the secondary survey, clinical information was obtained through a structured interview with the patient, physical

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examination, laboratory tests, and review of medical records. Medications were determined by combining the information provided by patients and medical records. Laboratory data on all parameters were obtained at the time when the maximum serum ferritin was detected. Articular X-rays were taken and reviewed in all patients.

Statistical analysis

Demographic characteristics are presented as mean ± SD (median) for continuous variables and as frequencies and percentages for categorical variables. The associations between serum ferritin level and other variables such as leukocyte count, lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and albumin were examined in ASD patients using Spearman's rank correlation analysis. Comparison of patients who achieved remission with patients who did not achieve remission after treatment with tocilizumab (TCZ), anti-IL-6 receptor antibody, was performed by Mann–Whitney U test, Fisher's exact test, and Cochran–Armitage test. We defined remission as the absence of articular, systemic, and laboratory evidence of disease activity under the current therapy [8]. Logistic regression analysis was employed to assess the association between clinical features and induction therapy with the risk of relapse in patients with ASD. For this analysis, variables of clinical features were age at onset (per 10 years old), gender, typical rash, lymphadenopathy, splenomegaly, disseminated intravascular coagulation (DIC), macrophage activation syndrome, abnormal liver function, and ferritin level (≧3,000 or not). Variables in relation to medications for induction therapy were administration of oral glucocorticoid only, pulse glucocorticoid therapy, MTX, CyA, non-steroidal anti-inflammatory drugs (NSAIDs), and TCZ. For treating missing data, we used the multiple imputation method. Two hundred imputed datasets were generated using the multiple imputation by chained equations method and their results were synthesized using the ordinary Rubin's rule [9]. We also evaluated potential predictive factors which associate with complication of MAS by logistic regressions. All analyses were performed using SPSS for Windows, version 18.0 (IBM Japan Inc., Tokyo, Japan) and R ver. 3.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Primary survey

A total of 7,999 Departments of Internal Medicine and 936 Departments of Rheumatology were subsequently stratified into the following seven categories according to the hospital size and number of beds: university hospitals, hospitals with ≥500 beds (excluding university hospitals), 400–499 beds, 300–399 beds, 200–299 beds, 100–199 beds, and ≤99 beds (Table 1). We randomly selected hospitals from among 100% of the university hospitals, the hospitals with 500 beds or over, and the departments of rheumatology, as well as from among 80% of the hospitals with 400–499 beds, 40% of the hospitals with 300–399 beds, 20% of the hospitals with 200–299 beds, 10% of the hospitals with 100–199 beds, and 5% of the hospitals with 99 beds or less. Among the 8,935 departments, we sent the primary questionnaire to 2,586 departments and we received 500 replies (19%). The reported number of patients was 956 (Table 1). The estimated number of patients with ASD across Japan was calculated by the following formula: Sum of [number of reported patients (C)/Returns (B) X Total number of departments (A) in each category]. The total number of ASD patients in 2011 was estimated to be 4,760 in Japan. According to the census conducted in 2010, the population of Japan on December 1, 2011 was 127,799,000; hence, the estimated prevalence rate of ASD was 3.9 patients per 100,000 people.

Secondary survey

Clinical characteristics

We received 40 replies to the secondary questionnaire survey and obtained clinical information on 169 patients, including 121 females, with a male:female ratio of 1:2.57 (Table 2). The information about 94 (55.6%) out of 169 patients with ASD was obtained from university hospitals in secondary survey. Estimated number of ASD patients in university hospitals was 1,516 (31.8%) out of 4,760 (estimated number of ASD patients in Japan). Thus, prevalence of the patients in university hospitals was higher in the secondary survey than that in the primary survey.

All patients satisfied Yamaguchi's criteria. Eight of the one sixty-nine patients developed ASD when they were at the age of younger than sixteen, while the other one fifty-eight patients had adult-onset ASD. The mean age at onset was 46 ± 19 years (Table 2). Disease duration at presentation of the patients was 0.4 ± 1.6 years (mean ± SD; median: 0.1 year). Observation period of this study was 4.9 ± 4.6 years (mean ± SD; median: 3.3 years). Two (1.3%) out of one fifty-eight patients had a family history of juvenile Still's disease, while none had family history of ASD. Seven (4.5%) out of one fifty-seven patients had family

Table 1. Reported patients in primary survey.

Category by specialty and number of beds	Total number of departments (A)	Surveys for target departments	Returns (B)	Response rate (%)	Number of reported patients with ASD (C)	Estimated number of patients with ASD (D)
Internal medicine						
University hospital	147	147	34	23	122	527
500 beds and over	384	384	60	16	118	755
400–499 beds	321	256	39	15	13	107
300–399 beds	662	265	39	15	7	119
200–299 beds	1023	204	39	19	10	262
100–199 beds	2367	236	55	23	7	301
Under 100 beds	3095	158	35	22	0	0
Subtotal	7999	1650	301	18	277	2072
Rheumatology						
University hospital	48	48	15	31	309	989
500 beds and over	67	67	16	24	79	331
400–499 beds	48	48	10	21	6	29
300–399 beds	67	67	12	18	12	67
200–299 beds	130	130	28	22	257	1193
100–199 beds	270	270	52	19	10	52
Under 100 beds	306	306	66	22	6	28
Subtotal	936	936	199	21	679	2688
Total	8935	2586	500	19	956	4760

ASD adult Still's disease

Table 2. Clinical characteristics of patients with ASD .

	Present study ($n = 169$) Values (Frequency)	Previous survey in 1988 ($n = 90$) Values (Frequency)
General characteristics		
Infant onset:Adult onset	8 (4.8%):158 (95.2%)	NA
Age at onset, years	46 ± 19 (median 46)	NA
Female (%)	121/168 (72.0%)	60/90 (66.7%)
Family history		
Juvenile idiopathic arthritis	2/158 (1.3%)	NA
ASD	0/159 (0.0%)	NA
RA	7/157 (4.5%)	NA
Other autoimmune disease	3/157 (1.9%)	NA
Clinical characteristics		
Fever > 39.0°C, ≧ 1 week	152/166 (91.6%)	71/90 (78.9%)
Arthralgia > 2 weeks	138/166 (83.1%)	90/90 (100.0%)
Arthritis	77/152 (50.7%)	62/86 (72.1%)
Typical rash	102/164 (62.2%)	72/83 (86.7%)
Sore throat	96/162 (59.3%)	58/83 (69.9%)
Lymphadenopathy	72/161 (44.7%)	59/86 (68.6%)
Splenomegaly	52/161 (32.3%)	56/86 (65.1%)
Pericarditis	5/161 (3.1%)	9/87 (10.3%)
Pleuritis	6/161 (3.7%)	11/89 (12.4%)
Interstitial pneumonia	4/161 (2.5%)	NA
Myalgia	42/162 (25.9%)	50/89 (56.2%)
Drug allergy	29/165 (17.6%)	44/82 (53.7%)
Complications		
Amyloidosis	0/127 (0.0%)	NA
DIC	8/127 (6.3%)	NA
Macrophage activation syndrome	19/127 (15.0%)	NA

There were some missing data in the database of this study. Total number of enrolled patients in this study was 169. Information about disease onset and gender was not obtained from 3 patients and 1 patient, each.

DIC disseminated intravascular coagulation, *NA* not applicable

Infant onset was defined as ASD developed at the age of less than sixteen. Adult onset was defined as ASD developed at sixteen years old or older.

history of RA and 3 patients had family history of autoimmune diseases (Graves' disease, $n = 1$; Sjögren's syndrome, $n = 2$) (Table 2). Clinical manifestations detected in the 169 ASD patients were mainly fever (> 39°C for at least 1 week, 91.6%), arthralgia (persisting for at least 2 weeks, 83.1%), and typical rash (62.2%). The features corresponded to the results of previous survey in Japan [3].

Arthritis was found in 44.4% of patients with arthralgia ($n = 138$) (Table 3). The number of ASD patients with monoarthritis, oligoarthritis, and polyarthritis was 3, 33, and 41, respectively. Polyarthritis was the most common in patients with ASD. The involved joints were the wrists (27.0%), knees (27.0%), and shoulders (15.8%) in order of descending prevalence. Articular X-rays of suffered joints were carried out in each patient by the attending physician's decision. Because we assumed that joint destruction did not exist when no articular X-ray was taken in the patients, we count such patients as having no joint destruction. Fifteen patients (11.7%) showed joint destruction, such as bone erosion (11 patients, 8.6%), joint space narrowing (10 patients, 7.8%), and/or ankylosis (2 patients, 1.6%). Three patients showed other radiographic abnormalities (osteoporosis, spur formation at the distal interphalangeal joints, and unspecified changes in 1 case each), while 110 other patients (85.9%) showed no radiographic abnormalities. Of 128 ASD patients with available X-ray findings,

Table 3. Articular manifestations on each joints during 6 months after disease onset in patients with ASD.

	Patients who were assessed by X-ray ($N = 128$)							
Joint	No of Pts with erosion in each joint	Erosion (%)	No of Pts with JSN in each joint	JSN (%)	No of Pts with ankylosis in each joint	Ankylosis (%)	No of Pts who had each joint destruction (erosion or JSN or ankylosis)	each joint destruction (%)
TMJ	0	0.0	0	0.0	0	0.0	0	0.0
Shoulder	0	0.0	1	0.8	0	0.0	1	0.8
SCJ	0	0.0	0	0.0	0	0.0	0	0.0
Elbow	2	1.6	1	0.8	0	0.0	2	1.6
Wrist	7	5.5	7	5.5	1	0.8	10	7.8
Hip	3	2.3	1	0.8	0	0.0	3	2.3
Knee	3	2.3	3	2.3	1	0.8	6	4.7
Ankle	2	1.6	1	0.8	0	0.0	3	2.3
MCP	1	0.8	4	3.1	0	0.0	4	3.1
PIP	3	2.3	6	4.7	0	0.0	8	6.3
DIP	1	0.8	3	2.3	0	0.0	3	2.3
ITJ	0	0.0	1	0.8	0	0.0	1	0.8
MTP	0	0.0	1	0.8	0	0.0	1	0.8

Pts patients, *JSN* joint space narrowing, *No* number, *TMJ* Temporomandibular joint, *SCJ* Sternoclavicular joint, *MCP* Metacarpophalangeal, *PIP* Proximal interphalangeal, *DIP* Distal interphalangeal, *ITJ* Intertarsal joints, *MTP* Metatarsophalangeal

Several joints were affected at the same time in some of the patients.

the radiographic abnormalities of erosion, joint space narrowing, and ankylosis were commonly seen in the wrists, knees, and proximal interphalangeal joints (Table 3). Several joints were affected at the same time in some of the patients. Erosions and joint space narrowing were detected in several joints in 8 patients and in 6 patients, each. Other findings included sore throat (57.3%), lymphadenopathy (44.7%), splenomegaly (32.3%), and drug allergy (17.6%) (Table 1). With respect to complications, 15.8% of the patients had macrophage activation syndrome (MAS) and 6.3% developed DIC, while none of the patients had secondary amyloidosis (Table 2).

Laboratory findings

The results of various laboratory tests are summarized in Table 4. In general, the laboratory tests demonstrated an inflammatory response, with high leukocyte count ($\geq 10{,}000/mm^3$, 79.4%), polymorphonuclear cells ($\geq 80\%$, 71.5%), erythrocyte sedimentation rate (ESR; ≥ 40 mm/hr, 68.9%), and C-reactive protein (CRP; positive CRP, 91.5%), together with liver dysfunction (abnormal liver function tests, 73.9%) and hyperferritinemia (presence of hyperferritinemia, 88.5%). Severe hyperferritinemia (ferritin, $\geq 3{,}000$ ng/mL) was noted in 60% of the patients. Serum rheumatoid factor (RF) and antinuclear antibody were negative in 79.9% and 74.2% of the patients, respectively. Serum IL-6 levels were high in all of the 15 patients tested. Plasma IL-18 levels were also elevated in 11 out of the 12 patients tested (91.7%).

Various factors, such as leukocyte count ($r = 0.289$, $P < 0.001$), AST ($r = 0.561$, $P < 0.001$), LDH ($r = 0.677$, $P < 0.001$), and hypoalbuminemia ($r = -0.445$, $P < 0.001$), correlated significantly with serum ferritin level at time of peak hyperferritinemia.

Treatment

The drugs used to treat 166 ASD patients are listed in Table 5. The most common was oral glucocorticoid, which was used in 160 patients (96.4%), followed by NSAIDs in 73 patients (44.0%). With respect to immunosuppressants, MTX was used in 68 patients (41.0%), followed by CyA in 45 patients (27.1%). Fifty-two patients (31.3%) were treated with glucocorticoid pulse therapy (Table 5, left column). Biological drugs were used on 33 occasions in 27 patients (16.3%) (Table 5, left column, and Table 6). Among them, four patients received two biologic agents (Patients 1, 5, 15, and 24) and one patient received three biologics (Patient 14, Table 6). As induction therapy for ASD ($n = 161$), oral glucocorticoid alone was the most common choice and used in 82 patients (50.9%), among whom 47 (29.2%) were treated with glucocorticoid pulse therapy. MTX was combined with glucocorticoid in 37 patients (23.0%) and CyA was administered with steroids in 30 patients (18.6%) (Table 5, middle column).

With regard to treatment of relapses ($n = 67$), oral glucocorticoid monotherapy was the most common, being used in 28 patients (41.8%). Twelve patients (17.9%) were treated with steroid pulse therapy. MTX was combined with glucocorticoid in 17 patients (25.4%), and CyA was administered with glucocorticoid in 8 patients (11.9%) (Table 5, right column).

Table 6 lists the demographic profiles of 27 patients treated with biologic agents, comprising TNF inhibitors in 12 patients (infliximab in 7 patients, etanercept in 4 patient, and adalimumab in 1 patient) and TCZ in 21 patients. We obtained clinical information on 19 patients out of 21 patients who received TCZ. Nine patients were treated with TCZ for induction therapy; however, only 2 achieved remission and 5 patients stopped TCZ because of adverse events (allergic reaction, hypotension, MAS, rash, and infection). On the other hand, 10 patients were treated with TCZ for maintenance therapy (2 patients) or for relapse (8 patients), among whom 7 patients achieved remission and 1 developed fungal infection. Comparison of clinical features of patients who achieved remission ($n = 9$) with patients who did not achieve remission ($n = 10$) by TCZ indicated significantly longer disease duration (6.2 ± 5.6 years) in the former compared with the latter (1.9 ± 2.8 years, $P = 0.03$). Age, gender, treatment period, and prevalence of patients with oral glucocorticoid or with other immunosuppressant were not related to TCZ-treated patients who did or did not achieve remission.

Clinical outcome

Of the 146 patients with available data on the clinical course, 58 (39.7%) and 50 patients (34.2%) showed monocyclic and polycyclic systemic patterns, respectively, while 15 (10.3%) and 23 patients (15.8%) showed monocyclic and polycyclic systemic

Table 4. Laboratory findings in patients with ASD.

	Present survey in 2011 ($n = 169$) Values (Frequency)	Previous survey in 1988 ($n = 90$) Values (Frequency)
Leukocytosis (Leucocytes ≧ 10,000/μL)	131/165 (79.4%)	80/90 (88.9%)
Glanulocytosis (Neutrophils ≧ 80%)	118/165 (71.5%)	74/89 (83.1%)
Anemia (Hemoglobin ≦ 10 g/dL)	68/169 (40.2%)	53/90 (58.9%)
Thrombocytopenia (Platelets $< 15 \times 10^4$)	23/169 (13.6%)	NA
Elevated ESR (ESR ≧ 40mm/hr)	113/164 (68.9%)	85/89 (95.5%)
Hypoproteinemia[#]	32/169 (18.9%)	NA
Hypoalbuminemia[#]	107/139 (77.0%)	44%
Abnormal liver function[†]	122/165 (73.9%)	74/87 (85.1%)
Positive CRP	151/165 (91.5%)	NA
Hyperferritinemia[*]	146/165 (88.5%)	28/34 (82.4%)
Serum ferritin levels above 3,000 ng/mL	99/165 (60.0%)	NA
Positive RF	33/164 (20.1%)	5/89 (5.6%)
Positive ANA	42/163 (25.8%)	6/88 (6.8%)
Elevation of serum IL-6 (pg/mL)	15/15 (100.0%)	NA
Elevation of plasma IL-18 (pg/mL)	11/12 (91.7%)	NA

ESR erythrocyte sedimentation rate, *CRP* C-reactive protein, *RF* rheumatoid factor, *ANA* anti-nuclear antibodies, *IL-6* interleukin-6, *IL-18* interleukin-18, *NA* not applicable

[†]Abnormal liver function was defined as any elevated liver enzymes (aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase) above the normal range in each medical facility.

[#]Hypoproteinuria and hypoalbuminemia were defined as total protein and serum albumin levels lower than a normal reference value in each medical facility.

[*]Hyperferritinemia was defined as serum ferritin levels higher than a normal reference value in each medical facility.

Table 5. Treatment of patients with ASD.

Medication for ASD ($N = 166$)		Induction therapy for ASD ($N = 161$)		Therapy for relapse of ASD ($N = 67$)	
Drugs	Numbers of patients (%)	Therapy	Numbers of patients (%)	Therapy	Numbers of patients (%)
Medication	163 (98.2%)	Pulse GC therapy	47 (29.2%)	Pulse GC therapy	12 (17.9%)
Pulse GC therapy	52 (31.3%)	GC only	82 (50.9%)	GC only	28 (41.8%)
GC	160 (96.4%)	GC + MTX	21 (13.0%)	GC + MTX	10 (14.9%)
NSAIDs	73 (44.0%)	GC + CyA	20 (12.4%)	GC + CyA	8 (11.9%)
MTX	68 (41.0%)	GC + NSAIDs	9 (5.6%)	GC + TCZ	3 (4.5%)
CyA	45 (27.1%)	GC + MTX + CyA	6 (3.7%)	MTX + TCZ	2 (3.0%)
TAC	12 (7.2%)	GC + MTX + TAC	3 (1.9%)	GC + MTX + NSAID	2 (3.0%)
Salazosulfapyridine	4 (2.4%)	TCZ	3 (1.9%)	GC + MTX + TAC	2 (3.0%)
Mizoribine	3 (1.8%)	GC + MTX + NSAID	2 (1.2%)	Other therapy	10 (14.9%)
Azathioprine	3 (1.8%)	Other therapy	15 (9.3%)		
Cyclophosphamide	2 (1.2%)				
Leflunomide	1 (0.6%)				
Auranofin	2 (1.2%)				
Gold	1 (0.6%)				
Etoposide	1 (0.6%)				
Infliximab	7 (4.2%)				
Etanercept	4 (2.4%)				
Adalimumab	1 (0.6%)				
Tocilizumab	21 (12.7%)				

ASD adult Still's disease, *GC* glucocorticoid, *MTX* methotrexate, *CyA* cyclosporine, *NSAIDs* non-steroidal anti-inflammatory drugs, *TAC* tacrolimus, *TCZ* tocilizumab.

Other therapy means the therapies carried out in only one patient, respectively.

As induction therapy, the group of other therapy includes 5 patients treated with MTX + GC+ some other drugs and 2 patients treated with CyA + GC+ some other drugs. As therapy for relapse, the group of other therapy includes 3 patients treated with MTX + GC+ some other drugs.

patterns with chronic articular involvement, respectively. Furthermore, a self-limited pattern was seen in 11 patients. During the observation period, 66 out of 169 patients (39.1%) experienced relapse. However, there were no deaths during the observation period. At the last medical examination, 145 out of 164 patients (88.4%) had achieved remission.

Relationship between clinical features/induction therapy and risk of relapse

Association of clinical features at baseline and induction therapy with risk of relapse was investigated by logistic regression analysis. Relapse was observed in 66 out of 169 patients with ASD. According to the previous report [10], serum ferritin levels of above 3000 ng/mL in a patient with compatible symptoms should lead to suspicion of ASD in the absence of a bacterial or viral infection. Therefore, we defined cutoff levels of ferritin as more than 3000 ng/ml. Univariate analysis showed that lymphadenopathy (odds ratio [OR] = 1.99, 95% confidence interval [CI]: 1.04–3.78; $p = 0.037$), and complication of MAS (OR = 2.88, 95%CI: 1.04–8.00; $p = 0.043$) were associated with risk of relapse in patients with ASD; however, marked hyperferritinemia (≥3,000 ng/mL), complication of DIC, and medications of induction therapy were not. Multivariate analysis identified lymphadenopathy as the only significant factor for risk of relapse after adjustment for age, sex, other clinical features, and medications for induction therapy (OR = 2.40, 95%CI: 1.08–5.33; $p = 0.032$).

Potential predictive factors for complication of MAS in patients with ASD

We conducted logistic regression analyses to explore potential predictive factors for complication of MAS in ASD. Here, the number of MAS events was small (only 19), so we evaluated univariate associations. As a result, AST (OR = 1.84, 95%CI: 1.24–2.74; $p = 0.003$), LDH (OR = 5.07, 95%CI: 1.98–12.97; $p = 0.001$), and hyperferritinemia (OR = 4.36, 95%CI: 1.30–14.68; $p = 0.017$) were significantly associated with complication of MAS. These factors have been known to be included in laboratory findings of MAS and the strong associations were also observed in the clinical data.

Discussion

According to the 1994 nationwide survey conducted by the research group of the Ministry of Health and Welfare of Japan [4], the prevalence rate of ASD was approximately 2 patients per 100,000 and the male:female ratio was 1:2. The current survey conducted in 2010 showed a prevalence rate of 3.7 per 100,000 people in Japan. The tendency for ASD to show female predominance was still noted. A French retrospective study published in 1995 showed an estimated prevalence rate of ASD of 0.16 cases per 100,000 people and no difference in prevalence between males and females [11]. These results suggest a higher prevalence rate in Japan than that in France. Yamaguchi's classification criteria were used in the 1994 survey, but serum ferritin was not included at that time [4]. The research group of the Ministry of Health and Welfare of Japan assessed the diagnostic value of serum ferritin and concluded that the inclusion of ferritin did not improve the diagnostic accuracy. Hence, serum ferritin is only used for reference in Yamaguchi's criteria. In the present survey, 89% of ASD patients showed hyperferritinemia and serum ferritin level was more than 3,000 ng/mL in 60% of patients. In the 1988 epidemiological survey of ASD, the major clinical manifestations consisted of fever (100%), high fever (81%), arthralgia (100%), typical rash (87%), sore throat (70%), lymphadenopathy (69%), splenomegaly (65%), pleuritis (12%), and pericarditis (10%) (Table 2) [3]. Laboratory findings included leukocytosis (89%), liver dysfunction (85%), negative RF (94%), and negative antinuclear antibody (93%) (Table 4). In the present survey, fewer patients were positive for each of these items (Table 4). Since earlier diagnosis would have been possible due to the wide-spreading knowledge of this disease, the prevalence of clinical and laboratory findings decreased in ASD patients of this study in 2011 compared with the 1988 epidemiological survey of ASD. Also, drug allergy was only found in 18% of the patients in this survey compared with 54% in the 1988 survey (Table 2). Initiation of steroids at an early stage of the disease might have resulted in a decrease in the number of ASD patients with drug allergy.

Recent advances have demonstrated the major role of proinflammatory cytokines, such as IL-6 and IL-18, in the pathogenesis of ASD [12,13]. IL-6 levels are associated with disease activity, and IL-18 levels are thought to be a marker of disease severity and

Table 6. Demographic profile of 27 patients treated with biologic agents.

Patient	Age (years)	Gender	Disease duration (years)	Biologic agent	Therapy	Treatment period (months)	Adverse events	Treatment progress	With GC	With other immunosuppressant
1–1	21	M	0.3	TCZ	Induction therapy	5	Allergy	Withdrawal (adverse event)	Yes	None
1–2	21	M	0.3	ADA	Induction therapy	NA		Ongoing (active)	NA	NA
2	20	M	0.1	TCZ	Induction therapy	10	Hypotension	Withdrawal (adverse event)	No	None
3	47	M	0.1	TCZ	Induction therapy	6		Withdrawal (remission)	Yes	CyA
4	26	F	0.1	TCZ	Induction therapy	1	MAS	Withdrawal (adverse event)	Yes	None
5–1	48	M	0.1	TCZ	Induction therapy	7		Withdrawal (unavailable)	No	None
5–2	48	M	1.2	IFX	Therapy at relapse	9		Ongoing (remission)	Yes	MTX
6	36	F	1.7	TCZ	Induction therapy	38		Ongoing (remission)	No	None
7	48	F	0.2	TCZ	Induction therapy	2	Generalized rash	Withdrawal (adverse event)	No	CyA
8	71	F	5.7	TCZ	Therapy at relapse	12		Ongoing (remission)	Yes	MTX
9	57	F	9.4	TCZ	Therapy at relapse	5		Ongoing (remission)	Yes	None
10	23	M	8.5	TCZ	Therapy at relapse	12		Ongoing (active)	Yes	None
11	78	F	1.8	TCZ	Therapy at relapse	18	Fungal infection	Ongoing (remission)	Yes	None
12	65	F	NA	IFX	Maintenance therapy	NA		NA	Yes	MTX
13	46	M	4.6	TCZ	Maintenance therapy	10		Ongoing (remission)	Yes	MTX
14–1	35	F	1.4	IFX	Induction therapy	17		Withdrawal (unavailable)	Yes	MTX
14–2	35	F	2.8	TCZ	Induction therapy	20	Infection	Withdrawal (adverse event)	Yes	MTX
14–3	35	F	4.8	ETN	Maintenance therapy	3		Withdrawal (unavailable)	Yes	MTX
15–1	34	F	NA	IFX	NA	NA		NA	NA	NA
15-2	34	F	15.1	TCZ	Therapy at relapse	22		Ongoing (remission)	Yes	MTX
16	39	F	NA	TCZ	Therapy at relapse	10		Ongoing (remission)	Yes	TAC
17	72	F	4.3	TCZ	Therapy at relapse	96		Ongoing (active)	None	MTX
18	42	M	1.7	IFX	Maintenance therapy	41		Withdrawal (remission)	Yes	MTX
19	50	F	0.2	TCZ	Induction therapy	8		Withdrawal (economic reason)	Yes	MTX
20	76	M	NA	TCZ	NA	NA		NA	NA	NA
21	32	F	NA	ETN	NA	NA		NA	NA	NA
22	52	M	NA	ETN	Maintenance therapy	NA		Withdrawal (remission)	Yes	CyA
23	60	F	NA	TCZ	NA	NA		NA	NA	NA
24–1	49	F	3.9	TCZ	Therapy at relapse	11		Withdrawal (unavailable)	Yes	MTX
24–2	49	F	4.8	IFX	Therapy at relapse	10		Ongoing (remission)	Yes	MTX
25	50	F	NA	IFX	Maintenance therapy	NA		Ongoing (remission)	Yes	MTX
26	37	F	2.5	ETN	Therapy at relapse	NA		Ongoing (active)	Yes	TAC
27	24	F	7.3	TCZ	Maintenance therapy	40		Ongoing (remission)	Yes	None

M male, *F* female, *GC* glucocorticoid, *MTX* methotrexate, *CyA* cyclosporine A, *TAC* tacrolimus, *ETN* etanercept, *IFX* infliximab, *ADA* adalimumab, *TCZ* tocilizumab, *MAS* macrophage activation syndrome, *NA* not applicable.

Patient 1 was treated with TCZ and ADA. Patients 5, 15, and 24 were treated with TCZ and IFX. Patient 14 was treated with IFX, TCZ, and ETN.

MAS in patients with ASD [12,13]. Serum IL-6 and plasma IL-18 were elevated in patients with ASD in this study, despite the small number of patients tested for these parameters.

Pouchot et al. [8] reported that 41% (16 of 39 patients) of ASD patients had abnormal X-ray findings of joint space narrowing at the carpometacarpal or intercarpal joints of the wrist, which progressed to ankylosis in 25% of the patients. In the present study, 12% of patients exhibited radiographic abnormalities (erosion, 9%; joint space narrowing, 8%), and only 2% showed ankylosis. With respect to the radiographic joint damage, the prognosis of joints in ASD seems to be relatively better than that before 1990s. A study of 90 ASD patients in 1990 reported that the polycyclic systemic pattern was the most common, being seen in 41% of patients, while more than half of the patients experienced relapse (55%) and 4 patients died (4%) [3]. At that time, glucocorticoid and NSAIDs were used for treating 92% and 79% of the patients, respectively, while only 10% were treated with immunosuppressants (cyclophosphamide and azathioprine) and neither MTX nor CyA was used. In the present survey, the monocyclic systemic pattern was the most common (in 40% of the patients), while 39% of patients experienced relapse and none of the patients died. The majority of the large observational studies of ASD were performed before MTX became widely used and before the marketing of biologic agents. The long-term benefits of MTX in limited joint destruction have been demonstrated in RA but not in ASD. One possible reason for the improved prognosis is that ASD was better controlled by steroid therapy combined with immunosuppressants (MTX or CyA) or biological agents, all of which have become available after the previous survey. ASD patients occasionally develop severe complications such as MAS or reactive hemophagocytotic syndrome (RHS). According to a retrospective study of 50 patients with ASD, 6 patients (12%) experienced RHS [14]. Another retrospective observational study showed that RHS was complicated in 8 out of 57 patients (14.0%) in ASD patients [15]. In our survey, 16% of patients developed MAS or RHS, which was similar to that in the previous reports. We evaluated potential predictive factors which were associated with complication of MAS in ASD. In our study patients with ASD, AST, LDH, and hyperferritinemia were associated with complication of MAS. Although the above factors have been already known to be included in laboratory findings of MAS and might not be novel findings, the information obtained in this epidemiological study would be meaningful. Secondary amyloidosis was also identified as a complication of ASD in previous surveys, but was not recognized in any of the patients in the present survey [2,15,16]. This could be due to early treatment, which resulted in inhibition of production and deposition of amyloid proteins associated with chronic inflammation.

Glucocorticoids were the most commonly used medications on the treatment of ASD. Furthermore, glucocorticoid pulse therapy, DMARDs, or biologic agents were added to control the disease based on the degree of disease activity and severity. The prognosis of ASD is known to be relatively good; however, 39% of the patients with ASD experienced relapse while on therapy or after discontinuation of treatment during the observation period (the mean observation period between the first and last examinations was 4.9 years in this survey). We investigated whether clinical features and induction therapy were associated with risk of relapse. Previous studies reported significant correlation between hyperferritinemia and disease activity, and recommended the use of hyperferritinemia as a marker to monitor the response to treatment in ASD [17,18]. Analysis of our survey data showed no relation between serum ferritin levels above 3,000 ng/mL and risk of relapse. Furthermore, pulse glucocorticoid therapy, and MTX, CyA, NSAIDs, and TCZ for the induction therapy did not reduce the risk of relapse. Univariate analysis showed that lymphadenopathy and MAS were associated with increased risk of relapse in patients with ASD, suggesting that these two complications could be considered as risk factors for relapse in patients with ASD.

Twenty-one ASD patients in the present survey were treated with TCZ, and clinical information was available for 19 of these 21 patients. Patients who achieved remission after treatment with TCZ had longer duration of disease compared with those who did not. A few studies have reported the efficacy of TCZ in refractory ASD [6,7]. Furthermore, 94% of 35 patients with ASD reported in the literature were resistant to other immunosuppressive agents, such as MTX, TNF blockers, and anakinra. TCZ induced remission and allowed reduction of the dose or discontinuation of corticosteroids [19]. Based on the above results and those of the present survey, we recommend the use of TCZ for treatment in patients with refractory and long-standing ASD.

There have been some papers about successful treatment experience with TCZ in patients with ASD [6,7,20,21]. MAS is a life-threatening syndrome with excess immune activation. MAS occurs either in ASD or in systemic juvenile idiopathic arthritis (sJIA) [14,22]. Major findings are fever, hepatosplenomegaly, rash, lymphadenopathy, neurologic symptoms, cytopenias, hyperferritinemia, and abnormal liver function. The propriety of TCZ therapy in MAS complicated with ASD patients has been controversial. In some cases of ASD and sJIA, MAS aggravated during TCZ therapy [6,19,21,23]. Although the contribution of TCZ to occurrence of MAS has not been determined, careful observation should be required during TCZ therapy in patients with active ASD.

Conclusion

We conducted a nationwide survey of ASD, which showed changes in treatment and improvement of prognosis compared with previous surveys. Our findings suggest that lymphadenopathy and MAS are potential risk factors for relapse in patients with ASD. The use of immunosuppressants like MTX or CyA and biologics for ASD has increased in recent years. We also recommend the use of TCZ for treatment of relapse in patients with long-standing ASD.

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Conflict of interest

None.

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