Table 1. Characteristics of the members of the expert panel*

Total	42
Specialty	
Gastroenterology	18
Rheumatology	13
Ophthalmology	3
Pulmonary–critical care	2
Hematology/oncology	2
Internal medicine	2
Nephrology	1
Endocrinology	1
Country	
Japan	24
US	9
Italy	2
Canada	1
UK	1
Germany	1
France	1
Sweden	1
South Korea	1
China	1
Years of experience with IgG4-RD patients	
<2	1
2–5	9
>5–10	11
>10	21
No. of IgG4-RD patients cared for	
5–10	5
>10–50	13
>50–100	9
>100	15

* Values are the number of individuals. IgG4-RD = IgG4-related disease.

retroperitoneum, and lymph nodes (4,5). The epidemiology of the disease remains poorly described, because of both the relative novelty of this diagnosis and continued underrecognition, but the medical literature pertaining to the diagnosis has shown a major expansion over the past 5 years, from 63 publications in 2008 to 729 in 2013. The disease is often mistaken for cancer, an infection, or another immune-mediated condition, e.g., Sjögren's syndrome, granulomatosis with polyangiitis (Wegener's), giant cell arteritis, and others.

Multiple approaches to the management of IgG4-RD have been reported, including surgical resection of affected tissues and treatment with systemic glucocorticoids, "steroid-sparing" immunosuppressive drugs, or biologic agents, but no randomized clinical trials have been conducted and there are no formal treatment guidelines. In preparation for the Second International Symposium on IgG4-RD and Associated Conditions (February 16–19, 2014), we assembled an international panel of experts to develop recommendations for the management of IgG4-RD. This consensus process involved a series of web-based questionnaires, face-to-face discussions, and a literature review.

Methods

Expert panel. Forty-two IgG4-RD experts were invited to participate by the Symposium Organizing Committee. The experts were selected because of their history of publication on IgG4-RD and their recognized clinical expertise pertaining to the condition. The goals in assembling the panel were to ensure that all organ systems and body regions frequently affected by IgG4-RD were represented by experts in those areas, and that the panel had representation by physician-investigators from all countries in Asia, North America, and Europe who have contributed to the medical literature on IgG4-RD. The panel was composed of 18 gastroenterologists, 13 rheumatologists, and 11 other specialists and subspecialists, representing a total of 8 medical specialties. Experts from Asia, North America, and Europe participated. More detailed information on the experts' specialties, nationalities, and experience in caring for patients with IgG4-RD is shown in Table 1.

Procedures. Panel members completed 2 internet-based surveys about their approaches to the diagnosis and management of IgG4-RD. The survey statements were composed by the Core Writing Committee (AK, ZSW, JLC, TC, JHS); each survey included >40 questions addressing all stages in the disease process from the diagnosis of IgG4-RD to the induction and maintenance of remission to the treatment of patients following disease flare. (The complete survey instruments are available as supplementary material on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.39132/abstract.) In recognition of the fact that some experts focus primarily on single-organ systems and seldom treat multiorgan disease, the panel members were permitted to complete either a general survey about disease management, an organ-specific survey, or both.

Following completion of the survey exercises by all panel members, the Core Writing Committee organized the results into 7 topics, each associated with 1 statement relevant to a particular phase of IgG4-RD (e.g., Remission Induction, Remission Maintenance, Treatment of Disease Flares). These 7 individual statements were then redistributed to the panel members to assess their level of agreement. Participants indicated the strength of their agreement or disagreement with each statement on a scale of 1–5 (1 = strongly disagree, 2 = disagree, 3 = neutral, 4 = agree, 5 = strongly agree). Panel members were also permitted to provide open-response answers elaborating on their reason for agreement or disagreement with the statement or providing other input that they considered meaningful.

Table 2. International consensus guidance statements on the treatment of IgG4-related disease (IgG4-RD), voting agreement, level of evidence, and citations

Statement	% agreement	Evidence level/grade of recommendation	References
1. The most accurate assessment of IgG4-RD are based on a full clinical history, physical examination, selected laboratory investigations, and appropriate radiology studies.	96	4/C	37, 51, 53, 54, 66, 67
2. Diagnostic confirmation by biopsy is strongly recommended for the exclusion of malignancies and other IgG4-RD mimics.	94	5/D	26, 57, 68
3. All patients with symptomatic, active IgG4-RD require treatment, some urgently. A subset of patients with asymptomatic IgG4-RD require treatment.	87	4/C	39, 47, 48, 51, 55, 67, 69–74
4. Glucocorticoids are the first-line agent for remission induction in all patients with active, untreated IgG4-RD unless contraindications to such treatment are present.	94	2b/B	38, 39, 47, 50, 51, 53, 54, 57, 66, 67, 69, 70, 72–77
5. Some but not all patients require the combination of glucocorticoids and a steroid-sparing immunosuppressive agent from the start of treatment. This is because glucocorticoid monotherapy will ultimately fail to control the disease and long-term glucocorticoid toxicities pose a high risk to patients.	46	4/C	38, 55, 56, 66, 78
6. Following a successful course of induction therapy, certain patients benefit from maintenance therapy.	94	2b/B	38, 47, 48, 50, 51, 54, 55, 67, 73
7. Re-treatment with glucocorticoids is indicated in patients who relapse off of treatment following successful remission induction. Following relapse, the introduction of a steroid-sparing agent for continuation in the remission maintenance period should be considered.	81	4/C	45, 56, 57, 59, 60, 77, 78

We performed an initial analysis of the consensus statement responses, modifying the wording to ensure clarity of meaning as appropriate based on the panel members' open-ended responses. Controversial topics were discussed by the experts at the symposium, during a 2-hour panel discussion devoted to disease management. Following the symposium, revised statements were distributed, and the experts completed 2 rounds of scoring these revised statements in the interest of maximizing consensus.

Literature review. A systematic search of PubMed for original and review articles in English published from March 2001 to February 2014 was performed using key words and text words related to IgG4-RD, including "IgG4-related disease," "autoimmune pancreatitis," "inflammatory pseudotumor," "sclerosing cholangitis," "glucocorticoids," "azathioprine," "mycophenolate mofetil," "6-mercaptopurine," "cyclophosphamide," "rituximab," "diagnosis," and "treatment," and specific to the 7 statements developed by the panel. Case reports and articles focusing only on pathology issues were excluded. Data relevant to each statement regarding the management or treatment of IgG4-RD were extracted, and the articles' methodologic quality was graded according to the levels of evidence described by the Oxford Centre for Evidence-Based Medicine (www.cebm.net, March 2009).

Results

Thirty-eight of the invited experts participated in all parts of the consensus exercise. Twenty-two (58%) completed the general survey, 18 (47%) completed the organ-specific survey, and 9 (24%) completed both. Table 2 shows a summary of each statement, the corresponding level of evidence, and the degree of consensus among the experts. The statements are discussed in detail below.

Patient evaluation (statement 1): The most accurate assessment of IgG4-RD is based on a full clinical history, physical examination, selected laboratory investigations, and appropriate radiology studies. (96% agreement). A thorough clinical history guides the initial evaluation of a patient with possible IgG-RD and subsequent

decisions regarding laboratory testing, imaging, and biopsies. A detailed review of past medical problems often reveals unrecognized manifestations of IgG4-RD (6). A complete physical examination may reveal involvement of an organ that is accessible for biopsy (e.g., major salivary gland swelling). Reexamination of archived biopsy samples with immunostaining for IgG4+ plasma cells may provide additional supportive diagnostic evidence.

Neither clinical nor pathologic findings alone are sufficient to diagnose IgG4-RD in most cases. The rigorous exclusion of diseases that mimic IgG4-RD both clinically and pathologically, through clinicopathologic correlation, is essential. A variety of diseases can be mistaken for IgG4-RD, and the list of disorders to consider varies by organ involvement. The clinicopathologic correlation required to make the correct diagnosis is determined most effectively with both clinicians and pathologists reviewing and discussing the patient's clinical features and pathologic findings. Table 3 summarizes conditions that can present with tumefactive lesions and increased IgG4+ plasma cells on tissue biopsy.

Serologic testing. In 2001, an association of type 1 autoimmune pancreatitis (AIP) with elevated serum IgG4 concentrations was reported (7). Subsequent diagnostic criteria for both IgG4-RD and type 1 (IgG4-related) AIP have typically included an elevated serum IgG4 concentration (8–10). Other studies have demonstrated variability in the sensitivity of serum IgG4 elevation for the diagnosis of IgG4-RD. Between 3% and 30% of IgG4-RD patients have normal serum IgG4 concentrations (8–12). The sensitivity and other test characteristics of the serum IgG4 concentration depend on a variety of factors, including the means of case identification, the diagnosis of "definitive" IgG4-RD, the type of assay used to measure serum IgG4 levels, the number of organs involved, and possibly the geographic origin of the patient.

Measurements of the serum IgG4 concentration remain important in the evaluation and longitudinal assessment of patients with possible IgG4-RD, but elevated levels are neither necessary nor sufficient for the diagnosis of IgG4-RD (9). Elevated serum IgG4 concentrations have been observed in patients with a variety of other disorders, making it a poor stand-alone diagnostic test for this condition (13,14). However, the degree of serum IgG4 elevation correlates with the number of organs involved: the greater the extent of disease, the higher the likelihood of an elevated serum IgG4 level (15–18).

Other laboratory markers. Recent studies indicate that IgG4-RD patients have substantial elevations of circulating plasmablasts and that plasmablast levels correlate with disease activity (18,19). Additional studies of circulating plasmablasts and IgG4+ plasmablasts as biomarkers are needed before their broad use can be endorsed.

Table 3. Conditions that can mimic IgG4-related disease clinically and histopathologically

Antineutrophil cytoplasmic antibody–associated vasculitides
Granulomatosis with polyangiitis (Wegener's)
Microscopic polyangiitis
Eosinophilic granulomatosis with polyangiitis (Churg-Strauss)
Adenocarcinoma and squamous cell carcinoma, peritumoral infiltrate
Castleman's disease (multicentric or localized)
Cutaneous plasmacytosis
Erdheim-Chester disease
Inflammatory myofibroblastic tumor
Inflammatory bowel disease
Lymphoproliferative diseases
Extranodal marginal zone lymphomas
Lymphoplasmacytic lymphomas
Follicular lymphomas
Perforating collagenosis
Primary sclerosing cholangitis
Rhinosinusitis
Rosai-Dorfman disease
Sarcoidosis
Sjögren's syndrome
Splenic sclerosing angiomatoid nodular transformation
Xanthogranuloma

Complement levels are a helpful indicator of disease activity in some IgG4-RD patients, particularly those with renal disease. Most patients with IgG4-related tubulointerstitial nephritis have hypocomplementemia at the time of disease relapse (19).

Radiology. Radiologic studies are often obtained early in the evaluation of a patient with possible IgG4-RD. Indeed, the diagnosis is often suggested by incidental findings on radiologic studies performed for reasons related or unrelated to IgG4-RD. Computed tomography (CT), CT performed with positron emission tomography (PET), magnetic resonance (MR) imaging, MR cholangiopancreatography, and endoscopic ultrasound are modalities that are commonly used to evaluate IgG4-RD. Selection of the imaging modality appropriate to the assessment of IgG4-RD is based on the organ under evaluation, local radiology expertise, and availability, as well as considerations such as radiation exposure and cost. Studies of fluorodeoxyglucose (FDG)–PET/CT imaging in IgG4-RD have emphasized its potential role at the time of initial evaluation (20,21). However, the utility of serial FDG-PET studies in gauging disease activity and guiding treatment decisions has not been demonstrated. Therefore, decisions regarding the use of PET must be made according to the individual patient's clinical features.

Many of the considerations relevant to diagnosis apply equally to longitudinal disease assessment. No single means of assessing disease response is currently adequate for all patients with IgG4-RD. An IgG4-RD Responder Index (IgG4-RD RI) has been developed as a quantitative means of assessing overall response to treatment (22). The instrument has been used successfully in clinical studies of IgG4-RD (15,16).

Tissue confirmation prior to treatment (statement 2): Diagnostic confirmation by biopsy is strongly recommended for the exclusion of malignancies and other IgG4-RD mimics. (94% agreement). Although comprehensive diagnostic criteria for IgG4-RD, AIP, and IgG4-related kidney disease have been proposed (6,23–25), the results of clinical assessment, laboratory evaluation, and imaging studies are often insufficient to distinguish the tumefactive lesions of IgG4-RD from cancer, and biopsy is typically necessary to exclude malignancy. International consensus guidelines outline the histopathologic and immunohistochemistry features that support the diagnosis of IgG4-RD and, in the proper clinical setting, can be viewed as diagnostic (26,27). Needle biopsy is usually inadequate for the histopathologic diagnosis of IgG4-RD, but generally yields quantities of tissue large enough to exclude malignancy with some confidence. In some settings, e.g., isolated submandibular gland enlargement, a small open biopsy as opposed to complete glandular excision may suffice (28). Archived tissue samples from previous biopsies may be diagnostic if reviewed along with immunostaining for IgG4+ plasma cells; immunostaining can be performed on paraffin-embedded specimens.

The presence of significant IgG4+ plasma cell infiltrates in biopsy specimens is not specific to IgG4-RD. Extensive IgG4+ plasma cell infiltration has been described in other conditions that commonly mimic IgG4-RD, including malignancy, granulomatosis with polyangiitis (Wegener's), eosinophilic granulomatosis with polyangiitis (Churg-Strauss), and multicentric Castleman's disease (29–35) (Table 3). Findings of storiform fibrosis and obliterative phlebitis heighten diagnostic specificity, but clinicopathologic correlation is always essential (26).

In cases of type 1 (IgG4-related) AIP, characteristic radiologic findings along with an elevated serum IgG4 concentration are typically sufficient to establish a diagnosis. However, fine-needle aspiration to exclude malignancy is often needed (27,36,37). Definitive diagnosis in patients with biliary disease is frequently challenging because diagnostic tissue is difficult to obtain in the absence of surgical resection. This is particularly true in the setting of IgG4-related sclerosing cholangitis involving intrahepatic and the proximal extrahepatic common bile duct. Biliary brushings and fine-needle biopsies are usually sufficient to exclude cholangiocarcinoma but are generally inadequate to distinguish IgG4-RD from primary sclerosing cholangitis (10,38–40). Endobiliary biopsy sometimes yields diagnostic tissue (41,42). Percutaneous liver biopsy may provide a diagnosis if biliary tract abnormalities are evident radiologically.

Indications for therapy (statement 3): All patients with symptomatic, active IgG4-RD require treatment, some urgently. A subset of patients with asymptomatic IgG4-RD require treatment. (87% agreement). *Treatment of asymptomatic disease.* Subclinical disease can lead to severe, irreversible sequelae in the biliary tree, kidney, aorta, mediastinum, retroperitoneum, mesentery, and other organs (43). However, not all manifestations of IgG4-RD require immediate treatment. "Watchful waiting" may be appropriate, for example, in patients with asymptomatic lymphadenopathy or mild submandibular gland enlargement.

Involvement of certain organs may be relatively asymptomatic until the late stages of disease, by which time chronic inflammation and fibrosis may have caused irreversible damage. Patients with AIP who are not treated with induction immunosuppression are less likely to achieve remission and more likely to experience disease complications (38,39). The importance of early intervention to prevent complications related to progressive fibrosis in the salivary glands has also been demonstrated (40).

Urgent treatment. A proportion of patients require treatment urgently because uncontrolled disease in certain organs can lead to irreversible damage (Table 4). Urgent treatment may include a combination of glucocorticoids at moderate-to-high doses, as well as other mechanical interventions in specific organs (e.g., stents for the biliary tract or ureter) (41). Rituximab (RTX), when available, may also be appropriate in some cases, if glucocorticoid treatment is contraindicated.

Other triggers for therapy. Spontaneous remissions of IgG-RD, or at least temporary remissions, without treatment have been reported (42,44), but the duration of followup in such cases has generally been short, and a relapsing-remitting pattern with progressive organ injury has been well described (45). Further, the metachronous nature of IgG4-RD suggests that although the disease may appear to improve at least temporarily in one organ, it may re-emerge months or years later at a different site (46).

Treatment leads to faster and more complete remission with fewer long-term complications of IgG4-RD than does waiting to treat (19,47). Treatment is therefore

Table 4. IgG4-related disease manifestations in which urgent treatment is recommended

Manifestation	Rationale for urgent treatment
Aortitis	Inflammatory aortic aneurysms can continue to enlarge and are at risk for dissection.
Retroperitoneal fibrosis	Progressive disease may lead to irreversible nerve damage/pain and/or ureteral obstruction/renal failure.
Proximal biliary strictures*	Untreated disease may lead to superimposed infectious cholangitis and eventually irreversible fibrosis and cirrhosis.
Tubulointerstitial nephritis	Untreated disease may lead to irreversible chronic kidney disease.
Pachymeningitis	Untreated disease puts the patient at risk for neurologic deficits and/or seizures.
Pancreatic enlargement	Untreated disease may lead to irreversible pancreatic exocrine and endocrine failure.
Pericarditis	Untreated disease may lead to tamponade or constrictive pericarditis

* "Proximal" denotes involvement of the intrahepatic bile ducts or extrahepatic portion of the common bile duct that is superior to the intrapancreatic portion.

justified in most cases in which laboratory or radiology findings suggest organ dysfunction (e.g., elevated serum levels of creatinine, hepatic transaminases, or bilirubin). Cosmetic concerns, particularly for periorbital or submandibular gland swelling, may also justify treatment in many cases.

Highly fibrotic lesions. In some cases, symptoms reflect fibrotic, "burnt-out" disease as opposed to active IgG4-RD. Longstanding, highly fibrotic lesions may respond poorly, if at all, to currently available pharmacologic agents. In such patients, the risk/benefit balance may not favor repeated courses of treatment. Surgical debulking is an option for IgG4-RD involvement of some organs, but the suitability of surgical interventions is governed by the anatomic regions and adjacent structures involved. Some cases of highly fibrotic orbital disease are more amenable to surgical interventions than to medical therapy. As examples, some fibrotic orbital pseudotumors and sclerosing mesenteritis respond best to surgical resection, when surgery is possible.

Remission induction with glucocorticoids (statement 4): Glucocorticoids are the first-line agent for remission induction in all patients with active, untreated IgG4-RD unless contraindications to such treatment are present. (94% agreement). Prednisone at a dosage of 30–40 mg/day is a common initial treatment for IgG4-RD (48). The dosage may be adjusted based on body weight or if the disease appears to be particularly aggressive. A lower dosage may be appropriate if the clinical IgG4-RD symptoms are mild. A review of the literature reveals a unified message that this condition responds well to initial glucocorticoid treatment. The responses to lower-dose treatment are variable, however, and glucocorticoid tapering and discontinuation are both associated with a high risk of disease relapse in many settings.

A nationwide survey by the Japanese Research Committee of Intractable Pancreatic Disease revealed no significant difference in results between prednisolone at 30 mg/day and prednisolone at 40 mg/day for the initial treatment of AIP (49). In a retrospective multicenter study of 978 patients with AIP, remission was achieved in nearly all patients (38).

In a prospective trial that included 28 patients with AIP, of whom 23 (82%) also had IgG4-related sclerosing cholangitis, patients were treated with prednisone monotherapy at an initial dosage of 30 mg/day. Remission was achieved in 82% of the patients, at a median of 5 months (50). A retrospective study by Ebbo et al showed improvement in 90% of patients treated with glucocorticoids at a mean dosage of 0.67 mg/kg/day (along with a steroid-sparing agent in 48% of the patients) (51). Improvement was regarded as the fulfillment of at least 2 of 3 criteria: improvement in overall clinical status, significant decrease in serum IgG4 concentration, and reduction of radiologic abnormalities.

Most experts agree that the initial glucocorticoid dosage should be maintained for 2–4 weeks, after which it can be tapered gradually. The tapering regimen has varied in different studies (47,52). One scenario is to taper the daily dosage by 10 mg every 2 weeks until a daily dosage of 20 mg is reached. After a short time (e.g., 2 weeks) of treatment at 20 mg/day, the tapering should resume by decreasing the daily dosage by 5 mg every 2 weeks. The goal of induction therapy at many centers is to discontinue glucocorticoid use 3–6 months after the start of treatment (53,54); many Japanese clinicians, however, recommend the use of low-dose glucocorticoid maintenance therapy for up to 3 years (48).

The use of steroid-sparing agents (statement 5): Some but not all patients require the combination of glucocorticoids and a steroid-sparing immunosuppressive agent from the start of treatment. This is because glucocorticoid monotherapy will ultimately fail to control the disease and long-term glucocorticoid toxicities pose a high risk to patients. (46% agreement). Opinion among the experts was split on this statement. Practice styles vary significantly across countries with regard to the use of a second immunosuppressive agent in addition to

glucocorticoids from the start of treatment. Eighty percent of the physicians from Japan (16 of 20) disagreed with addition of an immunosuppressive agent to glucocorticoids at the beginning of treatment. Conversely, 76% (13 of 17) of the participants from other countries (Korea, China, and countries in North America and Europe) agreed that this practice is appropriate in some patients. Among the subspecialties, gastroenterologists were the least likely to add another agent to glucocorticoids for initial treatment.

The variation in practice style across countries probably relates in part to the lack of universal access to certain steroid-sparing agents, particularly B cell depletion therapies. Twenty-seven percent of the panel members, mostly gastroenterologists, reported having no experience with the use of steroid-sparing agents of any kind. RTX is not available in Japan for the treatment of IgG4-RD.

Both prospective and retrospective studies demonstrate that, although glucocorticoids are effective initially for most patients, they are often tolerated poorly, and disease recurrences during or after glucocorticoid tapering are common. In the retrospective study by Ebbo and colleagues, only 30% of the 25 patients studied were able to discontinue glucocorticoid therapy, despite the fact that nearly half of the patients also received conventional steroid-sparing agents (51). Similar findings have been observed in other retrospective series, including 2 large cohorts of 563 and nearly 1,000 patients with AIP (38,47,55).

A retrospective study of AIP patients showed relapse in 38 (40%) of 96 patients who received maintenance therapy with low-dose glucocorticoids. Relapses occurred despite a maintenance prednisolone dosage of >5 mg/day in 10 (26%) of the patients whose disease relapsed (48). In another study, relapse occurred in 14 of 26 patients (54%) after discontinuation of maintenance prednisolone treatment (54).

Most experts agree that the addition of a steroid-sparing agent is appropriate when the glucocorticoid dosage cannot be tapered due to persistently active disease. In certain circumstances, providers may consider adding steroid-sparing agents during induction therapy, with plans to continue the agent as maintenance therapy. This is especially true when the there is risk that recurrent flares during glucocorticoid tapering might precipitate irreversible organ damage.

Conventional steroid-sparing medications. Azathioprine (AZA), mycophenolate mofetil (MMF), 6-mercaptopurine (6-MP), methotrexate, tacrolimus, and cyclophosphamide have all been used as steroid-sparing agents (38,50,53,56–58). However, the efficacies of these agents have not been evaluated in prospective trials, and there are few data overall to support the notion that conventional steroid-sparing agents are effective in IgG4-RD. Hart et al retrospectively compared the results of treatment of patients at their center who had relapsing AIP with AZA, MMF, or 6-MP versus glucocorticoid monotherapy (38), and found that relapse-free survival was not significantly different between the 2 groups.

B cell depletion as a steroid-sparing approach. Data from retrospective studies suggest that B cell depletion with RTX is effective, even in many patients in whom treatment with conventional steroid-sparing agents has been unsuccessful (59–62). Patients treated with RTX often require no glucocorticoid therapy during the remission induction period beyond that received as part of the infusion regimen (typically 100 mg of methylprednisolone with each RTX infusion). Among patients who are on a glucocorticoid regimen at the time RTX is initiated, the glucocorticoid dosage can often be tapered rapidly, following RTX administration (56). Hart and colleagues reported an 83% rate of complete remission following RTX treatment (3 weekly doses of 375 mg/m^2) in a group of patients with AIP whose disease had been resistant to, or who had contraindications to, steroids or conventional steroid-sparing agents (38).

A recently completed open-label trial of RTX (2 doses of 1 gm administered intravenously) in 30 patients with IgG4-RD showed encouraging results (https://clinicaltrials.gov/NCT01584388) (63). Nearly 90% of the patients in this trial were treated with RTX alone, and disease response was observed in 97% of the patients at 6 months. At baseline the mean ± SD IgG4-RD RI and physician's global assessment scores were 11 ± 7 and 63 ± 22 mm (100-mm scale), respectively. These declined to 1 ± 2 and 11 ± 17 mm at 6 months ($P < 0.001$ for both). The primary outcome measure, defined as improvement in the IgG4-RD RI of at least 2 points and absence of disease flares without glucocorticoid treatment at 6 months, was achieved in 23 (77%) of the patients.

Mechanistic studies performed in conjunction with RTX treatment have demonstrated that patients with IgG4-RD have increased levels of circulating plasmablasts and that serial plasmablast measurements may help estimate the risk of disease flare (15,16). In addition, B cell depletion appears to target the IgG4 subclass, which declines disproportionately to the decreases observed in the concentrations of other IgG subclasses (56). Because the CD20 marker is not present on the surface of either plasmablasts or plasma cells, this suggests that the plasmablasts and plasma cells producing IgG4 in IgG4-RD are short-lived and that the effect of RTX is due at least in part to a failure of repletion. Plasmablast

measurement is not yet widely available in general clinical care, limiting its use as a biomarker at this time.

The use of maintenance therapy following remission induction (statement 6): Following a successful course of induction therapy, certain patients benefit from maintenance therapy. (94% agreement). The concept of maintenance therapy following the achievement of remission received broad support from the panel. Anecdotal reports (55,64) and one study of 10 patients (65) suggest that patients with multiorgan disease, significantly elevated serum IgG4 concentrations, involvement of the proximal bile ducts, or a history of disease relapse are at higher risk of early recurrence following remission induction. Patients with organ-threatening IgG4-RD manifestations and those with an elevated risk of relapse will likely benefit from maintenance therapy in an effort to minimize morbidity.

Maintenance therapy may consist of low-dose glucocorticoids or any of the steroid-sparing agents discussed above. RTX has been useful as maintenance therapy but the optimal frequency and duration of treatment have not been clearly defined. When used as maintenance therapy, RTX is often administered when there is evidence of disease flare rather than at a predetermined time interval (e.g., every 6 months). This practice requires further evaluation and might vary depending on the potential risk of organ damage associated with a disease flare.

Regardless of the agent used, the optimal duration of maintenance therapy has not been evaluated rigorously and probably depends on a number of patient-specific factors. Most of these variables remain poorly understood and require further study.

The Japanese consensus guideline for AIP recommends maintenance therapy with low-dose steroids (prednisolone 2.5–5 mg/day) for patients who are at increased risk of relapse (48). The optimal duration of maintenance therapy has not been studied. In a retrospective, multicenter study of 459 AIP patients in Japan, it was found that 82% of the patients received glucocorticoids as maintenance therapy (47). A maintenance oral prednisone dosage of 5 mg/day was most common (63%), followed by 2.5 mg/day (21%). Relapse rates were significantly lower during maintenance glucocorticoid therapy (23%) than after glucocorticoid discontinuation (34%). Similar observations have been made in other studies (19,38).

Glucocorticoid monotherapy is often less effective than desired. Kamisawa et al reported that, despite the use of maintenance glucocorticoid therapy, nearly one-quarter of patients had a disease relapse (63 of 273 [23%]); however, the percentage was significantly higher among patients who stopped maintenance treatment (375 of 1,104 [34%]) (47). Similar findings have been observed in other retrospective series (55).

Continuous treatment with glucocorticoids for years, even at low doses, is associated with treatment-related morbidity, particularly in a disease such as IgG4-RD that often targets the pancreas and affects a patient population that is middle-aged to elderly (39).

Managing disease relapse (statement 7): Re-treatment with glucocorticoids is indicated in patients who relapse off of treatment following successful remission induction. Following relapse, the introduction of a steroid-sparing agent for continuation in the remission maintenance period should be considered. (81% agreement). Practices with regard to the use of remission maintenance treatments following disease flares vary among the experts. The evidence presented thus far highlights the fact that relapses of IgG4-RD are common, even with the use of glucocorticoid maintenance therapy. In a large AIP registry from Japan, 30% of patients had relapses while receiving glucocorticoid maintenance therapy, and 43% of those relapses involved organs beyond the pancreas (49). Hart and colleagues observed that in 32% of patients who had relapses, the relapse occurred while the glucocorticoid dosage was being tapered or the patient was receiving some other remission maintenance therapy (38). A history of relapse appears to be a strong predictor of future relapse.

In the vast majority of patients who experience flares of IgG4-RD, the flares respond well to glucocorticoid-based strategies for reinduction. If a short initial course

Table 5. Research priorities for advances in the management and treatment of IgG4-related disease

Management
1. Validation of clinical diagnostic criteria
2. Further evaluation of the relative value of various biomarkers (e.g., serum IgG4 concentration, circulating plasmablast levels) for diagnosis and monitoring disease activity
3. Large cohort studies that can identify clinically useful disease subgroups and clarify the natural history of the condition
4. Evaluation of the relative utility of various imaging modalities to identify and monitor disease activity at different anatomic sites
Treatment
1. Randomized controlled trials comparing glucocorticoids and steroid-sparing agents
2. Mechanistic studies designed to clarify aspects of disease pathophysiology and identify specific targets for therapy
3. Longitudinal cohort studies that clarify risk factors for disease flares
4. Studies clarifying the optimal timing of re-treatment to prevent disease flares

of glucocorticoid treatment yields prolonged disease remission, then a repeat glucocorticoid course without additional therapy may be the most prudent strategy in the setting of a flare.

Discussion

Recognition of IgG4-RD has expanded across medical specialties and around the world over the past decade. Early in the history of a newly recognized disease, before the availability of rigorous evidence obtained from prospective, controlled trials, guidance from clinicians who are experienced in the management of the disease is helpful in discerning the appropriate approach to the treatment of individual patients. This consensus statement, representing the collaborative efforts of 42 experts from 10 different countries and representing 8 medical subspecialties, is the first approach to summarizing treatment strategies used in different parts of the world. The goal is to provide guidance to clinicians with regard to questions that are important in the field today (Table 5).

IgG4-RD presents a challenge to physicians' full complement of skills because of its multiorgan nature and the necessity of close clinicopathologic correlation in disease management. Although IgG4-RD has pathologic characteristics that are highly suggestive of the diagnosis, most of its organ manifestations cannot be diagnosed definitively in the absence of input from the clinician about the patient's clinical phenotype. Conversely, although a growing number of typical clinical features are now recognized, clinicians who see patients with IgG4-RD are seldom comfortable making the diagnosis without histopathologic confirmation and the exclusion of potentially dangerous mimics (e.g., malignancy). Clinical experts from a variety of medical subspecialties believe that proof of the diagnosis through biopsy of an affected organ is essential in the great majority of cases. The sole exception includes some cases of AIP, assuming that the nearly diagnostic imaging features are accompanied by a compatible clinical scenario.

As clinical experience with IgG4-RD has grown in recent years, the pendulum has swung decisively away from reliance on serum IgG4 concentrations for the purposes of diagnosis and longitudinal assessment of disease activity. As treatment approaches are refined, identification of biomarkers that are more reliable than serum IgG4 levels becomes important for the assessment of longitudinal disease activity. Flow cytometry–based assays that measure plasmablasts appear to have important potential for this purpose but, as noted above, are not yet widely available.

The consensus among experts in IgG4-RD is that the threshold for initiating treatment in patients with active disease is low. Irreversible injury to some organs can occur within weeks or months if effective therapy is not initiated. The prevention of fibrosis and its potentially destructive impact on organs is a major aim of treatment. Once fibrosis is established, therapeutic options are currently limited.

Glucocorticoids remain the preference among experts as the initial therapy. It is increasingly clear, however, that in many patients, disease response is not maintained as glucocorticoids are tapered. There is no consensus approach regarding the use of remission maintenance agents, and in fact there are few data to suggest that conventional steroid-sparing agents are effective in IgG4-RD.

Approaches to both remission induction and remission maintenance vary significantly from country to country, based partly on the availability of B cell depletion therapy. Most countries' health insurance structures do not pay consistently (or at all) for RTX treatment of IgG4-RD. As a result, experience with B cell depletion in IgG4-RD differs across countries and even across subspecialties. Gastroenterologists in many countries are less experienced with the use of RTX than are rheumatologists or oncologists. This consensus exercise highlighted the tendency of Japanese experts to rely upon glucocorticoid-based treatment regimens—usually glucocorticoid monotherapy. In contrast, North American and European panel members tend to emphasize the early introduction of glucocorticoid-sparing agents, including B cell–depleting strategies.

Many questions with regard to general approaches to the treatment of IgG4-RD, as well as treatment approaches for individual organ manifestations, remain to be answered in randomized, controlled clinical trials. As knowledge of the pathophysiology of this fibroinflammatory disorder advances, treatment algorithms are also likely to evolve to include therapies that target the problematic fibrotic lesions as well as the highly cellular lymphoplasmacytic inflammation present in earlier phases of the disease.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Stone had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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REFERENCES

1. Stone JH, Zen Y, Deshpande V. IgG4-related disease. N Engl J Med 2012;366:539–51.
2. Carruthers MN, Stone JH, Khosroshahi A. The latest on IgG4-RD: a rapidly emerging disease. Curr Opin Rheumatol 2012;24:60–9.
3. Stone JH, Khosroshahi A, Deshpande V, Chan JK, Heathcote JG, Aalberse R, et al. Recommendations for the nomenclature of IgG4-related disease and its individual organ system manifestations. Arthritis Rheum 2012;64:3061–7.
4. Kamisawa T, Funata N, Hayashi Y, Eishi Y, Koike M, Tsuruta K, et al. A new clinicopathological entity of IgG4-related autoimmune disease. J Gastroenterol 2003;38:982–4.
5. Cheuk W, Chan JK. IgG4-related sclerosing disease: a critical appraisal of an evolving clinicopathologic entity. Adv Anat Pathol 2010;17:303–32.
6. Kawano M, Saeki T, Nakashima H, Nishi S, Yamaguchi Y, Hisano S, et al. Proposal for diagnostic criteria for IgG4-related kidney disease. Clin Exp Nephrol 2011;15:615–26.
7. Hamano H, Kawa S, Horiuchi A, Unno H, Furuya N, Akamatsu T, et al. High serum IgG4 concentrations in patients with sclerosing pancreatitis. N Engl J Med 2001;344:732–8.
8. Ghazale A, Chari ST, Smyrk TC, Levy MJ, Topazian MD, Takahashi N, et al. Value of serum IgG4 in the diagnosis of autoimmune pancreatitis and in distinguishing it from pancreatic cancer. Am J Gastroenterol 2007;102:1646–53.
9. Carruthers MN, Khosroshahi A, Augustin T, Deshpande V, Stone JH. The diagnostic utility of serum IgG4 concentrations in IgG4-related disease. Ann Rheum Dis 2015;74:14–8.
10. Takahira M, Ozawa Y, Kawano M, Zen Y, Hamaoka S, Yamada K, et al. Clinical aspects of IgG4-related orbital inflammation in a case series of ocular adnexal lymphoproliferative disorders. Int J Rheumatol 2012;2012:635473.
11. Tanaka A, Tazuma S, Okazaki K, Tsubouchi H, Inui K, Takikawa H. Nationwide survey for primary sclerosing cholangitis and IgG4-related sclerosing cholangitis in Japan. J Hepatobiliary Pancreat Sci 2014;21:43–50.
12. Saeki T, Nishi S, Imai N, Ito T, Yamazaki H, Kawano M, et al. Clinicopathological characteristics of patients with IgG4-related tubulointerstitial nephritis. Kidney Int 2010;78:1016–23.
13. Ebbo M, Grados A, Bernit E, Vely F, Boucraut J, Harle JR, et al. Pathologies associated with serum IgG4 elevation. Int J Rheumatol 2012;2012:602809.
14. Ryu JH, Horie R, Sekiguchi H, Peikert T, Yi ES. Spectrum of disorders associated with elevated serum IgG4 levels encountered in clinical practice. Int J Rheumatol 2012;2012:232960.
15. Wallace ZS, Mattoo H, Carruthers M, Mahajan VS, Della Torre E, Lee H, et al. Plasmablasts as a biomarker for IgG4-related disease, independent of serum IgG4 concentrations. Ann Rheum Dis 2015;74:190–5.
16. Mattoo H, Mahajan VS, Della-Torre E, Sekigami Y, Carruthers M, Wallace ZS, et al. De novo oligoclonal expansions of circulating plasmablasts in active and relapsing IgG4-related disease. J Allergy Clin Immunol 2014;134:679–87.
17. Kamisawa T, Okamoto A, Funata N. Clinicopathological features of autoimmune pancreatitis in relation to elevation of serum IgG4. Pancreas 2005;31:28–31.
18. Tabata T, Kamisawa T, Takuma K, Egawa N, Setoguchi K, Tsuruta K, et al. Serial changes of elevated serum IgG4 levels in IgG4-related systemic disease. Intern Med 2011;50:69–75.
19. Saeki T, Kawano M, Mizushima I, Yamamoto M, Wada Y, Nakashima H, et al. The clinical course of patients with IgG4-related kidney disease. Kidney Int 2013;84:826–33.
20. Ebbo M, Grados A, Guedj E, Gobert D, Colavolpe C, Zaidan M, et al. Usefulness of 2-[^{18}F]-fluoro-2-deoxy-D-glucose-positron emission tomography/computed tomography for staging and evaluation of treatment response in IgG4-related disease: a retrospective multicenter study. Arthritis Care Res (Hoboken) 2014;66:86–96.
21. Takahashi H, Yamashita H, Morooka M, Kubota K, Takahashi Y, Kaneko H, et al. The utility of FDG-PET/CT and other imaging techniques in the evaluation of IgG4-related disease. Joint Bone Spine 2014;81:331–6.
22. Carruthers MN, Stone JH, Deshpande V, Khosroshahi A. Development of an IgG4-RD Responder Index. Int J Rheumatol 2012;2012:259408.
23. Umehara H, Okazaki K, Masaki Y, Kawano M, Yamamoto M, Saeki T, et al. Comprehensive diagnostic criteria for IgG4-related disease (IgG4-RD), 2011. Mod Rheumatol 2012;22:21–30.
24. Shimosegawa T, Chari ST, Frulloni L, Kamisawa T, Kawa S, Mino-Kenudson M, et al. International consensus diagnostic criteria for autoimmune pancreatitis: guidelines of the International Association of Pancreatology. Pancreas 2011;40:352–8.
25. Kawa S, Hamano H. Autoimmune pancreatitis and bile duct lesions. J Gastroenterol 2003;38:1201–3.
26. Deshpande V, Zen Y, Chan JK, Yi EE, Sato Y, Yoshino T, et al. Consensus statement on the pathology of IgG4-related disease. Mod Pathol 2012;25:1181–92.
27. Okazaki K, Kawa S, Kamisawa T, Ito T, Inui K, Irie H, et al. Amendment of the Japanese Consensus Guidelines for Autoimmune Pancreatitis, 2013. I. Concept and diagnosis of autoimmune pancreatitis. J Gastroenterol 2014;49:567–88.
28. Moriyama M, Furukawa S, Kawano S, Goto Y, Kiyoshima T, Tanaka A, et al. The diagnostic utility of biopsies from the submandibular and labial salivary glands in IgG4-related dacryoadenitis and sialoadenitis, so-called Mikulicz's disease. Int J Oral Maxillofac Surg 2014;43:1276–81.
29. Brenner I, Roth S, Puppe B, Wobser M, Rosenwald A, Geissinger E. Primary cutaneous marginal zone lymphomas with plasmacytic differentiation show frequent IgG4 expression. Mod Pathol 2013;26:1568–76.
30. Wallace ZS, Carruthers MN, Khosroshahi A, Carruthers R, Shinagare S, Stemmer-Rachamimov A, et al. IgG4-related disease and hypertrophic pachymeningitis. Medicine (Baltimore) 2013;92:206–16.
31. Strehl JD, Hartmann A, Agaimy A. Numerous IgG4-positive plasma cells are ubiquitous in diverse localised non-specific chronic inflammatory conditions and need to be distinguished from IgG4-related systemic disorders. J Clin Pathol 2011;64:237–43.
32. Chang SY, Keogh KA, Lewis JE, Ryu JH, Cornell LD, Garrity JA, et al. IgG4-positive plasma cells in granulomatosis with polyangiitis (Wegener's): a clinicopathologic and immunohistochemical study on 43 granulomatosis with polyangiitis and 20 control cases. Hum Pathol 2013;44:2432–7.

33. Vaglio A, Strehl JD, Manger B, Maritati F, Alberici F, Beyer C, et al. IgG4 immune response in Churg-Strauss syndrome. Ann Rheum Dis 2012;71:390–3.
34. Yamamoto M, Takahashi H, Suzuki C, Tabeya T, Ohara M, Naishiro Y, et al. Analysis of serum IgG subclasses in Churg-Strauss syndrome: the meaning of elevated serum levels of IgG4. Intern Med 2010;49:1365–70.
35. Sato Y, Kojima M, Takata K, Morito T, Mizobuchi K, Tanaka T, et al. Multicentric Castleman's disease with abundant IgG4-positive cells: a clinical and pathological analysis of six cases. J Clin Pathol 2010;63:1084–9.
36. Okazaki K, Kawa S, Kamisawa T, Shimosegawa T, Tanaka M, working members of Research Committee for Intractable Pancreatic Disease and Japan Pancreas Society. Japanese consensus guidelines for management of autoimmune pancreatitis. I. Concept and diagnosis of autoimmune pancreatitis. J Gastroenterol 2010;45:249–65.
37. Chari ST, Takahashi N, Levy MJ, Smyrk TC, Clain JE, Pearson RK, et al. A diagnostic strategy to distinguish autoimmune pancreatitis from pancreatic cancer. Clin Gastroenterol Hepatol 2009;7:1097–1103.
38. Hart PA, Topazian MD, Witzig TE, Clain JE, Gleeson FC, Klebig RR, et al. Treatment of relapsing autoimmune pancreatitis with immunomodulators and rituximab: the Mayo Clinic experience. Gut 2013;62:1607–15.
39. Hirano K, Tada M, Isayama H, Yagioka H, Sasaki T, Kogure H, et al. Long-term prognosis of autoimmune pancreatitis with and without corticosteroid treatment. Gut 2007;56:1719–24.
40. Shimizu Y, Yamamoto M, Naishiro Y, Sudoh G, Ishigami K, Yajima H, et al. Necessity of early intervention for IgG4-related disease: delayed treatment induces fibrosis progression. Rheumatology (Oxford) 2013;52:679–83.
41. Mizushima I, Inoue D, Yamamoto M, Yamada K, Saeki T, Ubara Y, et al. Clinical course after corticosteroid therapy in IgG4-related aortitis/periaortitis and periarteritis: a retrospective multicenter study. Arthritis Res Ther 2014;16:R156.
42. Ohshima K, Sato Y, Yoshino T. A case of IgG4-related dacryoadenitis that regressed without systemic steroid administration. J Clin Exp Hematop 2013;53:53–6.
43. Stone JH, Patel VI, Oliveira GR, Stone JR. Case 38-2012—a 60-year-old man with abdominal pain and aortic aneurysms. N Engl J Med 2012;367:2335–46.
44. Seki N, Yamazaki N, Kondo A, Nomura K, Himi T. Spontaneous regression of lung lesions after excision of the submandibular gland in a patient with chronic sclerosing sialadenitis. Auris Nasus Larynx 2012;39:212–5.
45. Takayama M, Hamano H, Ochi Y, Saegusa H, Komatsu K, Muraki T, et al. Recurrent attacks of autoimmune pancreatitis result in pancreatic stone formation. Am J Gastroenterol 2004;99:932–7.
46. Miura H, Miyachi Y. IgG4-related retroperitoneal fibrosis and sclerosing cholangitis independent of autoimmune pancreatitis: a recurrent case after a 5-year history of spontaneous remission. JOP 2009;10:432–7.
47. Kamisawa T, Shimosegawa T, Okazaki K, Nishino T, Watanabe H, Kanno A, et al. Standard steroid treatment for autoimmune pancreatitis. Gut 2009;58:1504–7.
48. Kamisawa T, Okazaki K, Kawa S, Ito T, Inui K, Irie H, et al. Amendment of the Japanese Consensus Guidelines for Autoimmune Pancreatitis, 2013. III. Treatment and prognosis of autoimmune pancreatitis. J Gastroenterol 2014;49:961–70.
49. Kanno A, Nishimori I, Masamune A, Kikuta K, Hirota M, Kuriyama S, et al, Research Committee on Intractable Diseases of Pancreas. Nationwide epidemiological survey of autoimmune pancreatitis in Japan. Pancreas 2012;41:835–9.
50. Sandanayake NS, Church NI, Chapman MH, Johnson GJ, Dhar DK, Amin Z, et al. Presentation and management of post-treatment relapse in autoimmune pancreatitis/immunoglobulin G4-associated cholangitis. Clin Gastroenterol Hepatol 2009;7:1089–96.
51. Ebbo M, Daniel L, Pavic M, Seve P, Hamidou M, Andres E, et al. IgG4-related systemic disease: features and treatment response in a French cohort: results of a multicenter registry. Medicine (Baltimore) 2012;91:49–56.
52. Ghazale A, Chari ST. Optimising corticosteroid treatment for autoimmune pancreatitis. Gut 2007;56:1650–2.
53. Ghazale A, Chari ST, Zhang L, Smyrk TC, Takahashi N, Levy MJ, et al. Immunoglobulin G4-associated cholangitis: clinical profile and response to therapy. Gastroenterology 2008;134:706–15.
54. Raina A, Yadav D, Krasinskas AM, McGrath KM, Khalid A, Sanders M, et al. Evaluation and management of autoimmune pancreatitis: experience at a large US center. Am J Gastroenterol 2009;104:2295–306.
55. Hart PA, Kamisawa T, Brugge WR, Chung JB, Culver EL, Czako L, et al. Long-term outcomes of autoimmune pancreatitis: a multicentre, international analysis. Gut 2013;62:1771–6.
56. Khosroshahi A, Carruthers MN, Deshpande V, Unizony S, Bloch DB, Stone JH. Rituximab for the treatment of IgG4-related disease: lessons from 10 consecutive patients. Medicine (Baltimore) 2012;91:57–66.
57. Bosco JJ, Suan D, Varikatt W, Lin MW. Extra-pancreatic manifestations of IgG4-related systemic disease: a single-centre experience of treatment with combined immunosuppression. Intern Med J 2013;43:417–23.
58. Buechter M, Klein CG, Kloeters C, Schlaak JF, Canbay A, Gerken G, et al. Tacrolimus as a reasonable alternative in a patient with steroid-dependent and thiopurine-refractory autoimmune pancreatitis with IgG4-associated cholangitis. Z Gastroenterol 2014;52:564–8.
59. Witzig TE, Inwards DJ, Habermann TM, Dogan A, Kurtin PJ, Gross JB Jr, et al. Treatment of benign orbital pseudolymphomas with the monoclonal anti-CD20 antibody rituximab. Mayo Clin Proc 2007;82:692–9.
60. Plaza JA, Garrity JA, Dogan A, Ananthamurthy A, Witzig TE, Salomao DR. Orbital inflammation with IgG4-positive plasma cells: manifestation of IgG4 systemic disease. Arch Ophthalmol 2011;129:421–8.
61. Khosroshahi A, Bloch DB, Deshpande V, Stone JH. Rituximab therapy leads to rapid decline of serum IgG4 levels and prompt clinical improvement in IgG4-related systemic disease. Arthritis Rheum 2010;62:1755–62.
62. Topazian M, Witzig TE, Smyrk TC, Pulido JS, Levy MJ, Kamath PS, et al. Rituximab therapy for refractory biliary strictures in immunoglobulin G4-associated cholangitis. Clin Gastroenterol Hepatol 2008;6:364–6.
63. Carruthers MN, Topazian M, Khosroshahi A, Witzig T, Wallace ZS, Hart P, et al. Rituximab for IgG4-related disease: a prospective, open-label trial. Ann Rheum Dis 2015. E-pub ahead of print.
64. Ghazale AH, Chari ST, Vege SS. Update on the diagnosis and treatment of autoimmune pancreatitis. Curr Gastroenterol Rep 2008;10:115–21.
65. Yoo JJ, Park JJ, Kang EH, Lee EB, Song YW, Go HJ, et al. Risk factors for the recurrence of IgG4-related sclerosing disease without autoimmune pancreatitis. J Clin Rheumatol 2011;17:392–4.
66. Chen H, Lin W, Wang Q, Wu Q, Wang L, Fei Y, et al. IgG4-related disease in a Chinese cohort: a prospective study. Scand J Rheumatol 2014;43:70–4.
67. Patel H, Khalili K, Kyoung KT, Yazdi L, Lee E, May G, et al. IgG4 related disease: a retrospective descriptive study highlighting Canadian experiences in diagnosis and management. BMC Gastroenterol 2013;13:168.
68. Kawakami H, Zen Y, Kuwatani M, Eto K, Haba S, Yamato H, et al. IgG4-related sclerosing cholangitis and autoimmune pancreatitis: histological assessment of biopsies from Vater's ampulla and the bile duct. J Gastroenterol Hepatol 2010;5:1648–55.

69. Liu B, Li J, Yan LN, Sun HR, Liu T, Zhang ZX. Retrospective study of steroid therapy for patients with autoimmune pancreatitis in a Chinese population. World J Gastroenterol 2013;19:569–74.
70. Kawa S, Okazaki K, Kamisawa T, Kubo K, Ohara H, Hasebe O, et al. Amendment of the Japanese Consensus Guidelines for Autoimmune Pancreatitis, 2013. II. Extrapancreatic lesions, differential diagnosis. J Gastroenterol 2014;49:765–84.
71. Kamisawa T, Chari ST, Lerch MM, Kim MH, Gress TM, Shimosegawa T. Recent advances in autoimmune pancreatitis: type 1 and type 2. Gut 2013;62:1373–80.
72. Kamisawa T, Okamoto A, Wakabayashi T, Watanabe H, Sawabu N. Appropriate steroid therapy for autoimmune pancreatitis based on long-term outcome. Scand J Gastroenterol 2008;43: 609–13.
73. Kim HM, Chung MJ, Chung JB. Remission and relapse of autoimmune pancreatitis: focusing on corticosteroid treatment. Pancreas 2010;39:555–60.
74. Takuma K, Kamisawa T, Tabata T, Inaba Y, Egawa N, Igarashi Y. Short-term and long-term outcomes of autoimmune pancreatitis. Eur J Gastroenterol Hepatol 2011;23:146–52.
75. Uchida K, Yazumi S, Nishio A, Kusuda T, Koyabu M, Fukata M, et al. Long-term outcome of autoimmune pancreatitis. J Gastroenterol 2009;44:726–32.
76. Song Y, Liu QD, Zhou NX, Zhang WZ, Wang DJ. Diagnosis and management of autoimmune pancreatitis: experience from China. World J Gastroenterol 2008;14:601–6.
77. Tomiyama T, Uchida K, Matsushita M, Ikeura T, Fukui T, Takaoka M, et al. Comparison of steroid pulse therapy and conventional oral steroid therapy as initial treatment for autoimmune pancreatitis. J Gastroenterol 2011;46:696–704.
78. Caputo C, Bazargan A, McKelvie PA, Sutherland T, Su CS, Inder WJ. Hypophysitis due to IgG4-related disease responding to treatment with azathioprine: an alternative to corticosteroid therapy. Pituitary 2014;17:251–6.

Plasmacytoid Dendritic Cell Activation and IFN-α Production Are Prominent Features of Murine Autoimmune Pancreatitis and Human IgG4-Related Autoimmune Pancreatitis

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The abnormal immune response accompanying IgG4-related autoimmune pancreatitis (AIP) is presently unclear. In this study, we examined the role of plasmacytoid dendritic cell (pDC) activation and IFN-α production in this disease as well as in a murine model of AIP (MRL/Mp mice treated with polyinosinic-polycytidylic acid). We found that the development of AIP in treated MRL/Mp mice occurred in parallel with pancreatic accumulation of pDCs producing IFN-α, and with pDC depletion and IFN-α-blocking studies, we showed that such accumulation was necessary for AIP induction. In addition, we found that the pancreas of treated MRL/Mp mice contained neutrophil extracellular traps (NETs) shown previously to stimulate pDCs to produce IFN-α. Consistent with these findings, we found that patients with IgG4-related AIP also exhibited pancreatic tissue localization of IFN-α–expressing pDCs and had significantly higher serum IFN-α levels than healthy controls. In addition, the inflamed pancreas of these patients but not controls also contained NETs that were shown to be capable of pDC activation. More importantly, patient pDCs cultured in the presence of NETs produced greatly increased levels of IFN-α and induced control B cells to produce IgG4 (but not IgG1) as compared with control pDCs. These data suggest that pDC activation and production of IFN-α is a major cause of murine AIP; in addition, the increased pDC production of IFN-α and its relation to IgG4 production observed in IgG4-related AIP suggest that this mechanism also plays a role in the human disease. ***The Journal of Immunology*****, 2015, 195: 3033–3044.**

Immunoglobulin G4–related disease (IgG4-RD) is a newly established chronic inflammatory disorder characterized by massive infiltration of IgG4-expressing plasma cells into multiple organs, elevated serum IgG4 levels, and storiform fibrosis (1, 2). Various inflammatory disorders, such as those formerly diagnosed as autoimmune pancreatitis (AIP), sialadenitis, and autoimmune cholangitis, are now regarded as organ-specific manifestations of systemic IgG4-RD (1–4). Although elevated serum IgG4 levels define the presence of IgG4-RD–associated inflammation in a variety of organs, and thus it is essential for disease diagnosis, the pathophysiological role of this elevated Ig subtype is poorly understood. On one hand, elevated IgG4 levels may be an epiphenomenon associated with IgG4-RD–associated chronic inflammation, because this IgG subtype exhibits limited ability to bind to complement and FcγRs (5, 6) and has been shown to have anti-inflammatory activity in experimental myasthenia gravis (7). On the other hand, enhanced IgG4 levels may play a vital immunopathologic role in this disease, because IgG4 Abs against autoantigens have also been shown to participate in the pathogenesis of autoimmunity (8, 9).

Adaptive immune responses involving T cell–derived cytokines such as IL-4, IL-10, and IL-13 that have been found to promote IgG4 production by B cells (5, 10, 11) may be involved in IgG4-RD–associated IgG4 production, because the number of Th2 cells and regulatory T cells (Tregs) producing these cytokines are increased in IgG4-RD patient lesions and peripheral blood (12–14). However, innate immune responses that activate B cells in a T cell–independent manner to induce Ig production upon exposure to microbial-associated molecular patterns (MAMPs) and damage-associated molecular patterns (15) may also be involved in the enhanced IgG4 production. This possibility is suggested by our previous studies in which we reported that TLR and nucleotide-binding oligomerization domain-like receptor activation in monocytes (16) and basophils (17) from patients with IgG4-RD enhance IgG4 production by B cells from healthy control individuals via production of BAFF.

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Abbreviations used in this article: AIP, autoimmune pancreatitis; BDCA, blood dendritic cell Ag; IFNAR, type I IFN receptor; IgG4-RD, IgG4-related disease; LF, lactoferrin; MAMP, microbial-associated molecular pattern; MPO, myeloperoxidase; MSU, monosodium urate; NET, neutrophil extracellular trap; pDC, plasmacytoid dendritic cell; PDCA, pDC Ag; poly (I:C), polyinosinic-polycytidylic acid; Siglec, sialic acid–binding Ig-like lectin; SLE, systemic lupus erythematosus; Treg, regulatory T cell.

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Plasmacytoid dendritic cells (pDCs) are another potential source of BAFF in IgG4-RD, because these cells produce IFN-α, a cytokine known to enhance BAFF production (18, 19). The involvement of pDCs in IgG4-RD immunopathogenesis aligns with the fact that pDC activation leading to increased IFN-α production has been postulated to be a major mechanism of autoantibody production in systemic lupus erythematosus (SLE). In this disease, FcR-mediated endocytosis of DNA/RNA-containing immune complexes are thought to activate pDCs expressing TLR7 and TLR9 and the pDCs so activated then induce autoantibodies (20, 21). However, little is known regarding the potential involvement of pDC-mediated IFN-α production in IgG4-RD development.

In the current study, we show that increased number of pDCs producing IFN-α populate the inflamed pancreas in both the murine model of AIP and patients with IgG4-related AIP. In the murine model, we demonstrate by pDC depletion and IFN-α receptor blocking that these cells are a major cause of the pancreatitis. Furthermore, in both the murine model and humans with IgG4-related AIP, the inflamed pancreas contained neutrophil extracellular traps (NETs) that could be shown to activate pDCs from patients with IgG4-related AIP to produce IFN-α and BAFF, which leads to production of IgG4 by B cells. Overall, these data provide strong evidence that pDC activation and IFN-α production are prominent features of IgG4-related AIP.

Materials and Methods

Induction of AIP in MRL/Mp mice

Female MRL/Mp mice were purchased from Japan SLC (Shizuoka, Japan). All mice were bred at Kyoto University animal facility under specific pathogen-free conditions. This study was approved by the Kyoto University ethics review boards for animal experiments. Six-week-old female MRL/Mp mice were treated by i.p. injections with 100 μg polyinosinic-polycytidylic acid [poly (I:C); InvivoGen, San Diego, CA] twice a week for a total of 14 or 16 injections to induce experimental AIP (22, 23). In some experiments, mice were treated with Ab against bone marrow stromal cell Ag 2 (100 μg, 120G8; Bioceros, Utrecht, the Netherlands) and Ab against IFN-αβ receptor (100 μg, anti–type I IFN receptor [IFNAR] Ab, MAR1-5A3; BD Biosciences, San Jose, CA) (24) prior to each poly (I:C) injection to deplete pDCs and inhibit type I IFN–mediated signaling pathways, respectively. Control mice were treated with either 100 μg rat IgG (Sigma-Aldrich, St. Louis, MO) or mouse IgG (Sigma-Aldrich).

Cytokine and chemokine array analysis

Pancreatic lysates were prepared as described previously (25). Pancreatic lysates (100 μg) and serum (100 μl) were applied to a commercial membrane with bound mouse cytokine array panel A (R&D Systems, Minneapolis, MN) to screen for cytokine and chemokine profiles.

Flow cytometry analysis of the intrapancreatic immune cells

The pancreas was minced with scissors and shaken in RPMI 1640 buffer containing 10% FBS, 25 mmol/l HEPES, and 3 mg/ml collagenase (Wako, Osaka, Japan) at 37°C and 180 rpm for 30 min. The cell suspension was spun at 50 × *g* for 30 s to remove debris, followed by centrifugation with 30% Percoll solution (GE Healthcare, Tokyo, Japan) at 2000 rpm for 30 min at room temperature. Isolated pancreatic immune cells were stained with FITC, PE, or allophycocyanin-conjugated B220 Ab (eBioscience, San Diego, CA), pDC Ag (PDCA)-1 (eBioscience), Gr-1 Ab (eBioscience), CD3 Ab (eBioscience), CD11b Ab (BD Biosciences), CD19 Ab (BioLegend, San Diego, CA), sialic acid–binding Ig-like lectin (Siglec)-H Ab (BioLegend), CD11c Ab (BioLegend), rat IgG (BioLegend), or hamster IgG (BioLegend). Flow cytometric analysis was performed using an Accuri C6 flow cytometer (BD Biosciences) and CFlow Plus software (BD Biosciences).

Serum cytokine assay

Serum was subjected to separate ELISAs for a murine cytokine Ab array, IFN-α, IFN-β, BAFF (R&D Systems), and dsDNA Ab titer (Shibayagi Co., Gunma, Japan). Serum levels of anti-lactoferrin (LF) Ab titer were measured as previously described (26).

Histology and immunohistochemistry

H&E staining was performed on paraffin-embedded pancreas samples. Histopathological evaluation of pancreatic lesions was performed to assess the degree of inflammatory cell infiltration (22, 23, 27). Inflammatory infiltrates were scored as follows: 0, pancreas without mononuclear cell infiltration; 1, mononuclear cell aggregation and/or infiltration within the interstitium, with no parenchymal destruction; 2, focal parenchymal destruction with mononuclear cell infiltration; 3, diffuse parenchymal destruction but some intact parenchymal residue retained; and 4, almost all pancreatic tissue, except the pancreatic islets, destroyed or replaced with fibrosis or adipose tissue (22). Immunofluorescence staining was performed using anti-amylase (Sigma-Aldrich), anti–PDCA-1 (BioLegend), and anti-myeloperoxidase (MPO; Abcam, Eugene, OR) Abs followed by the incubation with matched secondary Abs (anti-rabbit or rat IgG–Alexa Fluor 488/546; Life Technologies, Carlsbad, CA) (28). Depletion of pDCs was confirmed by the immunohistochemical analysis by using anti–PDCA-1 Ab (BioLegend) or anti-Siglec H Ab (Abcam). In some analyses, dsDNA in NETs and nuclei were stained with Sytox Orange (Life Technologies) (28, 29).

Patient characteristics

We enrolled patients who were recently diagnosed with IgG4-RD and exhibited clinical features of AIP at Kyoto University Hospital and Kansai Medical University Hospital between January 2007 and September 2013. All human protocols were in compliance with the Declaration of Helsinki and approved by Kyoto University ethics committee. Written informed consent was obtained from each patient. Twenty patients were included (16 males and 4 females; age range, 42–83 y; median, 68 y). Resected pancreas samples were obtained from 4 patients, peripheral blood cell samples were obtained from 13 patients, and serum samples were obtained from 15 patients. All patients were diagnosed with IgG4-related AIP according to the Japanese diagnostic criteria (30). Patient serum IgG4 levels were much higher (range 226–1490 mg/dl; median 528.5 mg/dl) than the upper normal limit (135 mg/dl). In addition to healthy control samples, serum samples from patients with alcoholic chronic pancreatitis (n = 10) were also evaluated (all males; age range, 49–73 y; median 67 y).

Evaluation of serum IFN-α, BAFF, NETs, and anti-LF Ab titer

Serum samples from patients with IgG4-related AIP before therapeutic intervention (n = 11), healthy controls (n = 10), and patients with chronic pancreatitis (n = 10) were subjected to ELISA for measuring IFN-α (R&D Systems) and BAFF (Antigenix America, Huntington Station, NY). Serum NETs was quantified from the same samples using the Quant-iT PicoGreen dsDNA Reagent (Life Technologies) (28). Serum anti-LF Ab levels were also measured by ELISA (n = 15) (26).

Immunofluorescence staining of human pancreas samples

Paraffin-embedded pancreas tissue samples resected from patients with IgG4-related AIP (n = 4), patients with pancreatic cancer (as controls; sections were free of tumor invasion as confirmed by H&E; n = 8), and patients with alcoholic chronic pancreatitis (n = 9) were prepared. Immunofluorescence staining was performed using anti-CD123 (Miltenyi Biotec, Auburn, CA), anti-CD303 (blood DC Ag 2 [BDCA2]; Miltenyi Biotec), anti-IgG4 (Southern Biotechnology Associates, Birmingham, AL), anti–IFN-α (Miltenyi Biotec), anti-BAFF (R&D Systems), and anti-MPO (Abcam) Abs, followed by incubation with matched secondary Abs (anti-mouse/rabbit IgG–Alexa Fluor 488/546; Life Technologies) (28). In some analyses, dsDNA in NETs and nuclei were stained with Sytox Orange (Life Technologies) (28, 29).

Observation and measurement of NET formation

Human neutrophils (2×10^7/ml) were isolated from peripheral blood, and NET formation was visualized after stimulation with monosodium urate (MSU) crystals (InvivoGen; 50 μg/ml), rabbit anti-human LF IgG Ab (10 μg/ml; Sigma-Aldrich), or control rabbit IgG (10 μg/ml; Sigma-Aldrich). Quantitative analysis was performed by counting the number of NET-forming neutrophils per field under confocal laser scanning fluorescence microscopy (data are the mean from five randomly selected fields) (29).

In vitro coculture and evaluation of cytokines and Igs in culture supernatants

Neutrophils were isolated from the peripheral blood of healthy controls and patients with IgG4-related AIP. CD19-positive B cells and pDCs were isolated from PBMCs using CD19 and CD303 (BDCA2) MicroBeads

(Miltenyi Biotec), respectively. The purity of pDCs was >90% as assessed by flow cytometric analysis (data not shown). Neutrophils (1×10^6/ml) were incubated with MSU crystals (50 μg/ml) or rabbit anti-LF IgG Ab (10 μg/ml; Sigma-Aldrich) for 3 h. Purified B cells (1×10^6/ml) and pDCs (1×10^6/ml) were added to the wells and incubated for 7 d. In some experiments, DNase (3 U/μl; Sigma-Aldrich), anti-IFNAR Ab (1 μg/ml; PBL Biomedical Laboratories, Piscataway, NJ), or control IgG (1 μg/ml; Sigma-Aldrich) was added to the coculture. Culture supernatants were collected and subjected to ELISAs for measuring IFN-α, BAFF, and IgG1, IgG4, or IgA (16, 17).

Statistical analyses

For statistical analyses, *t* tests were performed using GraphPad Prism (GraphPad Software, La Jolla, CA). Differences with $p < 0.05$ were considered statistically significant.

Results

Type I IFN production and pDC infiltration characterize the pancreatitis induced by poly (I:C) in MRL/Mp mice, a model of AIP

In our initial studies, we used a well-established animal model of human AIP to identify the type of immune cells and cytokines that might be involved in the development of human AIP (27, 31). Consistent with previous reports (27, 31), MRL/Mp mice treated with poly (I:C) exhibited several important histological features of pancreatitis that were similar to human AIP. Thus, after treatment with poly (I:C), the pancreatic tissue of MRL/Mp mice exhibited a massive infiltration of immune cells, destruction of the pancreatic acinar architecture, and pancreatic fibrosis (Fig. 1A); consistent with these changes, treated mice exhibited significantly higher pancreatitis inflammatory scores than untreated mice (Fig. 1A). In addition, treated mice exhibited a marked elevation in serum autoantibody levels, including anti-dsDNA Ab and anti-LF Ab (Fig. 1B). Accompanying flow cytometric analysis revealed that treated mice as compared with untreated mice had a marked increase in the number of intrapancreatic $CD3^+$ T cells, $B220^+$ B cells, Gr-1^+ neutrophils, and $CD11b^+$ macrophages (Fig. 1C).

In further studies, we evaluated the cytokines and chemokines produced in mice with murine AIP. For this purpose, we analyzed serum or pancreatic lysates obtained from MRL/Mp mice with or without poly (I:C) treatment with cytokine and chemokine arrays. This analysis showed, as expected, that serum levels of prototypical proinflammatory cytokines such as TNF-α, IFN-γ, and IL-6 were elevated in mice treated with poly (I:C). More interestingly, this analysis also showed that poly (I:C) injection was accompanied by a marked increase in a group of five chemokines, CXCL9, CXCL10, CXCL11, CCL2, and CCL5 in both serum and pancreatic lysates that are tightly regulated by type I IFNs (Fig. 1D) (25, 32). These findings prompted us to measure IFN-α and IFN-β serum levels in mice treated with poly (I:C), and indeed, we found that poly (I:C) injection led to a marked elevation of IFN-α and IFN-β serum levels (Fig. 1E). These elevations also relate to the fact that serum levels of BAFF, a cytokine induced by type I IFN and considered to be involved in the immunopathogenesis of human IgG4-related AIP (16, 17), were also elevated upon injection of mice with poly (I:C).

We reasoned that pDCs in the inflamed pancreas of poly (I:C)–treated mice might be the source of the increased type I IFN production in these mice because pDCs are known to produce high levels of this cytokine (20). To examine this possibility, we conducted flow cytometric analysis of pancreatic infiltrates derived from treated and untreated mice and indeed found that pDCs, defined as $B220^{dim}$PDCA-1^+ cells (33, 34), were significantly increased in the pancreatic infiltrate of mice treated with poly (I:C) (Fig. 1F, *left* and *middle panels*). Consistent with previous reports regarding the cell-surface markers of pDCs (35, 36), triple-color stainings revealed that pancreatic $B220^{dim}$PDCA-1^+ pDCs were positive for both Siglec-H and CD11c and negative for CD19 (Supplemental Fig. 1). In addition, accompanying immunofluorescence analysis of pancreatic tissue from treated and untreated mice revealed increased accumulation of PDCA-1^+ pDCs in the pancreas of the treated mice (Fig. 1F, *right panel*). These findings led us to conclude that pDCs are greatly increased in the inflamed pancreas of mice treated with poly (I:C). In addition, as shown by pDC depletion studies described below, these pDCs are the major source of the greatly increased type I IFN produced by these treated mice.

Finally, we turned our attention to the possible mechanism of pDC stimulation in the inflamed pancreas of poly (I:C)–treated mice. Whereas poly (I:C) stimulation of pathogen recognition receptors in pDCs is likely to be one source of stimulation at least initially, two recent studies addressing the pathogenic roles of pDCs in SLE have provided evidence that neutrophil-associated NETs containing self-DNA as well as neutrophil-derived proteins are potent inducers of IFN-α production of pDC that is then involved in autoantibody production (37, 38). Based on these studies, plus the facts that the pancreatitis in MRL/Mp mice treated with poly (I:C) contains Gr-1^+ neutrophils and that serum Ab titer against LF, prototypical NET component (39), was elevated, we determined if NETs are present in the inflamed pancreatic tissue. In fact, we found that NETs as defined by colocalization of extracellular dsDNA and MPO were present in the pancreatic tissue of mice treated with poly (I:C), but not in control mice (Fig. 1G). These data thus suggest the possibility that pDCs producing IFN-α in the pancreas of MRL/Mp mice are being stimulated by NETs. Of interest, in the studies mentioned above (37, 38), type I IFN is an inducer of NETs so that the latter is part of a positive-feedback loop that is both involved in type I IFN induction and is itself induced by type I IFN.

Depletion of pDCs prevents AIP in MRL/Mp mice

The presence of pDCs and type I IFN in experimental AIP in MRL/Mp mice documented above led us to evaluate the role of these components in development of the AIP. To address this question, we determined the effect of multiple injections of 120G8 Ab, an Ab that has previously been shown to deplete pDCs (40), on AIP development. We found that repeated injections of 120G8 Ab were in fact effective in greatly reducing the number and percentages of pDCs in the pancreas of MRL/Mp mice as compared with mice treated with control Ab (Fig. 2A). The depletion of pDCs was further confirmed by the tissue-staining studies in which treatment with 120G8 Ab markedly reduced pancreatic infiltration of PDCA-1^+ or Siglec-H^+ cells (Supplemental Fig. 2). In addition, such depletion of pDCs prevented AIP development as assessed by pathological analysis (Fig. 2B) and reductions in the numbers of infiltrating T cells, B cells, and $CD11b^+$ macrophages in the pancreas (Fig. 2C). Perhaps more importantly, depletion of pDCs led to a marked decrease in systemic type I IFN–related responses, as assessed by greatly diminished production of IFN-α and BAFF (Fig. 2D). In addition, such depletion caused greatly reduced pancreatic NETs formation (Fig. 2E) as well as a significant reduction in serum Ab titers against LF, a prototypical NET component (Fig. 2F). These data thus suggested that pDCs play a critical role in the pathogenesis of AIP in MRL/Mp mice treated with poly (I:C).

IFN-α–mediated signaling pathway blockade prevents AIP in MRL/Mp mice

The pDC depletion studies above showed that depletion resulted in a significant reduction of type I IFN production in mice with experimental AIP but did not determine how such reduction of type I IFN led to reduced pancreatitis. To answer this question, we determined the effect of treating mice with neutralizing Ab against IFNAR (24) on the development of experimental AIP.

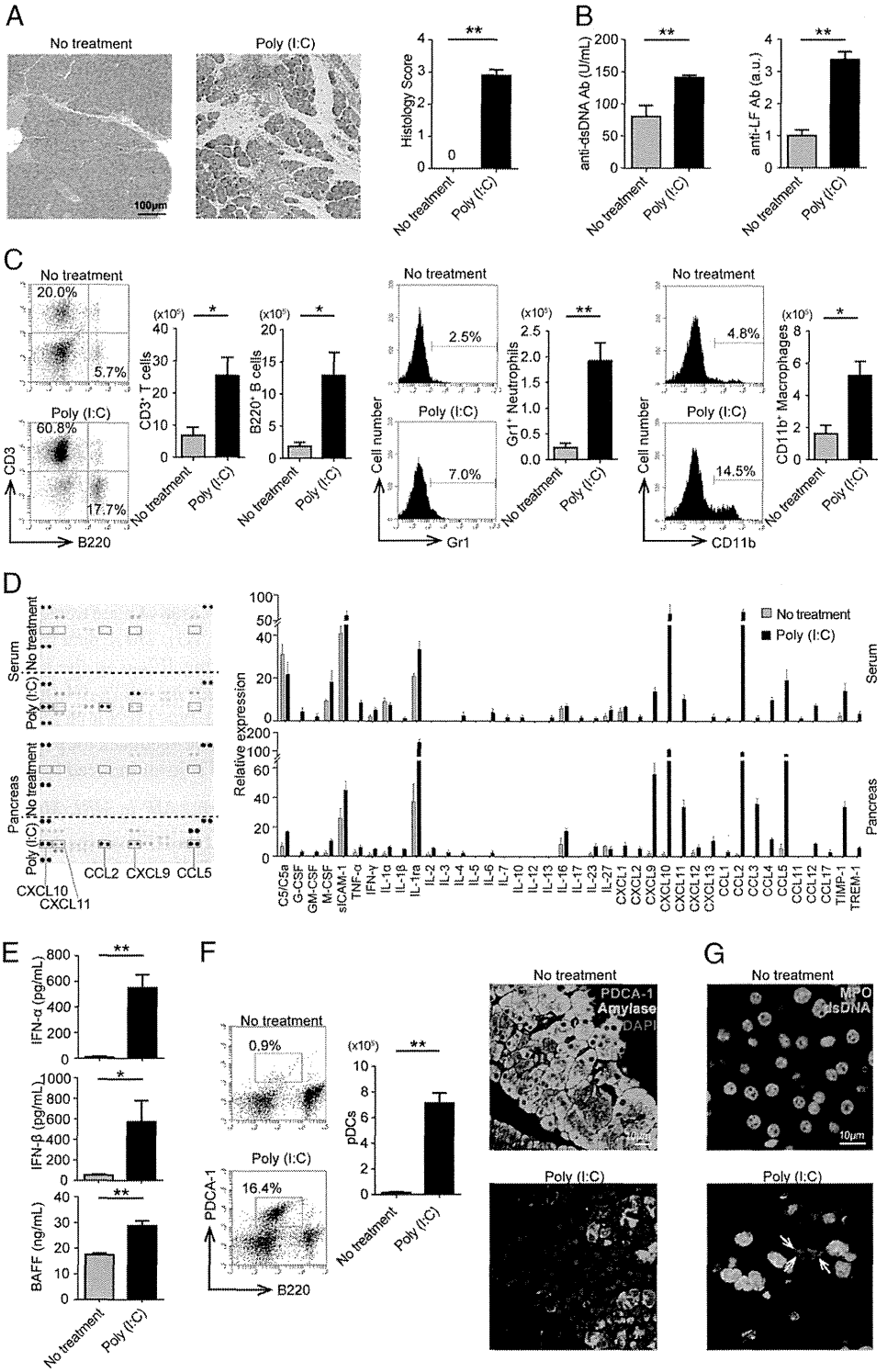

FIGURE 1. Massive increase in pDCs in the pancreas of MRL/Mp mice treated with poly (I:C). MRL/Mp mice ($n = 10$) received i.p. injections with 100 μg poly (I:C) twice a week for 8 wk (a total of 16 injections). Mice were sacrificed 3 h after the last injection. Nontreated mice ($n = 5$) were used as controls. (**A**) Pancreas pathology by H&E staining of nontreated MRL/Mp mice (*left panel*) and poly (I:C)–treated MRL/Mp mice (*middle panel*). Pancreatitis scores were assessed using the scoring system described in the *Materials and Methods* (*right panel*). (**B**) Serum autoantibody levels (anti-dsDNA Ab in the *left panel* and anti-LF Ab in the *right panel*) in mice treated with poly (I:C) and control mice ($n = 5$ each). The anti-LF Ab titer was defined as the ratio to the average control mouse titer (arbitrary units [a.u.]). (**C**) Flow cytometric analysis of pancreatic immune cells. The numbers and percentages of $CD3^+$ T cells, $B220^+$ B cells, $Gr\text{-}1^+$ neutrophils, and $CD11b^+$ macrophages are shown together with representative dot plots and histograms. The data shown were obtained from MRL/Mp mice treated with poly (I:C) ($n = 10$) and nontreated MRL/Mp mice ($n = 5$). (**D**) Serum and pancreatic lysate cytokine and chemokine arrays. Array analyses were performed using serum or pancreatic lysates. The relative expression of cytokines and chemokines in serum and pancreatic lysates are shown together with the representative images. The data presented were obtained from MRL/Mp mice treated with poly (I:C) ($n = 4$) and nontreated MRL/Mp mice ($n = 4$). Note that the duplicate dots and red squares indicate chemokines related to type I IFN production. (**E**) Serum levels of IFN-α, IFN-β, and BAFF in (*Figure legend continues*)

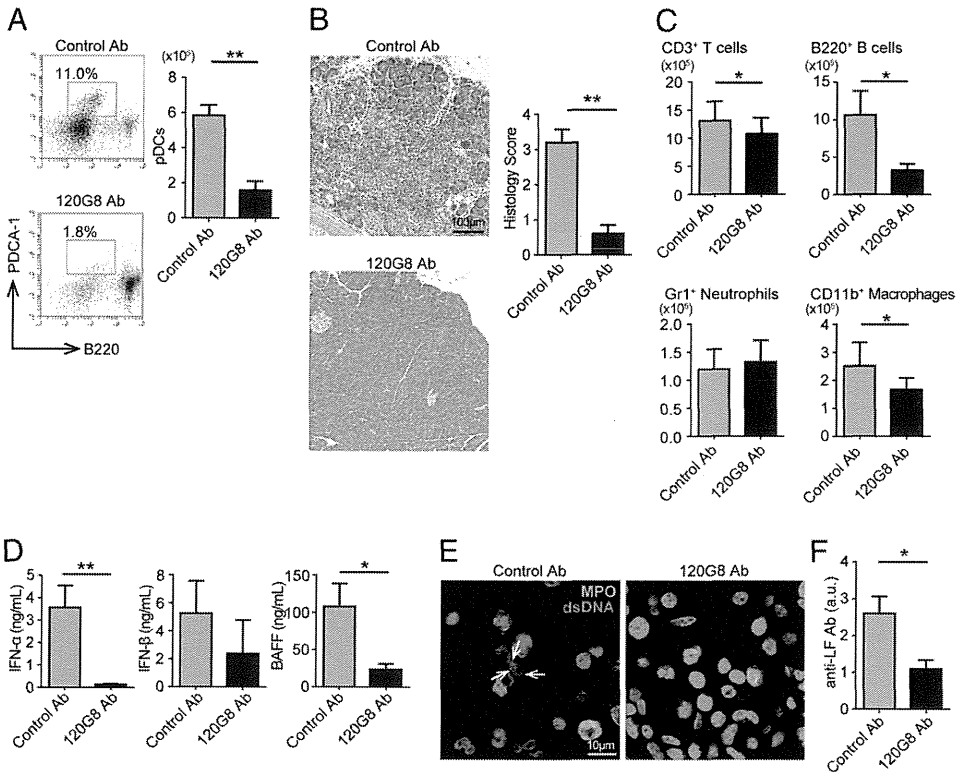

FIGURE 2. Depletion of pDCs prevents AIP in MRL/Mp mice treated with poly (I:C). MRL/Mp mice were treated with the pDC-depleting Ab (100 μg, 120G8; $n = 5$) or control Ab (100 μg; $n = 5$) prior to each poly (I:C) injection. Mice in each group received i.p. injections with 100 μg poly (I:C) twice a week for a total of 14 times. (**A**) Confirmation of pDC depletion by flow cytometry. Representative dot plots of flow cytometric analyses for the detection of pDCs are shown (*left panel*). The number of pDCs in the pancreas was determined as described in Fig. 1 (*right panel*). (**B**) Prevention of AIP development by the depletion of pDCs with 120G8 Ab. Representative images of H&E staining are shown in the *left panel*. The pancreatic pathology of MRL/Mp mice treated with 120G8 Ab or a control Ab was evaluated as described in Fig. 1 (*right panel*). (**C**) Flow cytometric analysis of pancreatic immune cells. The numbers and percentages of $CD3^+$ T cells, $B220^+$ B cells, $Gr\text{-}1^+$ neutrophils, and $CD11b^+$ macrophages were determined as described in Fig. 1. (**D**) Serum levels of IFN-α, IFN-β, and BAFF in mice treated with 120G8 Ab and control Ab. (**E**) Immunofluorescence detection of NETs visualized by extracellular colocalization of MPO and dsDNA. NET formation was indicated by white arrows. (**F**) Serum levels of anti-LF Ab titer were determined as described in Fig. 1 (arbitrary units [a.u.]). Results are expressed as the mean ± SE. $*p < 0.05$, $**p < 0.01$.

We found that such treatment of MRL/Mp mice inhibited AIP development to about the same extent as pDC depletion (Fig. 3A) and coincided with the fact that the number of pDCs in the pancreas was also significantly reduced, indicating that type I IFN production is involved in the pancreatic accumulation of pDCs (Fig. 3B). Consistent with this reduction in the number of pancreatic pDCs, serum levels of IFN-α and BAFF were significantly reduced in mice treated with the anti-IFNAR Ab, as was the case with mice treated with pDC-depleting Ab (Fig. 3C). Moreover, pancreatic NET formation and elevation of the serum anti-LF Ab titer was inhibited in mice treated with anti-IFNAR Ab (Fig. 3D). These data have several implications. First, they show that production of type I IFN (particularly IFN-α) is a critical factor in the development of experimental murine AIP. Second, they show that type I IFN production is required for the maintenance of the increased pancreatic infiltration of pDCs necessary for AIP development. The most likely explanation for this latter effect is that type I IFN is also necessary for NET generation, a possible activator of pDCs.

Pancreatic infiltration of IFN-α producing pDCs in patients with IgG4-related AIP

The studies of experimental AIP in MRL/Mp mice discussed above allowed us to probe the mechanism of human IgG4-related AIP; in particular, they allowed us to determine the relevance of pDC-mediated IFN-α signaling pathways in this disease.

In initial studies, we measured IFN-α levels in the serum of patients with IgG4-related AIP and control individuals. Serum IFN-α levels were markedly elevated in patients with IgG4-related AIP as compared with healthy controls or patients with chronic pancreatitis (Fig. 4A). Consistent with previous reports (41, 42), this elevation correlated with elevated serum BAFF levels (Fig. 4A).

We next conducted immunohistochemical studies of pancreatic tissue from patients with IgG4-related AIP who had undergone pancreatectomy ($n = 4$) as well as control pancreatic tissue from patients with pancreatic cancer (noncancerous tissue) ($n = 8$) and with alcoholic chronic pancreatitis ($n = 9$) to determine levels of pancreatic pDC infiltration in these diseases. The pancreatic tissues

mice treated with poly (I:C) and control mice ($n = 5$ each). (**F**) Pancreatic accumulation of pDCs. Representative dot plots of flow cytometric analyses; pancreatic immune cells were stained with B220 and PDCA-1. pDCs were defined as $B220^{dim}PDCA\text{-}1^+$ cells (*left panel*). The total number of pancreatic pDCs in mice treated with poly (I:C) and control mice ($n = 5$ each) (*middle panel*). Immunofluorescence stainings showing pancreatic infiltration of pDCs visualized as $PDCA\text{-}1^+$ cells. Pancreatic tissues were stained with amylase (green) and PDCA-1 (red) (*right panel*). Nuclei were counterstained with DAPI. (**G**) Immunofluorescence detection of NETs visualized by extracellular colocalization of MPO and dsDNA. NET formation was indicated by white arrows. Results are expressed as the mean ± SE. $*p < 0.05$, $**p < 0.01$.

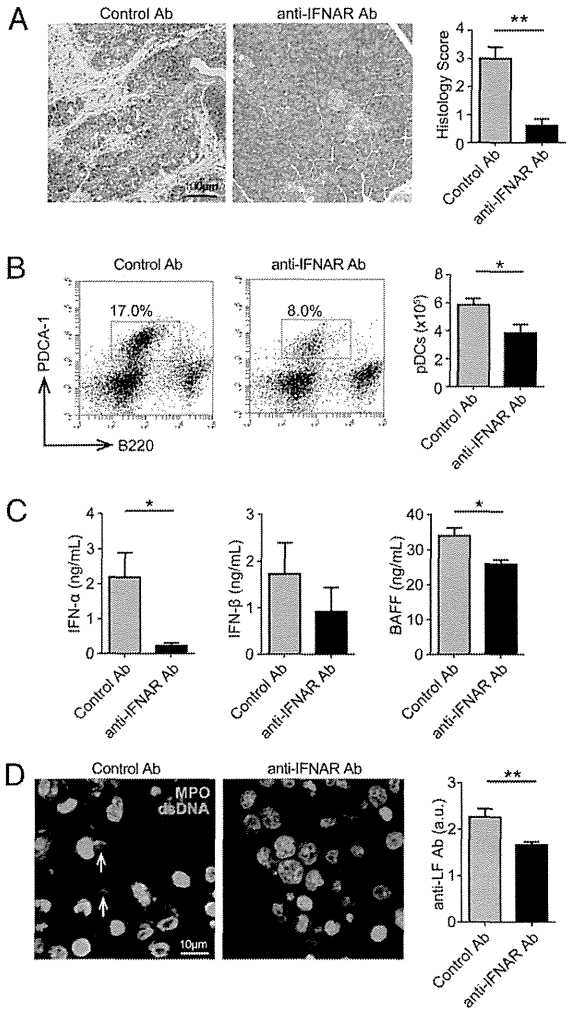

FIGURE 3. Blockade of IFN-α–mediated signaling pathways prevents AIP in MRL/Mp mice treated with poly (I:C). MRL/Mp mice were treated with a type I IFN receptor–neutralizing Ab (100 μg, anti-IFNAR Ab; $n = 5$) or a control Ab (100 μg; $n = 4$) prior to each poly (I:C) injection. Mice from each group received i.p. injections with 100 μg poly (I:C) twice a week for a total of 14 times. (**A**) Prevention of AIP development by anti-IFNAR Ab injection. Representative images by H&E staining are shown in the *left panel*. The pancreatic pathology of MRL/Mp mice treated with anti-IFNAR Ab or control Ab was evaluated as described in Fig. 1 (*right panel*). (**B**) Representative dot plots of flow cytometric analyses for the detection of pDCs (*left panel*). The number of pDCs in the pancreas was determined as described in Fig. 1 (*right panel*). (**C**) Serum levels of IFN-α, IFN-β, and BAFF in mice treated with anti-IFNAR Ab and control Ab. (**D**) Immunofluorescent detection of NETs visualized by extracellular colocalization of MPO and dsDNA. NET formation is indicated by white arrows. Serum levels of anti-LF Ab titer were determined as described in Fig. 1 (arbitrary units [a.u.]). Results are expressed as the mean ± SE. $*p < 0.05$, $**p < 0.01$.

of patients with IgG4-related AIP were distinguished from the control tissues by the fact that they displayed storiform fibrosis (Fig. 4B, *left panel*) as well as a massive infiltration of inflammatory cells enriched in IgG4$^+$ plasma cells (Fig. 4B, *right panel*). These characteristics were in line with the published Japanese criteria for the diagnosis of IgG4-related AIP (30). More importantly, the pancreatic tissues from patients with IgG4-related AIP, but not control pancreas tissues or chronic pancreatitis tissues, contained easily identified cells bearing CD123 and BDCA2, both of which are established markers of human pDCs (43) (Fig. 4C, Supplemental Fig. 3). In addition, the pancreatic BDCA2$^+$ pDCs from patients with IgG4-related AIP expressed both IFN-α and BAFF (Fig. 4D). These data suggest that pDCs are a major component of the pancreatic lesion in patients with IgG4-related AIP; in addition, because these cells express IFN-α, it is likely that pDC-derived IFN-α responses are one of the prominent features of IgG4-related AIP, as is the case in the murine AIP model described above.

NET formation in patients with IgG4-related AIP

Having confirmed that pDCs producing IFN-α are present in the inflamed pancreas of both experimental AIP and human IgG4-related AIP, we hypothesized that AIP in humans was similar to

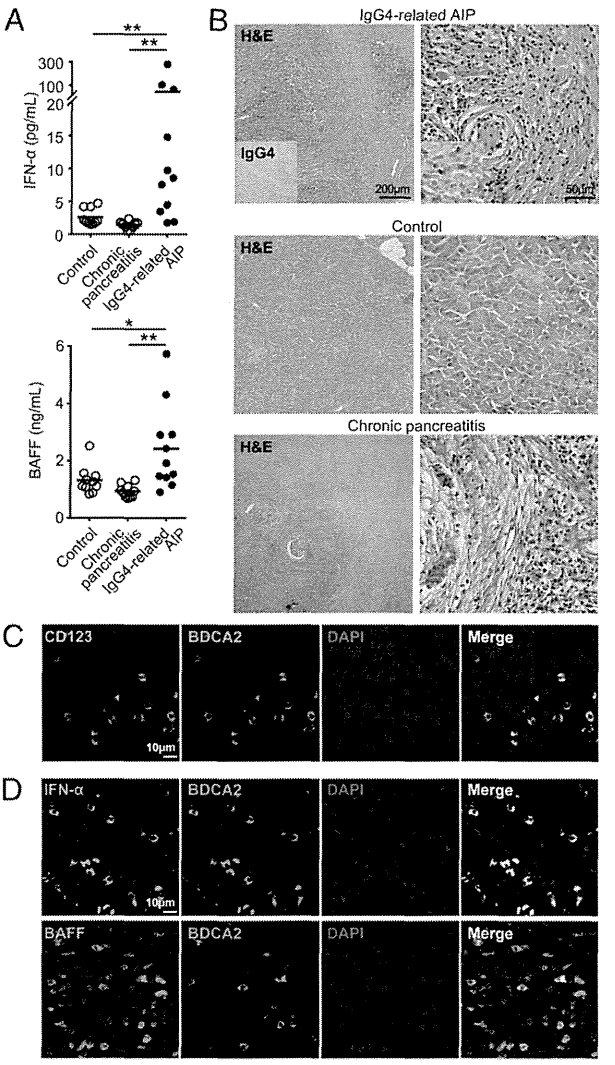

FIGURE 4. Serum levels of IFN-α and pancreatic localization of IFN-α–producing pDCs in patients with IgG4-related AIP. (**A**) Serum levels of IFN-α and BAFF in patients with IgG4-related AIP ($n = 11$), healthy controls ($n = 10$), and patients with chronic pancreatitis ($n = 10$). Each dot corresponds to the value of an assayed sample, and black bars indicate the mean values. (**B**) Massive infiltration of immune cells and storiform fibrosis in the pancreatic tissue specimens from patients with IgG4-related AIP (H&E staining, *top panel*). Accumulation of IgG4$^+$ plasma cells in the pancreas of patients with IgG4-related AIP was also observed (*inset in top panel*). (**C**) Localization of CD123$^+$BDCA2$^+$ pDCs in IgG4-related AIP pancreas tissue samples. (**D**) Localization of pDCs expressing IFN-α and BAFF as determined by double staining for BDAC2 with either IFN-α or BAFF in pancreatic tissues of patients with IgG4-related AIP. One representative image from four pancreas specimens from patients with IgG4-related AIP is presented (C and D). $*p < 0.05$, $**p < 0.01$.

that in mice in that NET formation in the pancreas also contributes to pDC activation and subsequent IFN-α production.

To examine this possibility, we initially performed immunohistochemical studies to determine whether NET formation occurs in the inflamed pancreas of IgG4-related AIP patients. To this end, we stained pancreatic tissue to identify both extracellular MPO and dsDNA using a method similar to that employed in the detection of NETs in the inflamed pancreas of MRL/Mp mice treated with poly (I:C). We found that pancreatic tissue from patients with IgG4-related AIP does contain extracellular NET structures characterized by dsDNA colocalized with MPO, whereas pancreatic tissues from controls and patients with chronic pancreatitis did not contain these structures (Fig. 5A). Serum NET concentrations, as determined by measuring serum dsDNA, were also higher in patients with IgG4-related AIP than in either healthy controls or patients with chronic pancreatitis, albeit with borderline significance (Fig. 5B). These data suggest that NET formation is in fact induced in pancreatic lesions of patients with IgG4-related AIP.

Recent studies have shown that neutrophils can be stimulated to produce NETs by immune complexes composed of proteins released by the neutrophils and autoantibodies recognizing these proteins or simply by the autoantibodies alone if they recognize neutrophil surface Ags (44). In addition, Okazaki et al. (26) have reported that Ab titers against LF, a NET component protein, were elevated in patients with AIP as well as in the mice with experimental AIP described above. With this information in hand, we next considered the possibility that NET formation in IgG4-related AIP pancreatic tissue is induced, at least in part, by LF–anti-LF Ab immune complexes. We found first that the serum anti-LF Ab titer was increased in the serum of patients with IgG4-related AIP and that this increase was especially prominent in Abs of the IgG4 subclass (i.e., the IgG subclass associated with this disease) (Fig. 5C). We then found that exposure of neutrophils from normal individuals to polyclonal anti-human LF Ab induced the formation of NETs to the same extent as exposure to MSU crystals, a well-established NET inducer (Fig. 5D, 5E). Thus, it seemed likely that NET formation in IgG4-related AIP pancreatic tissue is due, in part, to release of LF and the formation of LF–anti-LF Ab immune complexes (38, 45) (see further discussion below).

IgG4 production by NET-stimulated pDC

Having established that neutrophils in pancreatic lesions of patients with IgG4-related AIP produce NETs as well as the potential

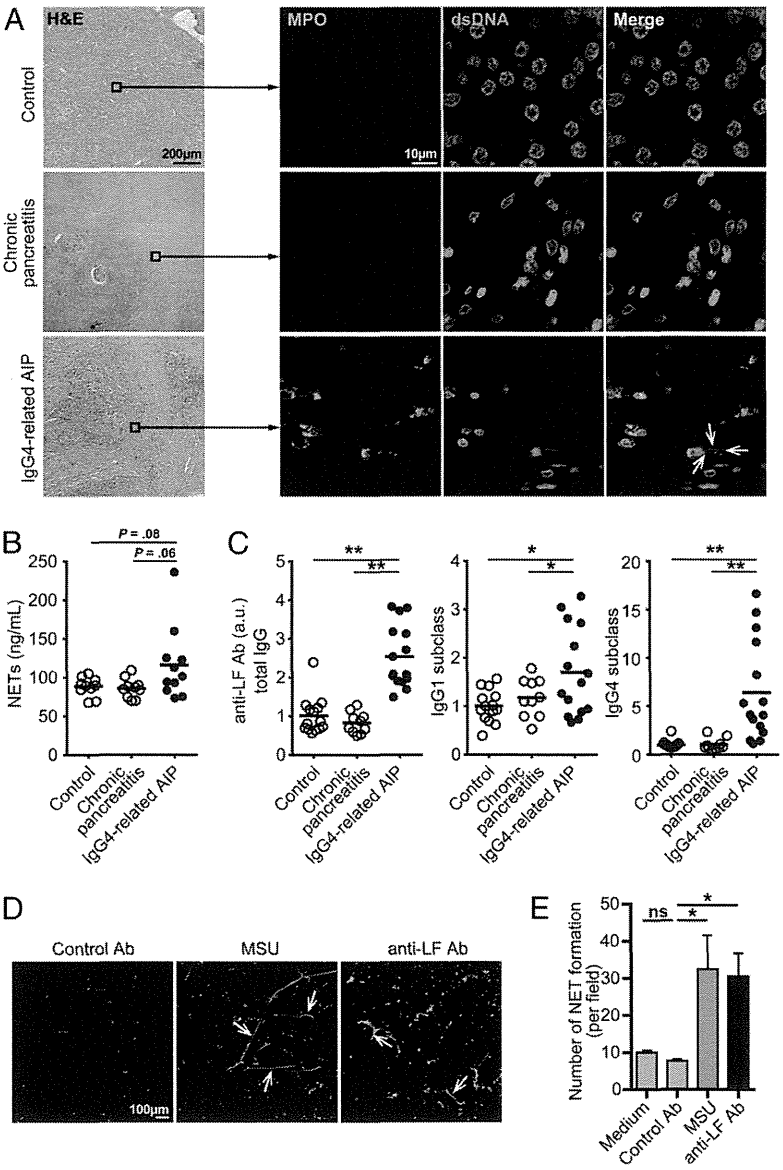

FIGURE 5. NET formation in patients with IgG4-related AIP. (**A**) Detection of pancreatic NET formation in samples obtained from patients with IgG4-related AIP. Resected pancreatic specimens from patients with IgG4-related AIP ($n = 4$), chronic pancreatitis ($n = 9$), and healthy controls ($n = 8$) were prepared. NETs were identified by immunofluorescence staining of MPO and dsDNA (Sytox Orange). White arrows show extracellular dsDNA (deposition of NETs). Note that NET formation was observed only in the IgG4-related AIP specimen (*bottom panels*). (**B**) Serum NET levels in samples from patients with IgG4-related AIP ($n = 11$), patients with chronic pancreatitis ($n = 10$), and healthy controls ($n = 10$). Each dot corresponds to the value of an assayed sample, and black bars indicate the mean values. (**C**) Serum anti-LF Ab titers in IgG4-related AIP ($n = 15$), patients with chronic pancreatitis ($n = 10$), and healthy controls ($n = 14$). LF-specific IgG, IgG1, and IgG4 Ab titers were determined by ELISA (arbitrary units [a.u.]). (**D** and **E**) NET induction by anti-LF Ab and MSU crystals. Neutrophils (2×10^7/ml) isolated from healthy controls ($n = 5$) were stimulated with MSU (50 μg/ml), anti-LF Ab (10 μg/ml), or control IgG (10 μg/ml) for 3 h to induce NET formation. NET release was visualized by confocal microscopy (white arrows) (D) and quantified by counting the number of NET-forming cells per field (E). Results are expressed as the mean ± SE. $^*p < 0.05$, $^{**}p < 0.01$.

relation between such NET formation and patient anti-LF Ab production, we next turned our attention to the role of NETs in pDC function. In initial studies along these lines, we determined the capacity of peripheral blood $CD19^+$ B cells from healthy control individuals to produce IgG4 when cocultured with neutrophils and/or pDCs in the presence or absence of NETs induced by the addition of MSU crystals to the culture. The results showed that in the presence of MSU-induced NETs and pDCs, healthy control B cells produced significantly greater amounts of IgG4 (but not IgG1 or IgA) than when cultured with pDCs alone (Fig. 6A). This enhanced IgG4 B cell response was accompanied by a marked increase in both IFN-α and BAFF production (Fig. 6B). The latter were likely due to NET-induced IFN-α production, because the increases were blocked by DNase inhibition of NET formation or by neutralization of IFN-α–mediated signaling pathways by anti-IFNAR Ab (Fig. 6C). In addition, the NETs themselves were not directly involved in IgG4 production because addition of IFN-α or BAFF to cultures of B cells from control individuals in the absence of NETs or pDCs also led to enhanced IgG4 production (data not shown).

Very similar results were obtained with cocultures in which NETs were induced by a polyclonal anti-LF IgG Ab instead of MSU in the absence of the addition of exogenous LF (Fig. 6D, 6E). Presumably the latter can occur because, as shown previously, the neutrophils are capable of producing LF that can then react with anti-LF Ab to form NET-stimulating immune complexes (46, 47). It should be noted, however, that IgG4 subclass isolated from patient serum did not have the capacity to induce NETs in vitro, probably because the concentration of anti-LF Ab in this preparation was too low (data not shown).

Taken together, these coculture studies provide evidence that NETs induced by MSU or anti-LF Ab lead to IgG4 Ab production by control B cells through a pDC IFN-α–mediated signaling pathway. It should be noted that such IFN-α/BAFF mediated IgG4 production could be due to either expansion of a pre-existing IgG4 B cell subset or to selective IgG4 class-switch differentiation plus expansion.

pDC activation by NETs plays an essential role in IgG4 generation

Although the above coculture studies using healthy control samples identified the important role of the neutrophil–pDC interaction in IgG4 production, they did not specifically address the contributions of the patient and control neutrophils or pDCs to such production.

To answer this question, we evaluated IgG4 production in cocultures containing neutrophils, pDCs, and B cells from both controls and patients with IgG4-related AIP in various combinations. The results indicated that IgG4 production by control B cells was markedly enhanced by cocultures containing pDCs from patients with IgG4-related AIP in the presence of MSU-induced NETs as compared with cultures containing pDCs from control individuals, regardless of the source of the NET-producing neutrophils (Fig. 7A). In addition, IFN-α and BAFF production were also markedly

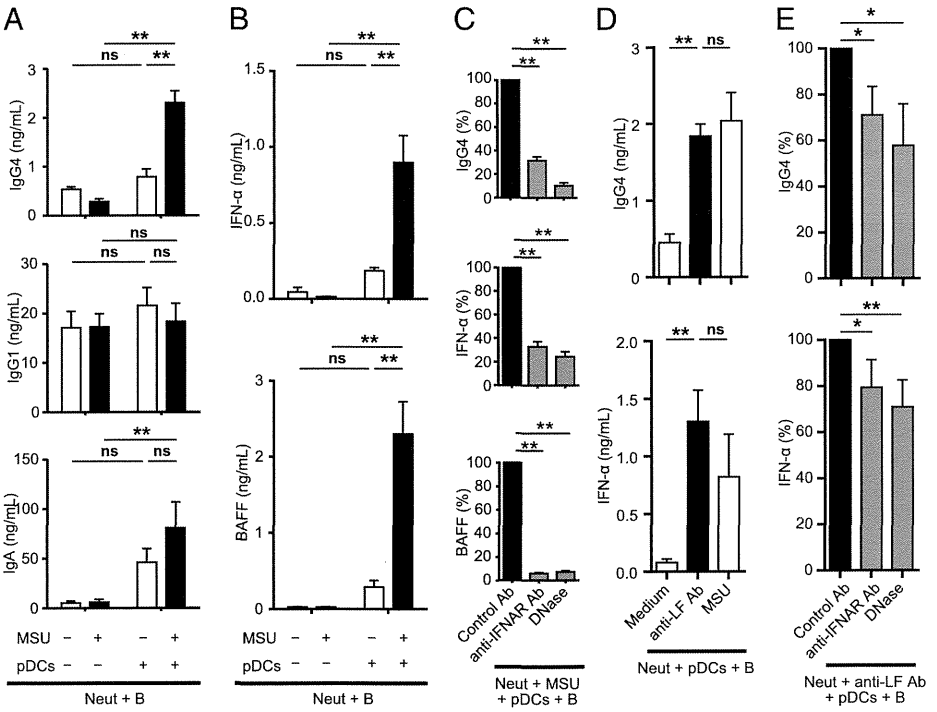

FIGURE 6. Enhanced IgG4 production by pDCs in response to NETs. (**A** and **B**) Ig production by B cells cocultured with NET-activated pDCs. Neutrophils (Neut), pDCs, and B cells were isolated from the peripheral blood of healthy controls ($n = 6$). Neutrophils (1×10^6/ml) were incubated with MSU crystals for 3 h to induce NET formation, after which pDCs (1×10^6/ml) and B cells (1×10^6/ml) were added to the coculture for 7 d and Ig production in the culture supernatants was measured (A). IFN-α and BAFF production in the culture supernatants was measured by ELISA (B). (**C**) Inhibition of IgG4 production by an anti–IFN-αβ receptor (IFNAR) Ab (1 μg/ml) and DNase (3 U/μl) in the coculture composed of MSU-induced NETs, pDCs, and B cells from healthy controls ($n = 6$). Production of IgG4, IFN-α, and BAFF in culture supernatants was measured by ELISA. Production of IgG4 or cytokines in the presence of control Ab and the absence of anti-IFNAR Ab or DNase was defined as 100% in each sample. (**D** and **E**) Ig production by B cells cocultured with pDCs activated by NETs, which were induced by an anti-LF Ab. (D) Neutrophils were incubated with anti-LF Ab (10 μg/ml) or MSU (50 μg/ml) for 3 h to induce NET formation, after which pDCs and B cells were added as described in (A). (E) Neutrophils were incubated with anti-LF Ab or MSU for 3 h to induce NET formation, after which pDCs and B cells were added in the presence of anti-IFNAR Ab and DNase for 7 d ($n = 4$). IgG4 and IFN-α production was measured in culture supernatants. Production of IgG4 or cytokines in the presence of control Ab and the absence of anti-IFNAR Ab or DNase was defined as 100% in each sample. Results are expressed as the mean ± SE. $*p < 0.05$, $**p < 0.01$.

elevated in supernatants from cocultures containing pDCs from patients with IgG4-related AIP, but not those containing pDCs from control individuals (Fig. 7A).

Consistent with the fact that control and patient neutrophils had an equal capacity to support IgG4 production in the coculture system, neutrophils isolated from healthy controls and patients with IgG4-related AIP exhibited equivalent amounts of NET formation upon exposure to MSU crystals (Fig. 7B). In addition, inhibition of NET formation by DNase or blockade of IFN-α–mediated signaling pathways by anti-IFNAR Ab significantly reduced IgG4 production in a coculture system comprised of control B cells, patient-derived neutrophils, and patient-derived pDCs (Fig. 7C). Finally, IgG4 production by control B cells was also enhanced in the presence of NETs induced by anti-LF Ab when the cells were cocultured with pDCs from patients with IgG4-related AIP (Fig. 7D), and this enhancement was again inhibited by the addition of DNase or by anti-IFNAR Ab (Fig. 7E). Taken together, these data suggest that pDCs, rather than neutrophils, have an indispensable role in the generation of IgG4 responses by B cells in patients with IgG4-related AIP and that NET-triggered pDC activation followed by IFN-α production is required for the development of IgG4-related AIP.

Discussion

The immunologic mechanisms underlying the pathogenesis of IgG4-RD as well as the increased production of the IgG4 subclass that characterizes this inflammatory disease are still poorly defined. In this study, we address this question with studies of mice with experimental AIP, MRL/Mp mice treated with poly (I:C), and studies of patients with IgG4-related AIP. The studies of the treated MRL/Mp mice showed that experimental AIP is characterized by a chronically inflamed pancreas exhibiting destruction of acinar architecture, massive infiltration of immune cells, and fibrosis; all findings were shared by human AIP. Although the pathogenic roles of proinflammatory cytokines such as IFN-γ, IL-6, and TNF-α are implicated in the development of such experimental AIP (31, 48), the roles of type I IFNs in this model had been poorly defined. In this study, we showed that experimental AIP is associated with the pancreatic infiltration of pDCs that have either migrated to or have

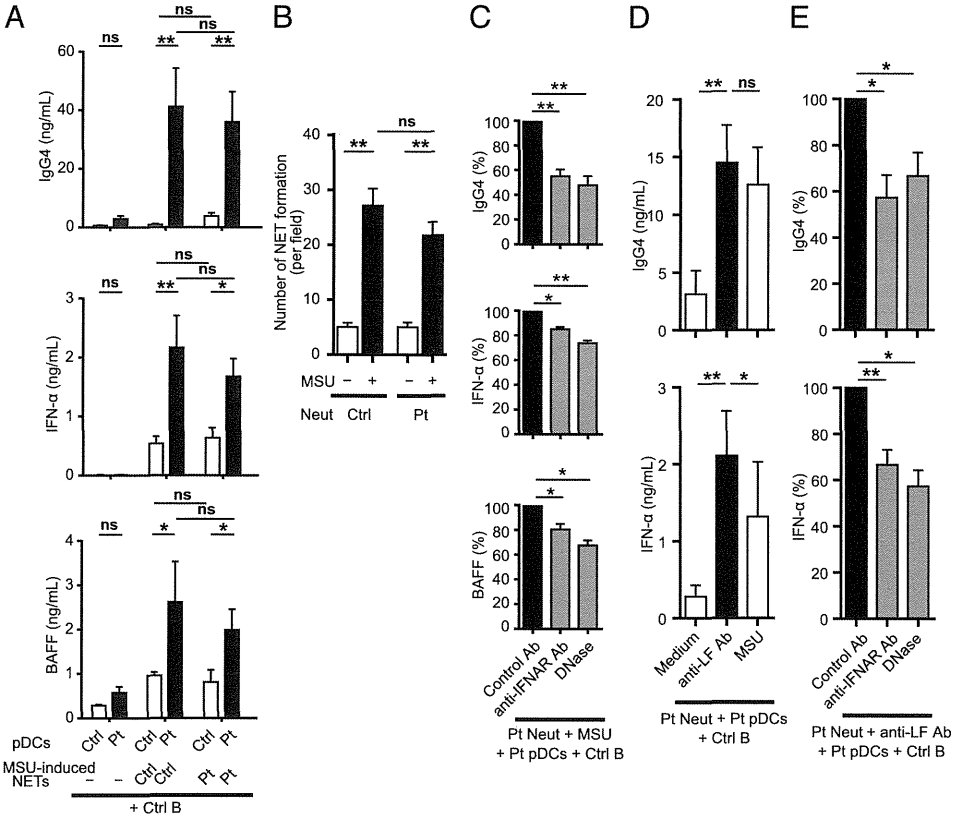

FIGURE 7. Enhanced NET-induced pDC activation in patients with IgG4-related AIP. (**A**) Coculture experiments composed of pDCs, B cells, and MSU-induced NETs were performed as described in Fig. 6A. Neutrophils isolated from healthy controls (Ctrl; $n = 6$) or patients with IgG4-related AIP (Pt; $n = 8$) were subjected to NET induction by MSU as described in Fig. 6. pDCs isolated from healthy controls (Ctrl) or patients with IgG4-related AIP (Pt) were subjected to the coculture assay together with B cells isolated from healthy controls (Ctrl B). IgG4, IFN-α, and BAFF production in the coculture supernatant was determined by ELISA. (**B**) Neutrophils (Neut) isolated from healthy controls (Ctrl; $n = 5$) and patients with IgG4-related AIP (Pt; $n = 5$) were subjected to NET induction by MSU, as described in Fig. 5. NET formation was determined by counting the number of NET-forming cells under high-power field. (**C**) Inhibition of IgG4 production by the addition of an anti–IFN-αβ receptor Ab (IFNAR Ab) and DNase. B cells from healthy controls (Ctrl B; $n = 6$) were cocultured with pDCs (Pt pDCs) and MSU-induced NETs (Pt Neut) derived from patients with IgG4-related AIP ($n = 6$) in the presence of anti-IFNAR Ab or DNase for 7 d, as described in Fig. 6C. Production of IgG4 or cytokines in the presence of control Ab and absence of anti-IFNAR Ab or DNase was defined as 100% in each sample. (**D** and **E**) Ig production by healthy control B cells (Ctrl B) cocultured with pDCs from patients with IgG4-related AIP (Pt pDCs) activated by NETs, which were induced by an anti-LF Ab. IgG4-related AIP patient-derived neutrophils (Pt Neut) were incubated with anti-LF Ab (10 μg/ml) or MSU (50 μg/ml) for 3 h to induce NET formation, after which Pt pDCs and Ctrl B cells were added (D). Pt Neut were incubated with anti-LF Ab for 3 h to induce NET formation, after which Pt pDCs and Ctrl B cells were added in the presence of the anti-IFNAR Ab and DNase for 7 d ($n = 4$) (E). IgG4 and IFN-α production in culture supernatants were measured as described in Fig. 6. Production of IgG4 or cytokines in the presence of control Ab and absence of anti-IFNAR Ab or DNase was defined as 100% in each sample. Results are expressed as the mean ± SE. $*p < 0.05$, $**p < 0.01$.