Clinical Immunology (2015) **156**, 9–18

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

Preferential M2 macrophages contribute to fibrosis in IgG4-related dacryoadenitis and sialoadenitis, so-called Mikulicz's disease

Sachiko Furukawa [a], Masafumi Moriyama [a,*], Akihiko Tanaka [a], Takashi Maehara [a], Hiroto Tsuboi [b], Mana Iizuka [b], Jun-Nosuke Hayashida [a], Miho Ohta [a], Takako Saeki [c], Kenji Notohara [d], Takayuki Sumida [b], Seiji Nakamura [a]

[a] *Section of Oral and Maxillofacial Oncology, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University, Fukuoka, Japan*
[b] *Department of Internal Medicine, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan*
[c] *Department of Internal Medicine, Nagaoka Red Cross Hospital, Nagaoka, Japan*
[d] *Department of Anatomic Pathology, Kurashiki Central Hospital, Kurashiki, Japan*

Received 20 August 2014; accepted with revision 21 October 2014
Available online 29 October 2014

KEYWORDS
IgG4-related dacryoadenitis and sialoadenitis;
M2 macrophage;
Fibrosis;
IL-10;
CCL18

Abstract IgG4-related dacryoadenitis and sialoadenitis (IgG4-DS) is characterized by bilateral swelling of glandular tissues with extensive fibrosis, and is immunologically considered a Th2-predominant disease. Recent studies reported that alternatively activated (M2) macrophages enhanced Th2 immune responses and fibrosis by production of pro-fibrotic factors (IL-10, IL-13 and CCL18). Therefore, we examined the association between M2 macrophages and fibrosis in submandibular glands from 7 patients with IgG4-DS, 10 patients with chronic sialoadenitis, 10 patients with Sjögren's syndrome, and 10 healthy subjects. The number of M2 macrophages in SMGs from patients with IgG4-DS was also significantly higher than in the other groups. Double immunofluorescence staining showed that IL-10 and CCL18 expression co-localized with M2 macrophage-marker (CD163). Furthermore, the SMG fibrosis score was positively correlated with the frequency of M2 macrophages in only IgG4-DS. These results indicate that IL-10 and CCL18 secreted by preferential M2 macrophages possibly play a key role in the development of severe fibrosis in IgG4-DS.

Abbreviations: MD, Mikulicz's disease; SMG, Submandibular gland; LG, lacrimal gland; SS, Sjögren's syndrome; IgG4-RD, IgG4-related disease; IgG4-DS, IgG4-related dacryoadenitis and sialoadenitis; Th2, helper T type 2; eGC, ectopic germinal center; OSCC, oral squamous cell carcinoma; CS, chronic sialoadenitis; MT, Masson's trichrome; Treg, regulatory T cell.

* Corresponding author at: 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. Fax: +81 92 642 6386.
E-mail address: moriyama@dent.kyushu-u.ac.jp (M. Moriyama).

http://dx.doi.org/10.1016/j.clim.2014.10.008

1. Introduction

Mikulicz's disease (MD), first reported by Mikulicz in 1888 [1], is characterized by firm swelling of submandibular glands (SMGs) and lacrimal glands (LGs), and has been considered to be a subtype of Sjögren's syndrome (SS) because of these histopathological similarities [2]. However, Yamamoto et al. reported that patients diagnosed with MD also had high serum levels of IgG4 and marked infiltration of IgG4-positive plasma cells in salivary glands [3]. Moreover, several reports also demonstrated that these findings were accompanied by autoimmune pancreatitis (AIP) [4], sclerosing cholangitis (SC) [5], tubulointerstitial nephritis (TIN) [6], interstitial pneumonia [7], Hashimoto's thyroiditis [8] and Küttner tumor [9]. These diseases are now collectively called "IgG4-related disease (IgG4-RD)" and we have described this concept and provided up-to-date information regarding this emerging disease entity in a recent review [10]. Furthermore, recent studies have also referred to MD as IgG4-related dacryoadenitis and sialoadenitis (IgG4-DS) [11].

Regarding the immunological aspect of IgG4-RD, it is well known that IgG4 is induced by T helper type 2 cell (Th2) cytokines such as interleukin (IL)-4 and IL-13. We previously reported that analysis of peripheral $CD4^+$ Th cells from patients with IgG4-DS revealed a deviation in the Th1/Th2 balance favoring Th2 [12] and that Th2 cells play a key role in the production of IgG4 and formation of ectopic germinal centers (eGCs) [13,14]. In addition, Watanabe et al. reported that abnormal immune responses might enhance the Th2 response via Toll-like receptors expressed by macrophages, contributing to the immunopathology of IgG4-RD [15]. Recent studies reported that rituximab targeting CD20 plasma cells appeared to be an effective treatment strategy for IgG4-RD, but plasmablasts lack surface expression of CD20 and thus demonstrate a resistance to direct depletion by rituximab and IgG4-positive plasmablast. Macrophage might play an effective role in IgG4 production produced by IgG4-positive plasmablast. Recently, innate immune system such as macrophages has received a lot of attention to the initiation of IgG4-RD [16]. At least two distinct subtypes of macrophages have been identified; the classically activated (M1) macrophage stimulated by Th1 responses and the alternatively activated (M2) macrophage stimulated by Th2 responses. M2 macrophages contribute to angiogenesis, suppression of adaptive immunity, and wound healing and fibrosis [17,18]. Histologically, the critical features of IgG4-RD are severe fibrosis with dense lymphoplasmacytic infiltration of the salivary glands and other lesion [19]. However, to our knowledge, no published reports have investigated the mechanism promoting severe fibrosis in IgG4-RD. In this study, we examined the distribution of macrophage subsets and the expression of pro-fibrotic factors in salivary glands to clarify the contribution of macrophages to the pathogenesis of IgG4-DS.

2. Material and methods

2.1. Patients

SMG samples were collected from 7 patients with IgG4-DS (five men and two women; mean age ± standard deviation

Table 1 Clinical characteristics of 7 patients with IgG4-related dacryoadenitis and sialoadenitis (IgG4-DS).

No.	Age	Sex	Disease duration	Complications	Swollen glands					Complaint		Histological findings		Serological test							
					LG	PG	SMG	SLG	PLG	LSG	Dry eye	Dry mouth	IgG4/IgG (%)	$IgG4^+$ cells (/HPF)	RF (U/ml)	ANA	IgG (mg/dl)	IgG4 (mg/dl)	IgA (mg/dl)	IgE (IU/ml)	IgM (mg/dl)	Anti-SS-A (U/ml)	Anti-SS-B (U/ml)
1	58	F	6 M	–	–	–	+	+	–	–	–	–	***73.0***	***28***	4	***80***	1188	***151***	193	***178***	56	–	–
2	68	F	9 M	AIP, TIN	–	–	+	+	–	+	–	–	***52.3***	***42***	5	***160***	***6758***	***1500***	78	13	81	–	–
3	39	M	2 Y	–	+	–	+	–	–	+	–	–	***64.2***	***77***	5	–	1534	***188***	170	***1619***	99	–	–
4	69	M	3 M	HT	+	–	+	+	–	+	–	–	***61.2***	***32***	ND	–	***1662***	***458***	97	60	79	–	–
5	74	M	4 M	AIP, Prostatitis	+	–	+	+	–	+	–	–	***50.0***	***85***	ND	40	***4217***	***524***	177	29	60	–	–
6	55	M	3 Y	AIP, IP	+	–	+	+	–	–	–	–	***70.0***	***18***	4	–	***2092***	***510***	148	ND	70	–	–
7	69	M	4 M	AIP	–	–	+	+	–	+	–	–	***63.2***	***79***	ND	–	***1675***	***484***	229	***283***	44	–	–

Abbreviations: LG, lachrymal gland; PG, parotid gland; SMG, submandibular gland; SLG, sublingual gland; PLG, palatine gland; LSG, labial salivary gland; TIN, tubulointerstitial nephritis; HT, higher tension; AIP, autoimmune pancreatitis; IP, interstitial pneumonitis; –, negative; ND, not done; bold italic means higher than normal values.