Pancreatology 14 (2014) 193–200

Contents lists available at ScienceDirect

Pancreatology

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

Original article

The role of $CD19^{+}CD24^{high}CD38^{high}$ and $CD19^{+}CD24^{high}CD27^{+}$ regulatory B cells in patients with type 1 autoimmune pancreatitis

Kimi Sumimoto, Kazushige Uchida, Takeo Kusuda, Toshiyuki Mitsuyama, Yutaku Sakaguchi, Toshiro Fukui, Mitsunobu Matsushita, Makoto Takaoka, Akiyoshi Nishio, Kazuichi Okazaki*

The Third Department of Internal Medicine, Division of Gastroenterology and Hepatology, Kansai Medical University, 2-5-1 Shinmachi, Hirakata, Osaka 573-1191, Japan

ARTICLE INFO

Article history:
Received 9 November 2013
Received in revised form
23 January 2014
Accepted 16 February 2014
Available online 25 February 2014

Keywords:
Type 1 autoimmune pancreatitis (AIP)
Regulatory B cells (Bregs)
Interleukin-10 (IL-10)

ABSTRACT

Background: Patients with type 1 autoimmune pancreatitis (AIP) have several immunologic and histologic abnormalities. It is known that depletion of B cells by rituximab is effective for treatment of IgG4-related disease (IgG4-RD) such as type 1 AIP, suggesting that B cells may be a key player in IgG4-RD. However, the role of regulatory B cells (Bregs) in type 1 AIP is unclear, and the objective of this paper is to clarify the role of Bregs in the pathophysiology of type 1 AIP by analyzing circulating Bregs.

Method: We recruited 21 patients with type 1 AIP as determined by the International Consensus Diagnostic Criteria for AIP (ICDC). No patients received corticosteroid treatments. For comparison, we recruited 14 patients with chronic pancreatitis (CP), 20 patients with pancreatic cancer, and 25 healthy subjects as controls. We analyzed Bregs as $CD19^{+}CD24^{high}CD38^{high}$ and $CD19^{+}CD24^{high}CD27^{+}$ from peripheral blood by flow cytometry.

Results: In peripheral blood, $CD19^{+}CD24^{high}CD38^{high}$ Bregs were significantly increased in type 1 AIP patients compared with CP, pancreatic cancer, and healthy controls. Although not significant different, $CD19^{+}CD24^{high}CD27^{+}$ Bregs of type 1 AIP were decreased compared to those of other groups. IL-10^{+} B cells were not significantly different from type 1 AIP patients and healthy controls. In untreated type 1 AIP patients, the number of $CD19^{+}CD24^{high}CD38^{high}$ Bregs and IgG4 were not correlated.

Conclusions: Our data suggested that $CD19^{+}CD24^{high}CD38^{high}$ Bregs seemed to increase reactively to suppress the disease activity, and are consistent with the hypothesis that $CD19^{+}CD24^{high}CD27^{+}$ Bregs might be involved in the development of type 1 AIP, although it still remains unclear whether the decrease of $CD19^{+}CD24^{high}CD27^{+}$ cells is cause or effect of AIP.

1. Introduction

Autoimmune pancreatitis (AIP) is a particular type of chronic pancreatitis due to autoimmune mechanism. In 1961, Sarles et al. [1] first observed a case of particular pancreatitis with hypergammaglobulinemia. In 1995, Yoshida et al. [2] proposed AIP as a new clinical entity. In 2001, Hamano et al. [3] reported that patients with AIP had high serum immunoglobulin G4 (IgG4) concentrations. In 2003, Kamisawa et al. [4] suggested that AIP was a systemic disease, based on the findings that the pancreas and other involved organs have abundant infiltration of IgG4-positive plasma cells. Many cases of AIP have been reported in Asia, Europe, and the United State [5–12]. There are several diagnostic criteria in each countries and areas. The diagnosis of AIP must be made on the presence of a unique set of characteristics [13–18]. IgG4-related AIP is characterized by irregular narrowing of the main pancreatic duct, enlargement of the pancreas, high serum levels of IgG4, other organ involvement, and steroid responsiveness. Recent studies suggested the existence of two subtypes of AIP; (a) type 1 AIP related with IgG4 showing lymphoplasmacytic sclerosing pancreatitis (LPSP) [19], and (b) type 2 AIP related with a granulocytic epithelial lesion (GEL) [20–22] showing idiopathic duct-centric chronic pancreatitis (IDCP) [23–25]. In 2011, the International Association of Pancreatology (IAP) proposed the

* Corresponding author. Tel.: +81 72 804 0101x2520; fax: +81 72 804 2061.
E-mail address: okazaki@hirakata.kmu.ac.jp (K. Okazaki).

http://dx.doi.org/10.1016/j.pan.2014.02.004
1424-3903/

International Consensus Diagnostic Criteria (ICDC) for AIP [26]. In the ICDC, AIP was classified into type 1 and type 2. Most of the Japanese AIP cases are type 1, whereas those concerning type 2 are very few. Japanese consensus clinical guidelines have focused on type 1 AIP [27].

We previously reported that induced $CD4^{+}CD25^{high}$ regulatory T cells (Tregs) were significantly increased and naïve Tregs were significantly decreased in the peripheral blood of the patients with type 1 AIP [28]. We suggest that decreased naïve Tregs may be pathogenetic. We also previously reported that Tregs were increased in the pancreas with type 1 AIP compared with control [28]. In the patients with type 1 AIP, the numbers of infiltrated Tregs were significantly positively correlated with IgG4-positive plasma cells. In type 1 AIP, inducible co-stimulatory molecule $(ICOS)^{+}$ and interleukin-10 $(IL\text{-}10)^{+}$ Tregs significantly increased compared with control groups [29]. The ratio of Foxp3-positive cells to infiltrated mononuclear cells and IgG4-positive cells to infiltrated mononuclear cells were positively correlated. We suggest that increased quantities of $ICOS^{+}$ Tregs may influence IgG4 production via IL-10 in type 1 AIP.

Recently, it has been reported that IL-10-producing B cell [30], T cell [28], dendritic cells (DCs) [31] and macrophage [32] are regulatory cells. In our previous studies, Miyoshi et al. [28] reported IL-10-producing Tregs in type 1 AIP and Hoshino et al. [33] reported IL-10-producing DCs in colitis models. Regarding B cells, great attention has been focused on relations between various diseases and IL-10-producing B cells, originally termed as regulatory B cells (Bregs) in mice by Mizoguchi et al. [30,34–38]. B cells terminally differentiate into plasma cells that are long-lived and secrete pathogenic antibody, promote immune responses by presenting antigen and providing co-stimulatory signals, and suppress immune responses though the production of IL-10 [39]. Recent studies suggest that human Bregs, in addition to mice Bregs, may be involved in the development of various autoimmune diseases such as systemic lupus erythematosus (SLE) [38], immune thrombocytopenia (ITP) [35], and sarcoidosis [36]. Recently, two novel phenotypes of human Bregs, $CD19^{+}CD24^{high}CD38^{high}$ [38] and $CD19^{+}CD24^{high}CD27^{+}$ [40], both of which can secrete IL-10 depending on various stimulations in vitro, have been identified. In IgG4-related disease (IgG4-RD), depletion of B cells by rituximab is effective for treatment [41]. However, it is unclear if Bregs are involved in the development of type 1 AIP. In the present study, to clarify the role of Bregs in the pathophysiology of type 1 AIP, we analyzed circulating Bregs.

2. Methods

2.1. Patients and controls

Peripheral blood was obtained from 21 untreated patients with type 1 AIP, diagnosed according to the ICDC for AIP proposed by the IAP [26], at Kansai Medical University and its affiliated hospitals (7 women and 14 men; mean age 67.0 years; range, 40–78 years). Twenty of the 21 patients were diagnosed as definitive type 1 AIP, and one patient was diagnosed as probable type 1 AIP. Fourteen patients with alcoholic or idiopathic pancreatitis (8 women, 6 men; mean age, 65.9 years; range, 48–79 years), 20 patients with pancreatic cancer (7 women, 13 men; mean age, 71.3 years; range, 59–86 years), and 25 healthy volunteers (7 women, 18 men; mean age, 65.6 years; range, 39–81 years) served as controls (Table 1). There was no difference in age and sex between type 1 AIP, chronic pancreatitis (CP), pancreatic cancer, and healthy controls (Table 1). IL-10 and each phenotype of Bregs were examined in some of the enrolled subjects; 16 out of 21 type 1 AIP patients and 18 out of 25 healthy volunteers for $CD19^{+}CD24^{high}CD38^{high}$ Bregs group, and 12 out of 13 type 1 AIP patients and 10 healthy volunteers for $CD19^{+}CD24^{high}CD27^{+}$ Bregs group. All the patients with CP were diagnosed according to the clinical diagnosis criteria of the Japan Pancreatic Society (JPS) [42]. Fifteen patients were histologically confirmed as having adenocarcinoma of the pancreas during surgery or by endoscopic ultrasonography-guided fine-needle aspiration (EUS-FNA), cytology of pancreatic juice, brushing cytology of pancreatic duct, bile cytology, or bile duct biopsy. One patient with peritoneal dissemination of pancreatic cancer and 3 patients with metastatic liver cancer were diagnosed as pancreatic cancer by the ascites cytology and liver biopsy. One patient was diagnosed as pancreatic cancer based on the clinical course. This study was approved by the Kansai Medical University's ethics committee, and all patients and healthy volunteers gave informed consents.

Serum IgG4 was significantly increased in type 1 AIP patients (mean 391.8 mg/dl, range 93.1–1010 mg/dl) compared with CP (mean 62.0 mg/dl, range 6.4–147 mg/dl), pancreatic cancer (mean 55.1 mg/dl, range 10.5–244 mg/dl) (Table 1). In this study, one group was studied $CD19^{+}CD24^{high}CD38^{high}$ Bregs phenotype and another group was studied $CD19^{+}CD24^{high}CD27^{+}$ Bregs phenotype, independently. There was no difference of age, sex, count of B cells, and serum IgG4 in each group of type 1 AIP, CP, pancreatic cancer, and healthy controls between $CD19^{+}CD24^{high}CD38^{high}$ Bregs group and $CD19^{+}CD24^{high}CD27^{+}$ Bregs group (Table 2).

2.2. Flow cytometry of Bregs in peripheral blood cells

Flow cytometry was performed according to the method described previously [28,29,43]. Briefly, cells were stained with fluorescein isothiocyanate (FITC)-conjugated anti-CD38 (eBioscience, San Diego, CA), FITC-conjugated anti-CD27 (eBioscience), phycoerythin (PE)-conjugated anti-CD24 (eBioscience), and peridinin chlorophyll protein (PerCP)-cyanine (Cy)5.5-conjugated anti-CD19 (eBioscience) at 4°°C for 30 min. After staining, the cells were fixed in 1% paraformaldehyde, and 3 color flow cytometric analyses were performed using FACS-Caliber (BD Biosciences, Franklin Lakes, NJ).

Table 1
Clinical profiles and characteristics of the patients and control groups.

	Type 1 AIP	CP	Pancreatic cancer	Healthy controls
N	21	14	20	25
Age (years)[a]	67.0 ± 10.6 (40–78)	65.9 ± 9.07 (48–79)	71.3 ± 7.34 (59–86)	65.6 ± 11.0 (39–81)
Sex (Female/Male)	7/14	8/6	7/13	7/18
Serum IgG4 (mg/dl)[a]	391.8 ± 281.9 (93.1–1010)	62.0 ± 43.1* (6.4–147)	55.1 ± 55.7* (10.5–244)	N.D.

Serum IgG4 were significantly increased in patients with type 1 AIP compared with CP and pancreatic cancer. *$P < 0.01$.
There was no difference of age and sex between type 1 AIP, CP, pancreatic cancer, and healthy controls.
AIP: autoimmune pancreatitis, CP: chronic pancreatitis.
[a] Values are given as the mean ± standard deviation (SD), with the range in parenthesis.