

Table 1 Lesions of various organs in IgG-related disease

Organ	Disease names	References
Pancreas	Autoimmune pancreatitis	5, 8, 20, 21
Salivary gland	Chronic sclerosing sialadenitis, Küttner tumour, Mikulicz disease	6
Lacrimal gland	Mikulicz disease	6, 19, 23
Biliary tract	Sclerosing cholangitis	7, 8
Peritoneum	Retroperitoneal fibrosis, ^a sclerosing mesenteritis	9
Pituitary gland	Autoimmune hypophysitis	11
Thyroid gland	Riedel thyroiditis, Hashimoto thyroiditis ^a	12
Lung	IgG4-related lung disease, interstitial pneumonia	13
Kidney	Tubulointerstitial nephritis	10
Prostate/testis	Epididymo-orchitis ^a	14
Aorta	Lymphoplasmacytic aortitis, inflammatory aneurysm	15, 16
Lymph node	Rosai-Dorfman disease ^a	17
Other	Orbital pseudotumour, eosinophilic angiocentric fibrosis	18

^aThese diseases are at least partly IgG4-related.

postulated to be IgG4-RDs. Macroscopically or radiologically, mass-forming, sclerosing lesions are commonly seen in the affected organs.^{1–4} Histopathologically, there are dense lymphoplasmacytic infiltrates, storiform fibrosis and moderate-to-moderate tissue eosinophilia.^{1,2} Systemic corticosteroids are effective for the conditions. Although IgG4-RD is prevalent in Asian countries and many reports have been reported from Japan, cases in white populations have also been documented.¹⁹

Some patients with IgG4-RD have skin lesions, and they are usually referred to dermatologists after the diagnosis of pancreatitis, Mikulicz disease or other conditions have been made. Alternatively, some patients present to dermatologists with skin lesions where the presence of IgG4-RD is not known. The skin manifestations of IgG4-RD have been poorly reported compared with those of other organs.² Although the skin lesions are called IgG4-related skin disease in some of the reports, there has been no comprehensive report on the relationship between the skin eruption types and pathogenesis. Furthermore, it is possible that some skin lesions of IgG4-RD have been historically diagnosed as other conditions showing plasma-cell infiltration or IgG deposition. For example, we have currently noticed that a considerable number of patients with cutaneous plasmacytosis can be diagnosed with IgG4-RD. In this review, we categorize IgG4-related skin disease based on the previous documentations and our experience, focusing on the aetiology and differential diagnoses.

History and definition of IgG4-related disease

Two different lines of organ-specific clinical observations for the pancreas and lacrimal/salivary glands initiated the concept of IgG4-RD as a novel clinical entity. Lymphoplasmacytic sclerosing pancreatitis was first described in 1991 for a tumorous lesion of the pancreas.²⁰ Subsequently, autoimmune pancreatitis was proposed to represent this condition. In 2001, high levels of serum IgG4 were found in patients with autoimmune pancreatitis.²¹

On the other hand, a swelling disorder of lacrimal and salivary glands was first described by Mikulicz in 1892. Since Morgan and Castleman's report in 1953, Mikulicz disease has been considered part of Sjögren syndrome.^{6,22} However, Mikulicz disease shows a unique persistent swelling of the lacrimal and salivary glands and a good therapeutic response to glucocorticoids. Mikulicz disease once again saw the light of day in 2004, when Yamamoto *et al.*²³ found serum IgG4 elevation in patients with Mikulicz disease. It is now accepted that IgG4-related Mikulicz disease is distinct from Sjögren syndrome.¹⁹ Autoimmune pancreatitis and Mikulicz disease triggered discovery of virtually the same disease occurring in various organs with high serum IgG4 levels, and this systemic disease has been termed IgG4-RD.

With regard to the diagnostic criteria, Umehara *et al.*¹ proposed interim criteria comprised of serum IgG4 levels > 35 mg dL⁻¹ and tissue infiltration of IgG4⁺ plasma cells in > 40% of total IgG⁺ plasma cells. It is noted that IgG4-RD is different from malignancies such as malignant lymphoma, Sjögren syndrome, primary biliary cirrhosis, Castleman disease, secondary retroperitoneal fibrosis, granulomatous polyangiitis and sarcoidosis.

Skin lesions of IgG4-related disease

Patients with IgG4-RD directly consulted us with skin lesions, or alternatively they were indirectly referred to us after the diagnosis of IgG4-RD was already made by other physicians. In 2012–13 we had 15 patients with IgG4-RD, but two patients were finally diagnosed with nodal lymphoma. Among 13 patients, four had no skin manifestation of IgG4-RD. Therefore, we analysed the remaining nine patients for IgG4-related skin disease (69% of all patients with IgG4-RD in our clinics). The frequency of the primary skin lesions was 56% (five of nine) and that of the second skin manifestations was 44% (four of nine). In this article, we present five of these nine cases.

Based on a review of literature and our own experience, we classified IgG4-related skin disease into seven types (Table 2). We first divided the skin lesions into primary eruptions with massive plasma-cell infiltrates and secondary eruptions without mass formation by plasma cells. The primary eruptions consisted of cutaneous plasmacytosis, pseudolymphoma, angiolymphoid hyperplasia with eosinophilia (ALHE) and Mikulicz disease. Cutaneous plasmacytosis has a distinguished clinical appearance with prominent plasma-cell infiltrates. Pseudolym-

Table 2 Types of skin lesions in IgG4-related disease

Type	Symptoms	Differential diagnoses	References
1 Cutaneous plasmacytosis	Multiple circular or ellipsoid patches with pigmentation	Multicentric Castleman disease	24–28, 30
2 Pseudolymphoma and angiolymphoid hyperplasia with eosinophilia	Plaques and papulonodules mainly on the periauricular and facial areas	B-cell pseudolymphoma, mucosa-associated lymphoid tissue syndrome	2, 31, 32, 37
3 Mikulicz disease or IgG4-related dacryoadenitis and sialadenitis	Palpebral swelling, sicca syndrome, exophthalmos	Sjögren syndrome	6, 19, 24, 38–40
4 Psoriasis-like eruption	Scaly erythematous plaques	Psoriasis vulgaris	41, 42
5 Unspecified maculopapular or erythematous eruptions	Multiple maculopapular or exudative erythematous lesions	Drug eruption, toxic erythema	45, 46
6 Hypergammaglobulinaemia, purpura and urticarial vasculitis	Bilateral palpable purpuric lesions, prolonged urticarial lesions	Anaphylactoid purpura, Sjögren syndrome, lupus erythematosus	47–49
7 Ischaemic digit	Raynaud phenomenon, digital gangrene	Systemic sclerosis, thrombosis, antiphospholipid syndrome	52

phoma and ALHE exhibit noncommittal types of plaques and papulonodules. Mikulicz disease may be first seen by dermatologists because of the presence of upper-eyelid swelling. The secondary eruptions include the following four heterogeneous manifestations. Psoriasis-like eruption strikingly mimics the plaque type of psoriasis, but plasma cells infiltrate the upper dermis. Unspecified maculopapular or erythematous eruption represents other plasma-cell-infiltrating lesions without mass formation. There have been purpuric eruptions reported in patients with IgG4-RD. The majority of patients show hypergammaglobulinaemic purpura with leucocytoclastic vasculitis, which may exhibit not only purpura but also urticaria. Finally, ischaemic digit is another secondary manifestation caused by vascular damage.

Based on the comprehensive criteria of IgG4-RD,¹ primary IgG4-related skin disease is defined as marked lymphocyte and plasmacyte infiltration with a ratio of IgG4⁺/IgG⁺ plasma cells > 40% and the number of IgG4⁺ plasma cells per high-power field > 10. Secondary IgG4-related skin disease is defined as plasmacyte infiltration with a ratio of IgG4⁺/IgG⁺ plasma cells > 40% and/or perivascular IgG4 deposition. Considering that fibrosis is less marked in skin lesions even in primary IgG4-related skin disease, fibrosis is not necessarily required.

Classification of IgG4-related disease

Type 1: cutaneous plasmacytosis

Cutaneous plasmacytosis is a representative, primary skin eruption of IgG4-RD. Even before the discovery of IgG4-RD, cutaneous plasmacytosis was known as a benign reactive proliferation of plasma cells, and cases of this disorder had been reported especially in Japan. Its common skin manifestation is

multiple red-brown papules/nodules and circular or ellipsoid indurations with prominent pigmentation, distributed over the trunk. It has recently been found that cutaneous plasmacytosis can be a skin manifestation of IgG4-RD (Fig. 1a), and at least four cases have been reported.^{24–27} An extremely high level of serum IgG4 and a marked skin infiltrate of plasma cells (Fig. 1b) positive for IgG4 (Fig. 1c) support the diagnosis of IgG4-RD.

In accordance with the higher incidence of IgG4-RD in Japan than in Western countries, cutaneous plasmacytosis affects mainly Asian subjects, especially Japanese middle-aged individuals.²⁸ In some cases of IgG4-RD presenting as cutaneous plasmacytosis, the involvement of organs other than the skin may be unremarkable,²⁶ suggesting that the skin is occasionally the primarily affected organ in IgG4-RD.

It is often difficult to distinguish IgG4-RD from multicentric Castleman disease.²⁹ While diagnosis of multicentric Castleman disease is usually based on hyperinterleukin (IL)-6 syndrome,²⁹ IgG4-RD shows normal or slightly high serum IL-6.³⁰ Involvement of the lymph nodes rather than pancreas and salivary/lacrimal glands suggests the diagnosis of multicentric Castleman disease.

Type 2: pseudolymphoma and angiolymphoid hyperplasia with eosinophilia

Sato *et al.*² have reported 10 cases of IgG4-RD presenting with erythematous plaques and nodules on the head and neck. We also experienced such a case of IgG4-related skin disease, showing papulonodules and indurative plaques (Fig. 2a) with mass-forming infiltration of lymphocytes and plasma cells (Fig. 2b). There are massive focal infiltrates (Fig. 2c) containing follicular germinal centres composed of CD20⁺ B cells (Fig. 2d). Such unspecified plaques and papulonodules can be

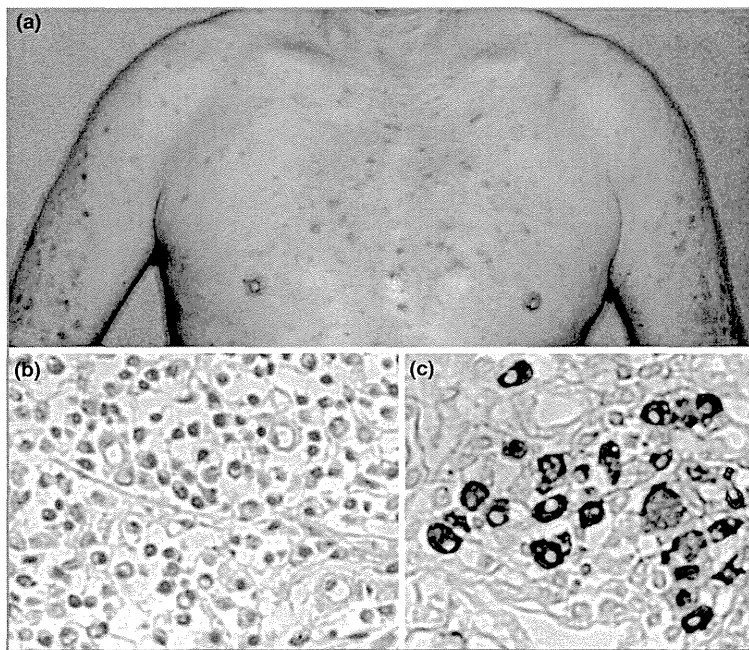


Fig 1. Cutaneous plasmacytosis. (a) A 63-year-old Japanese man had a 10-year history of papules and nodules on the trunk and four extremities. He had high levels of IgG (3629 mg dL^{-1} ; normal $870\text{--}1700$), IgG4 (1250 mg dL^{-1} ; normal $4.8\text{--}105$) and IgE (4591 kU L^{-1} ; normal < 160) and a high IgG4/IgG ratio (37.4%). Antinuclear antibody was negative, and serum interleukin-6 was normal (2.6 pg mL^{-1} ; normal, < 4.0). His lymph nodes were slightly enlarged, but there was no mass in the pancreas, kidneys or lung. (b) A skin biopsy specimen from a nodule showed infiltration of lymphocytes, plasma cells and eosinophils ($\times 200$, haematoxylin and eosin stain). (c) Immunostaining for IgG4 revealed that 54% of IgG⁺ cells were positive for IgG4 ($\times 100$). This case was reported by Yamaguchi *et al.*²⁶

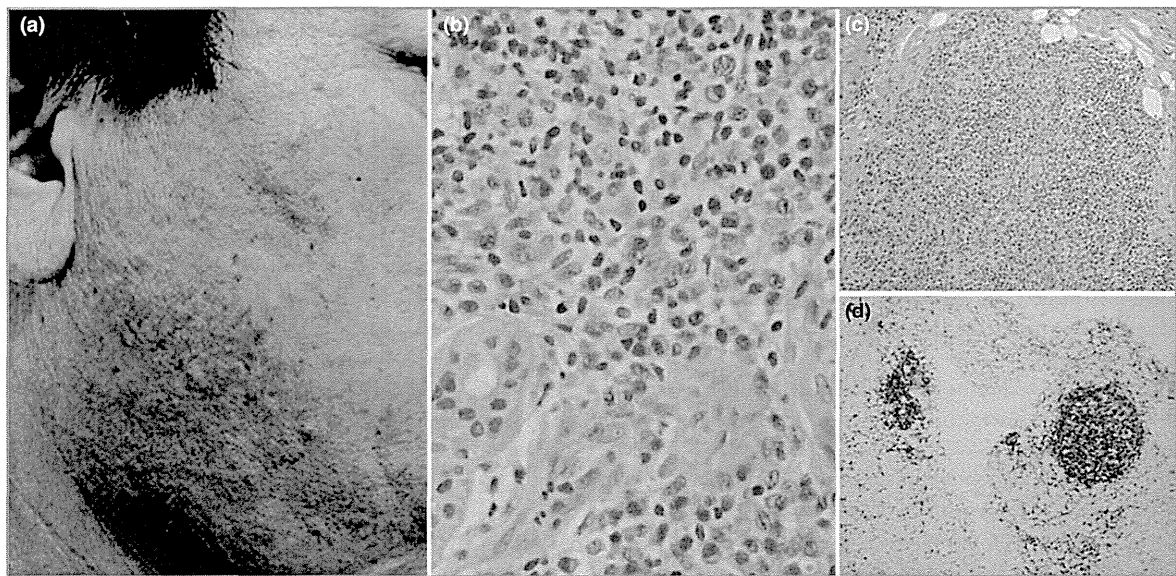


Fig 2. Pseudolymphoma. (a) A 64-year-old Japanese man had a 10-year history of nodular or indurative lesions on the cheeks, palpebrae, neck and upper limbs. The patient suffered from autoimmune pancreatitis. He had high levels of IgG (2127 mg dL^{-1}), IgG4 (1270 mg dL^{-1}) and IgE (466 kU L^{-1}) and a high IgG4/IgG ratio (59.7%). Serum interleukin-6 was normal (2.1 pg mL^{-1}). (b, c) Focal and massive infiltration of lymphocytes and plasma cells was observed around periadnexal and perifollicular areas. (d) Immunostaining for CD20 disclosed the presence of B-cell germinal centres with diffuse T-cell infiltration.

diagnosed as pseudolymphoma when they lack the monoclonality of infiltrating lymphocytes as assessed by immunoglobulin and T-cell-receptor gene rearrangements. When tissue-infiltrating plasma cells include IgG4⁺ cells as $\geq 40\%$ of total IgG⁺ cells, the diagnosis of IgG4-RD should be made.³¹ The most common sites of nodules/plaques of IgG4-RD include

the periauricular, cheek and mandible regions.² Ingen-Housz-Oro *et al.*³² reported two cases of cephalic nodules histologically characterized by pseudolymphoma with IgG4⁺ plasma-cell infiltrates. The patients had neither systemic involvement nor serum IgG4 elevation. Although these cases cannot fulfil the definite diagnostic criteria of IgG4-RD because of lack of

Fig 3. Mikulicz disease. (a) A 66-year-old Japanese woman suffered from renal dysfunction and presented with swelling of the bilateral upper eyelids. She had high levels of IgG (2470 mg dL^{-1}), IgG4 (811 mg dL^{-1}) and IgE (695 kU L^{-1}) with eosinophilia (12.1%) and a high IgG4/IgG ratio (32.8%). Serum creatinine was elevated (1.52 mg dL^{-1} ; normal, 0.4–0.8) and complement components were slightly decreased. 2-deoxy-2- ^{18}F fluoro-D-glucose positron emission tomography/computed tomography disclosed a diffuse inflammatory change in both kidneys. (b) A biopsy specimen from the patient's lacrimal gland revealed massive infiltration of lymphocytes and plasma cells (haematoxylin and eosin, $\times 400$). (c) Immunostaining for IgG4 showed infiltration of IgG4 $^{+}$ cells ($\times 400$). The ratio of IgG4 $^{+}$ /IgG $^{+}$ cells was approximately 70%.

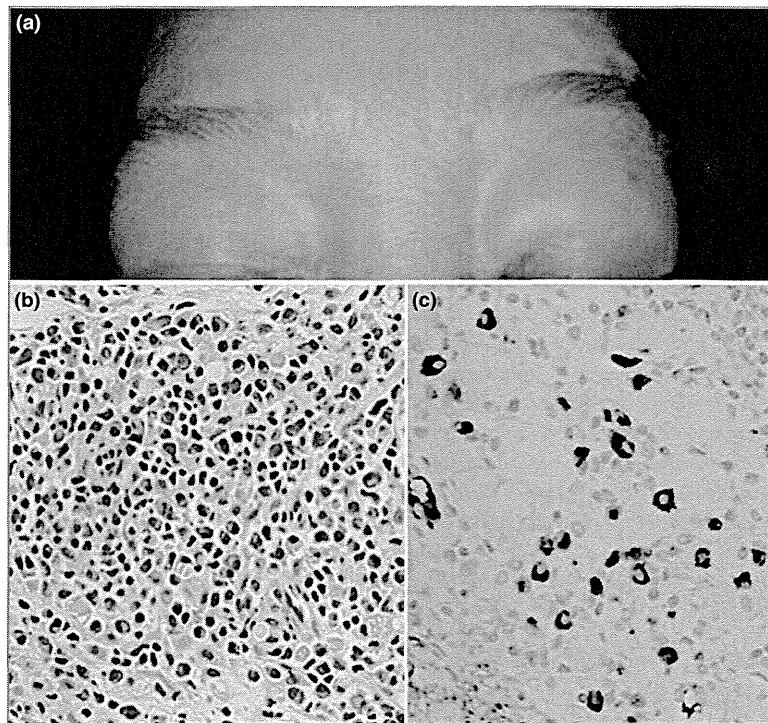
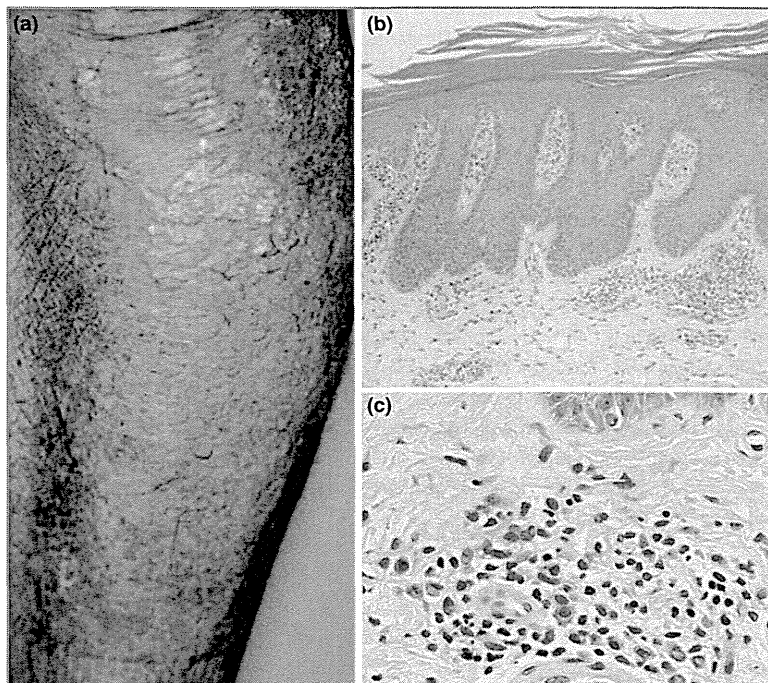


Fig 4. Psoriasis-like eruption. (a) A 65-year-old Japanese man was diagnosed as having cutaneous plasmacytosis, sclerosing kidney, and bile duct, liver and prostate tumours. Two years later, he developed scaly erythematous lesions on the lower limbs and trunk. He had high levels of IgG (3525 mg dL^{-1}), IgG4 (780 mg dL^{-1}) and IgE (2010 kU L^{-1}) and a high IgG4/IgG ratio (22.1%). Serum interleukin-6 was normal (4.4 pg mL^{-1}). (b) Histologically, there were hyperkeratosis, parakeratosis and marked elongation of rete ridges. Munro's microabscess was less remarkable than that of typical psoriasis (haematoxylin and eosin, $\times 50$). (c) Notably, both plasma cells and lymphocytes infiltrated perivascularly in the dermal papillae (haematoxylin and eosin, $\times 200$). Immunostaining for IgG and IgG4 disclosed an IgG4 $^{+}$ /IgG $^{+}$ ratio of approximately 60%.



serum IgG4 elevation, they can be diagnosed as probable IgG4-RD.¹ The authors suggested the presence of primary IgG4-related disease without other organ involvement.

Concerning the number of tissue-infiltrating IgG4 $^{+}$ plasma cells, elevated tissue IgG4 $^{+}$ to IgG $^{+}$ cell ratio is particularly important in the setting of relatively low tissue IgG4 $^{+}$ cell

density.³³ However, there is an opinion that the appropriate cut-off point may vary from organ to organ because of predominant fibrosis at the time when the diagnosis is made.³⁴ Thus, depending on the biopsied tissue, the number of IgG4 plasma cells per high-power field may vary from 10 to > 200.³⁵

A differential diagnosis is represented by the extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). In cutaneous MALT lymphoma, the infiltrate consists of lymphoplasmacytoid cells and monocytoid cells,³⁶ sharing histological features with IgG4-RD. The tumour cells show monoclonal gene rearrangement of immunoglobulin with the CD79a⁺, CD19⁺, CD20^{+/-} and cIg⁺ (κ or λ) phenotype.

ALHE, or Kimura disease, is characterized clinically by tumoral lesions on the head and neck. The histological features include proliferation of thick-walled blood vessels lined with prominent endothelial cells, infiltration of eosinophils, lymphocytes, histiocytes and plasma cells, and presence of lymphoid follicles with germinal centres. ALHE has been reported to be a skin lesion of IgG4-RD.³⁷ Kimura disease is characterized by eosinophilic infiltrate and healing-stage fibrosis, which share features with IgG4-RD lesions in other organs. However, not all cases of ALHE or Kimura disease are IgG4-RD, because we examined serum levels of IgG4 in some patients and found that the levels were normal.

Type 3: Mikulicz disease

Mikulicz disease is a representative of IgG4-RD, and many patients in Japan are treated by otolaryngologists. Dermatologists may see the patients because of palpebral swelling. This classical disease has been considered a subtype of Sjögren syndrome based on histopathological similarities. However, Mikulicz disease is unique in the persistent swelling of the lacrimal and salivary glands and the good therapeutic responsiveness to glucocorticoids. It is now recognized that Mikulicz disease is a subtype of IgG4-RD distinguishable from Sjögren syndrome, and is also called IgG4-related dacryoadenitis and sialoadenitis.⁶

Persistent bilateral swelling in the eyelids and submandibular region is commonly seen in Mikulicz disease (Fig. 3a).^{38,39} The patients may have sicca syndrome with lacrimal and parotid gland swelling. Histology shows infiltration of plasma cells (Fig. 3b) positive for IgG4 (Fig. 3c) around the small salivary glands. Sjögren syndrome is the most critical differential diagnosis, and laboratory examination including anti-SSA and anti-SSB antibodies is helpful for the diagnosis. It is also notable that palpebral swelling and exophthalmos mimic thyroid disease.⁴⁰

Type 4: psoriasis-like eruption

A couple of cases have shown that patients with IgG4-RD develop multiple hyperkeratotic erythematous plaques indistinguishable from chronic plaque psoriasis.^{41,42} There were typical histological findings, but plasma cells infiltrated perivascularly.⁴¹ We experienced a similar case of IgG4-RD, presenting with psoriatic lesions (Fig. 4a), psoriasiform epidermal changes (Fig. 4b) and a perivascular infiltrate of both plasma cells and lymphocytes (Fig. 4c). The reported case⁴¹ and ours are characterized by infiltration of plasma cells, some

of which bore IgG4, and deposition of IgG4 in the vascular endothelial cells. Notably, these and our own cases developed psoriasis-like eruption after long-standing sclerosing cholangitis and/or sclerosing kidney.^{41,42} As the frequency of psoriasis is generally high, it remains to be elucidated whether IgG4-RD and psoriasis are pathologically related or simply coincidental. Participation of IgG4⁺ plasma cells in the development of psoriatic lesions is supported by their infiltrate, but the psoriatic lesions are induced by T cells, especially T helper (Th) 17 cells.⁴³ The involvement of IgG4 in the pathogenesis still needs an explanation. It is well known that plasma cells

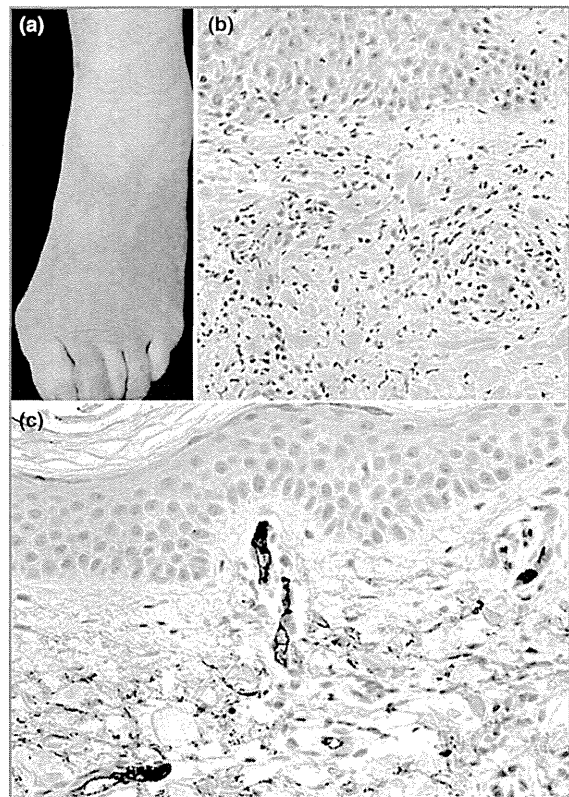


Fig 5. Hypergammaglobulinaemic purpura. (a) A 72-year-old Japanese woman suffered from arthralgia and photosensitivity and was tentatively diagnosed as having systemic lupus erythematosus approximately 30 years ago. She also had chronic pancreatitis and pancreatic tumour, diagnosed as autoimmune pancreatitis, and lacrimal and parotid gland swelling with sicca syndrome, diagnosed as Mikulicz disease. Three months before our first examination, she developed Raynaud phenomenon and gangrene of the fingers. Concomitantly, the patient exhibited a purpuric eruption on the lower legs and dorsum of the feet. She had high levels of IgG (2433 mg dL⁻¹) and IgG4 (1240 mg dL⁻¹) and a high IgG4/IgG ratio (51.0%). Antinuclear antibodies, antineutrophil cytoplasmic antibodies, cryoglobulin and lupus anticoagulant were all negative. (b) Histopathology showed leucocytoclastic vasculitis (haematoxylin and eosin, $\times 100$). (c) Immunostaining revealed deposition of IgG4 in the vessels of the upper dermis ($\times 100$). This case was reported by Ikawa *et al.*⁵²

infiltrate in psoriasiform eruption of secondary syphilis,⁴⁴ implying their role for acanthosis and hyperkeratosis.

Type 5: unspecified maculopapular or erythematous eruptions

This type of eruption tentatively encompasses nonmass-forming, plasma-cell-infiltrating dermatitis. The lesions are usually multiple, and histologically show upper-dermal infiltration of plasma cells positive for IgG4, but cases with an IgG4⁻ plasma-cell infiltrate have also been reported.⁴⁵ A characteristic histology of reactive perforating collagenosis was also documented.⁴⁶ Maculopapular or erythematous eruptions are usually associated with systemic involvement of the major organs, such as lacrimal and salivary glands, bile duct, lung and peritoneum.^{45,46}

Type 6: hypergammaglobulinaemic purpura and urticarial vasculitis

Leucocytoclastic vasculitis or urticarial vasculitis with IgG4 deposition has been reported to arise in patients with IgG4-RD.⁴⁷⁻⁴⁹ These cases can be diagnosed as hypergammaglobulinaemic purpura, and exhibit bilateral asymmetrical palpable purpuric lesions on the lower extremities (Fig. 5a). The symptom of urticarial vasculitis is a prolonged urticarial eruption occasionally with purpura. Urticarial vasculitis shares histology with hypergammaglobulinaemic purpura (Fig. 5b). Notably, hypergammaglobulinaemia is also caused by Sjögren syndrome or lupus erythematosus.⁵⁰ In addition, anaphylactoid purpura – also called Henoch–Schönlein purpura – also represents leucocytoclastic vasculitis and resembles IgG4-RD purpura. These diseases should be differentially diagnosed.

The tissue infiltration of IgG4⁺ plasma cells is one of the crucial diagnostic criteria of IgG4-RD. In the IgG4-related leucocytoclastic vasculitis, while IgG4 deposits in the vascular vessels (Fig. 4c), plasma cells do not infiltrate the purpuric

lesions. In IgG4-RD, purpura or petechiae may be induced alternatively by nonvasculitic diseases, represented by idiopathic or thrombotic thrombocytopenic purpura.^{10,51} An autoimmune mechanism has been postulated for the thrombocytopenia.

Type 7: ischaemic digit

Recent reports may indicate that IgG4-related large-vessel vasculitis is more common than widely realized, and the inflammation affects even thoracic and abdominal aortae, and iliac, mesenteric and splenic arteries.^{15,16} The luminal changes are mostly dilation but can be stenotic. Corticosteroid therapy rapidly diminished the arterial wall thickening in one report.¹⁵ IgG4-RD also affects arteries smaller than the aorta and its main branches, leading to ischaemic digits, the symptoms of which are Raynaud phenomenon and gangrenous fingers (Fig. 6a).⁵⁰ Stenosis of digital arteries was found (Fig. 6b). There was a report that both ischaemic digits and hypergammaglobulinaemic purpura were seen simultaneously in a patient with IgG4-RD.⁵² Differential diagnoses include systemic sclerosis and other autoimmune diseases, antiphospholipid syndrome and drug use.

Pathogenesis in relation to elevated IgG4 levels

In the seven types of cutaneous manifestations of IgG4-RD, cutaneous plasmacytosis, pseudolymphoma/ALHE and Mikulicz disease result from direct infiltrates of plasma cells and lymphocytes, and subsequent fibroinflammatory changes. Therefore, these three types are primary IgG4-related skin diseases as seen in the other organs.

Meanwhile, secondary IgG4-related skin disease shows inflammatory skin manifestations of systemic IgG4-RD. Psoriasis-like eruption may be the most difficult type to interpret in its mechanism. Although plasma cells infiltrate and IgG4

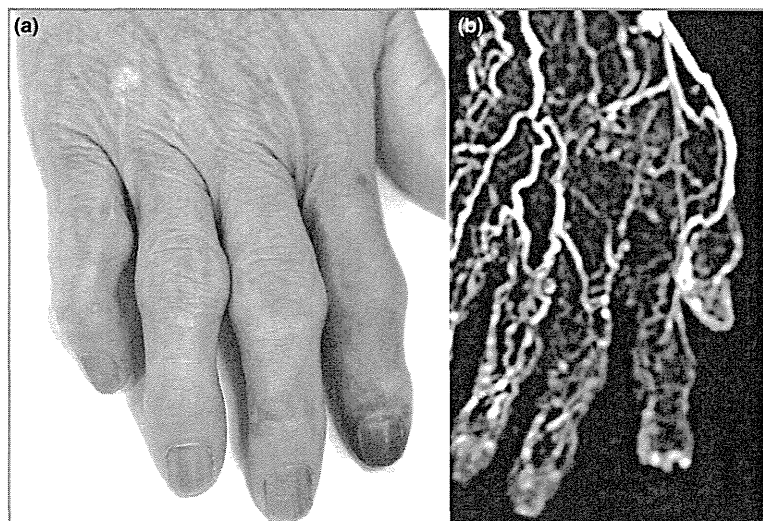


Fig 6. Ischaemic digit. (a) The same patient as shown in Figure 5. The patient had autoimmune pancreatitis, Mikulicz disease and hypergammaglobulinaemic purpura. In addition, she developed Raynaud phenomenon, cyanosis of the digits and gangrene of her right index finger. (b) Magnetic resonance angiography revealed ischaemic digital arteries of the hands, especially the index finger. This case was reported by Ikawa *et al.*⁵²

deposits in the vessels, it remains unclear how they can promote the Th17-mediated psoriatic lesion. Considering that plasma cells infiltrate in the psoriasiform eruption of secondary syphilis,⁴⁴ they have a potential role for the formation of acanthosis and hyperkeratosis. In hypergammaglobulinaemic purpura, IgG contributes to the formation of the immune complex, activating complement and leading to neutrophil accumulation and leucocytoclastic vasculitis. However, IgG4 is characterized by the very low ability to activate complement or to bind to the Fcγ receptor, indicating that IgG4 is less pathogenic than the other subclasses of IgG, such as IgG1 and IgG3.⁵³ Rather, IgG4 possibly may suppress the inflammatory responses. Thus, antigen binding by IgG4 likely has no or minimally harmful consequences. In pemphigus, binding of IgG to keratinocytes is sufficient to cause intraepidermal blisters without engaging innate immune effectors or complement, and IgG4 autoantibodies seem mainly to mediate acantholysis.⁵⁴ It seems that leucocytoclastic vasculitis is attributable to the other subclasses of IgG, such as IgG1 and IgG3, and immunohistochemical IgG4 reactivity in the vessels might indicate prominent deposition of all subclasses of IgG. Ischaemic digit is assumed to be a consequence of both primary and secondary changes. Given that the plasma-cell-mediated fibrotic tissue surrounding arteries causes the stenosis of arteries,^{15,16} it may be evaluated in the primary lesions.

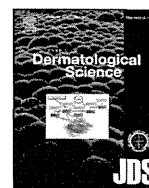
Similarly to IgE, the production of IgG4 is upregulated by Th2 cytokines, IL-4 and IL-13.²⁴ Accordingly, IgG4-RD usually shows elevated Th2 cytokines such as IL-4 and IL-5, leading to blood and tissue eosinophilia. In fact, approximately 40% of patients with IgG4-RD have elevated serum IgE and eosinophilia.² Furthermore, regulatory T cell (Treg) cytokines, IL-10 and transforming growth factor (TGF)-β, promote the IgG4 expression by B cells.^{55–57} In addition to the ability of TGF-β to produce IgG4, TGF-β is well known to induce fibrosis, potentially contributing to the pathological change in IgG4-RD.³² Given the poor functional activity of IgG4, it is tempting to speculate that IgG4 is a biomarker of IgG4-RD rather than a disease mediator. Exploring the roles of Th2 and Treg subsets in IgG4-RD and its skin manifestations is a highly promising field of investigation. A recent study suggested that circulating plasmablasts are elevated in active IgG4-RD, even in patients with normal serum IgG4 concentrations. Plasmablast counts are a potentially useful biomarker for diagnosis, assessing response to treatment, and determining the appropriate time for re-treatment.⁵⁸

We herein proposed comprehensive classification of IgG4-related skin disease with the differential diagnoses. Future study will further clarify each type and the diagnostic criteria in this newly established disease.

References

- Umehara H, Okazaki K, Masaki Y *et al.* Comprehensive diagnostic criteria for IgG4-related disease (IgG4-RD), 2011. *Mod Rheumatol* 2012; **22**:21–30.
- Sato Y, Takeuchi M, Takata K *et al.* Clinicopathologic analysis of IgG4-related skin disease. *Modern Pathol* 2013; **26**:523–32.
- Fernandez-Flores A. The role of IgG4 in cutaneous pathology. *Rom J Morphol Embryol* 2012; **53**:221–31.
- Cheuk W, Chan JK. IgG4-related sclerosing disease: a critical appraisal of an evolving clinicopathologic entity. *Adv Anat Pathol* 2010; **17**:303–32.
- Kamisawa T, Chari ST, Lerch MM *et al.* Recent advances in autoimmune pancreatitis: type 1 and type 2. *Gut* 2013; **62**:1373–80.
- Himi T, Takano K, Yamamoto M *et al.* A novel concept of Mikulicz's disease as IgG4-related disease. *Auris Nasus Larynx* 2012; **39**:9–17.
- Tanaka A, Tazuma S, Okazaki K *et al.* Nationwide survey for primary sclerosing cholangitis and IgG4-related sclerosing cholangitis in Japan. *J Hepatobiliary Pancreat Sci* 2014; **21**:43–50.
- Okazaki K, Uchida K, Ikeura T, Takaoka M. Current concept and diagnosis of IgG4-related disease in the hepato-bilio-pancreatic system. *J Gastroenterol* 2013; **48**:303–14.
- Laco J, Podhola M, Kamarádová K *et al.* Idiopathic vs. secondary retroperitoneal fibrosis: a clinicopathological study of 12 cases, with emphasis to possible relationship to IgG4-related disease. *Virchows Arch* 2013; **463**:721–30.
- Morimoto J, Hasegawa Y, Fukushima H *et al.* Membranoproliferative glomerulonephritis-like glomerular disease and concurrent tubulointerstitial nephritis complicating IgG4-related autoimmune pancreatitis. *Intern Med* 2009; **48**:157–62.
- Bando H, Iguchi G, Fukuoka H *et al.* The prevalence of IgG4-related hypophysitis in 170 consecutive patients with hypopituitarism and/or central diabetes insipidus and review of the literature. *Eur J Endocrinol* 2013; **170**:161–72.
- Watanabe T, Maruyama M, Ito T *et al.* Clinical features of a new disease concept, IgG4-related thyroiditis. *Scand J Rheumatol* 2013; **42**:325–30.
- Matsui S, Hebisawa A, Sakai F *et al.* Immunoglobulin G4-related lung disease: clinicoradiological and pathological features. *Respirology* 2013; **18**:480–7.
- Migita K, Miyashita T, Mizuno A *et al.* IgG4-related epididymo-orchitis associated with bladder cancer: possible involvement of BAFF/BAFF-R interaction in IgG4-related urogenital disease. *Mod Rheumatol* 2014; **24**:188–94.
- Inoue D, Zen Y, Abo H *et al.* Immunoglobulin G4-related periarteritis and periarteritis: CT findings in 17 patients. *Radiology* 2011; **261**:625–33.
- Stone JR. Aortitis, periarteritis, and retroperitoneal fibrosis, as manifestations of IgG4-related systemic disease. *Curr Opin Rheumatol* 2011; **23**:88–94.
- Liu L, Perry AM, Cao W *et al.* Relationship between Rosai-Dorfman disease and IgG4-related disease: study of 32 cases. *Am J Clin Pathol* 2013; **140**:395–402.
- Origuchi T, Yano H, Nakamura H *et al.* Three cases of IgG4-related orbital inflammation presented as unilateral pseudotumor and review of the literature. *Rheumatol Int* 2013; **33**:2931–6.
- Yao Q, Wu G, Hoschar A. IgG4-related Mikulicz's disease is a multiorgan lymphoproliferative disease distinct from Sjögren's syndrome: a Caucasian patient and literature review. *Clin Exp Rheumatol* 2013; **31**:289–94.
- Kawaguchi K, Koike M, Tsuruta K *et al.* Lymphoplasmacytic sclerosing pancreatitis with cholangitis: a variant of primary sclerosing cholangitis extensively involving pancreas. *Hum Pathol* 1991; **22**:387–95.
- Hamano H, Kawa S, Horiuchi A *et al.* High serum IgG4 concentrations in patients with sclerosing pancreatitis. *N Engl J Med* 2001; **344**:732–8.

- 22 Morgan WS, Castleman B. A clinicopathologic study of 'Mikulicz's disease'. *Am J Pathol* 1953; **29**:471–503.
- 23 Yamamoto M, Ohara M, Suzuki C *et al.* Elevated IgG4 concentrations in serum of patients with Mikulicz's disease. *Scand J Rheumatol* 2004; **33**:432–3.
- 24 Miyagawa-Hayashino A, Matsumura Y, Kawakami F *et al.* High ratio of IgG4-positive plasma cell infiltration in cutaneous plasmacytosis – is this a cutaneous manifestation of IgG4-related disease? *Hum Pathol* 2009; **40**:1269–77.
- 25 Honda R, Cerroni L, Tanikawa A *et al.* Cutaneous plasmacytosis: report of 6 cases with or without systemic involvement. *J Am Acad Dermatol* 2013; **68**:978–85.
- 26 Yamaguchi H, Moriki M, Ito T, Tokura Y. Cutaneous plasmacytosis as a skin manifestation of IgG4-related disease. *Eur J Dermatol* 2013; **23**:560–2.
- 27 Kato K, Satoh T, Tanaka-Fujimoto T *et al.* IgG4-positive cells in skin lesions of cutaneous and systemic plasmacytosis. *Eur J Dermatol* 2013; **23**:255–6.
- 28 Shimizu S, Tanaka M, Shimizu H, Han-yaku H. Is cutaneous plasmacytosis a distinct clinical entity? *J Am Acad Dermatol* 1997; **36**:876–80.
- 29 Haque M, Hou JS, Hisamichi K *et al.* Cutaneous and systemic plasmacytosis vs. cutaneous plasmacytic Castleman disease: review and speculations about pathogenesis. *Clin Lymphoma Myeloma Leuk* 2011; **11**:453–61.
- 30 Takeuchi M, Sato Y, Takata K *et al.* Cutaneous multicentric Castleman's disease mimicking IgG4-related disease. *Pathol Res Pract* 2012; **208**:746–9.
- 31 Cheuk W, Lee KC, Chong LY *et al.* IgG4-related disease: a potential new etiology of cutaneous pseudolymphoma. *Am J Surg Pathol* 2009; **33**:1713–19.
- 32 Ingen-Housz-Oro S, Ortonne N, Elhai M *et al.* IgG4-related skin disease successfully treated by thalidomide: a report of 2 cases with emphasis on pathological aspects. *JAMA Dermatol* 2013; **149**:742–7.
- 33 Lehman JS, Pittelkow MR, Smyrk TC. IgG4-related skin disease. *JAMA Dermatol* 2013; **149**:1439–40.
- 34 Deshpande V, Zen Y, Chan JK *et al.* Consensus statement on the pathology of IgG4-related disease. *Mod Pathol* 2012; **25**:1181–92.
- 35 Ingen-Housz-Oro S, Ortonne N, Chosidow O. IgG4-related skin disease – reply. *JAMA Dermatol* 2013; **149**:1440.
- 36 Suárez AL, Pulitzer M, Horwitz S *et al.* Primary cutaneous B-cell lymphomas: part I. Clinical features, diagnosis, and classification. *J Am Acad Dermatol* 2013; **69**:329e1–13.
- 37 Hamaguchi Y, Fujimoto M, Matsushita Y *et al.* IgG4-related skin disease, a mimic of angiolymphoid hyperplasia with eosinophilia. *Dermatology* 2011; **223**:301–5.
- 38 Nanke Y, Kobashigawa T, Yago T *et al.* A case of Mikulicz's disease, IgG4-related plasmacytic syndrome, successfully treated by corticosteroid and mizoribine, followed by mizoribine alone. *Intern Med* 2010; **49**:1449–53.
- 39 Aoki A, Sato K, Itabashi M *et al.* A case of Mikulicz's disease complicated with severe interstitial nephritis associated with IgG4. *Clin Exp Nephrol* 2009; **13**:367–72.
- 40 Inaba H, Hayakawa T, Miyamoto W *et al.* IgG4-related ocular adnexal disease mimicking thyroid-associated orbitopathy. *Intern Med* 2013; **52**:2545–51.
- 41 Kuboyama T, Nakamura A, Harada K *et al.* Co-existence of psoriasis-like lesions during treatment of IgG4-related sclerosing cholangitis. *Jpn J Dermatol* 2011; **121**:869–74.
- 42 Ramachandran R, Rajakumar V, Rawat A *et al.* IgG4-related tubulointerstitial nephritis presenting with psychiatric manifestations and skin lesions. *Int Urol Nephrol* 2014; **46**:235–8.
- 43 Tokura Y, Mori T, Hino R. Psoriasis and other Th17-mediated skin diseases. *J UOEH* 2010; **32**:317–28.
- 44 Pandhi RK, Singh N, Ramam M. Secondary syphilis: a clinicopathologic study. *Int J Dermatol* 1995; **34**:240–3.
- 45 Ikezawa Y, Kambara T, Matukura S *et al.* Two cases of IgG4-related disease accompanied by pruritic eruptions with an infiltration of plasmacytes and eosinophils into the dermis. *Jpn J Dermatol* 2013; **123**:17–24.
- 46 Shiomi T, Yoshida Y, Horie Y, Yamamoto O. Acquired reactive perforating collagenosis with the histological features of IgG4-related sclerosing disease in a patient with Mikulicz's disease. *Pathology Int* 2009; **59**:326–31.
- 47 Tamai R, Hasegawa Y, Hisano S *et al.* A case of IgG4-related tubulointerstitial nephritis concurrent with Henoch-Schönlein purpura nephritis. *Allergy Asthma Clin Immunol* 2011; **7**:5–9.
- 48 Naitoh I, Nakazawa T, Ohara H *et al.* Autoimmune pancreatitis associated with various extrapancreatic lesions during a long-term clinical course successfully treated with azathioprine and corticosteroid maintenance therapy. *Intern Med* 2009; **48**:2003–7.
- 49 Wakamatsu R, Watanabe H, Suzuki K *et al.* Hypocomplementemic urticarial vasculitis syndrome is associated with high levels of serum IgG4: a clinical manifestation that mimics IgG4-related disease. *Intern Med* 2011; **50**:1109–12.
- 50 Finder KA, McCollough ML, Dixon SL *et al.* Hypergammaglobulinemic purpura of Waldenström. *J Am Acad Dermatol* 1990; **23**:669–76.
- 51 Taniguchi T, Hamasaki A, Okamoto M. A case of suspected lymphocytic hypophysitis and organizing pneumonia during maintenance therapy for autoimmune pancreatitis associated with autoimmune thrombocytopenia. *Endocr J* 2006; **53**:563–6.
- 52 Ikawa T, Kasuya A, Hirakawa S, Tokura Y. Raynaud phenomenon, digital gangrene and hypergammaglobulinaemic purpura occurring in a patient with IgG4-related disease. *Br J Dermatol* 2011; **165**:1364–6.
- 53 Schroeder HW Jr, Cavacini L. Structure and function of immunoglobulins. *J Allergy Clin Immunol* 2010; **125** (Suppl. 2):S41–52.
- 54 Sitaru C, Mihai S, Zillikens D. The relevance of the IgG subclass of autoantibodies for blister induction in autoimmune bullous skin diseases. *Arch Dermatol Res* 2007; **299**:1–8.
- 55 Zen Y, Fujii T, Harada K *et al.* Th2 and regulatory immune reactions are increased in immunoglobulin G4-related sclerosing pancreatitis and cholangitis. *Hepatology* 2007; **45**:1538–46.
- 56 Nakashima H, Miyake K, Moriyama M *et al.* An amplification of IL-10 and TGF- β in patients with IgG4-related tubulointerstitial nephritis. *Clin Nephrol* 2010; **73**:385–91.
- 57 Takeuchi M, Sato Y, Ohno K *et al.* T helper 2 and regulatory T-cell cytokine production by mast cells: a key factor in the pathogenesis of IgG4-related disease. *Mod Pathol* 2014; **27**:1126–36.
- 58 Wallace ZS, Mattoo H, Carruthers M *et al.* Plasmablasts as a biomarker for IgG4-related disease, independent of serum IgG4 concentrations. *Ann Rheum Dis* 2014 [Epub ahead of print].



VEGF-A promotes IL-17A-producing $\gamma\delta$ T cell accumulation in mouse skin and serves as a chemotactic factor for plasmacytoid dendritic cells



Takahiro Suzuki^a, Satoshi Hirakawa^a, Takatoshi Shimauchi^a, Taisuke Ito^a, Jun-ichi Sakabe^a, Michael Detmar^b, Yoshiki Tokura^{a,*}

^aDepartment of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan

^bInstitute of Pharmaceutical Sciences, Swiss Federal Institute of Technology, ETH Zurich, Zurich, Switzerland

ARTICLE INFO

Article history:

Received 11 September 2013

Received in revised form 27 December 2013

Accepted 27 December 2013

Keywords:

VEGF

IL-17

$\gamma\delta$ T cell

Plasmacytoid dendritic cell

Chemotaxis

Psoriasis

ABSTRACT

Background: IL-17-producing CD4⁺ T (Th17) cells and their cytokines, IL-17A and IL-22, are deeply involved in the pathogenesis of psoriasis by stimulating epidermal keratinocytes to proliferate and to produce cytokines/chemokines and vascular endothelial growth factor (VEGF)-A. Plasmacytoid dendritic cells (pDCs), infiltrating in psoriatic lesions, are known to exacerbate the Th17-mediated pathogenesis of psoriasis.

Objective: To address the initiative role of VEGF-A in the development of psoriasis and the pDC accumulation.

Methods: Numerical changes and VEGF receptor 1 (VEGFR1) and VEGFR2 expressions were investigated in skin-infiltrating T cells and pDCs of K14-VEGF-A transgenic (Tg) and wild type (WT) mice. The chemotactic properties of VEGF-A for purified splenic pDCs were also evaluated by real-time chemotaxis assay.

Results: By flow cytometry and immunohistochemistry, we observed that the number of dermal IL-17A⁺ $\gamma\delta$ T cells, but not CD4⁺ T cells, was increased in VEGF-A Tg mice, suggesting that the main source of IL-17A was $\gamma\delta$ T cells. Moreover, we identified pDCs as 440c⁺ cells by immunohistochemistry and as PDCA-1⁺B220⁺ cells by flow cytometry, and found that pDCs infiltrated at a higher frequency in VEGF-A Tg than WT mice. pDCs, but not $\gamma\delta$ T cells, isolated from the skin expressed VEGFR1 and VEGFR2. Freshly isolated splenic pDCs expressed both receptors after 48-h cultivation. pDCs did not produce cytokines in response to VEGF-A, however, they had a strong velocity of chemotaxis toward VEGF-A at a comparable level to chemerin.

Conclusions: These findings suggest that VEGF-A functions as not only a downstream enhancer but also an upstream initiator by chemoattracting pDCs in psoriatic lesions.

© 2014 Japanese Society for Investigative Dermatology. Published by Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Recent accumulating evidence has demonstrated that psoriasis is mediated by interleukin (IL)-17-producing T helper (Th17) cells and their released IL-17 and IL-22 [1]. For maintenance of Th17 cells, IL-23 is essential and released from tumor necrosis factor- α (TNF- α) and inducible nitric oxide synthetase-producing inflammatory dendritic cells (DCs) [2]. The inflammatory DCs are activated via autocrine mechanism by virtue of TNF- α . Thus, the expression of IL-

17, IL-22, IL-23, and TNF- α is elevated in psoriatic skin compared to normal skin [1,3]. This cytokine network has been proven by the therapeutic effectiveness of cytokine blocking biologics [4], including antibody therapies to TNF- α , IL-23/IL-12p40, and IL-17 or its receptors. It is noted that IL-17 and IL-22 are mainly produced by Th17 cells in human, but they may be derived from $\gamma\delta$ T cells in mouse psoriatic or other inflammatory models [5–7].

IL-22 is the most effective cytokine for keratinocyte proliferation [8]. IL-17 and/or IL-22 are capable of stimulating keratinocytes to produce cytokines/chemokines and antimicrobial peptides [9]. Vascular endothelial growth factor (VEGF) is also produced by epidermal keratinocytes in synergistic stimulation with IL-17 and IL-22 [10]. Thus, proliferative keratinocytes in psoriatic skin are a major source of VEGFs [11], and VEGFs induce microvascular alterations in the dermal papillae essential for the development and persistence of the psoriatic lesions. Vasculature provides

* Corresponding author at: Department of Dermatology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu 431-3192, Japan. Tel.: +81 53 435 2303; fax: +81 53 435 2368.

E-mail addresses: tokura@hama-med.ac.jp, tamaeo@hama-med.ac.jp (Y. Tokura).

cellular and tissue nutrition to hyperplastic keratinocytes and promotes inflammatory cell migration [12].

VEGFs constitute a family of six polypeptides, VEGF-A, -B, -C, -D, -E and PlGF, which regulate blood and lymphatic vessel development. VEGFs specifically bind to VEGFR1, 2 and 3, and to coreceptor neuropilins (Nrp) [13]. VEGF-A, a major regulator for angiogenesis, binds and activates two tyrosine kinase receptors, VEGFR1 and VEGFR2. While VEGFR2 has strong tyrosine kinase activity and transduces the major signals for angiogenesis, VEGFR1 plays dual roles, a negative role in embryonic angiogenesis and a positive role in adulthood in a tyrosine kinase-dependent manner [14]. VEGFR1 is expressed not only in endothelial cells but also in macrophage/monocyte-lineage cells [15] and even leukemic cells [16], and promotes tumor growth, metastasis, and inflammation [15]. Therefore, VEGFR1 is also a therapeutic target for human diseases.

In addition to Th17 cells, inflammatory DCs, and their cytokines, plasmacytoid DCs (pDC) are deeply involved in the pathogenesis of psoriasis [17,18]. pDCs are matured from hematopoietic progenitor cells and migrate to the skin in various pathological conditions [19]. Upon stimulation with toll-like receptor ligands [20] and antimicrobial peptide-self DNA complex [21], pDCs produce type 1 interferon (IFN) such as IFN- α . Although the target cells of IFN- α remain to be elucidated, pDC-derived IFN- α is thought to promote the Th17- or Th1-based cascade of psoriasis.

Considering that VEGF is not only an angiogenic factor but also an inflammatory promoter, it is possible that keratinocyte-derived VEGF stimulates pDCs that infiltrate in the upper dermis. The aforementioned finding that monocyte/macrophage-lineage cells express VEGFR1 further supports this possibility. We therefore sought to investigate the role of VEGF-A for the activation of pDCs. We used K14-VEGF-A transgenic mice (Tg) [22,23], which exhibit spontaneous skin lesions resembling psoriasis [24,25]. Moreover, we studied *in vitro* the ability of VEGF-A to chemoattract freshly isolated pDCs.

2. Materials and methods

2.1. Mice

The K14-VEGF transgenic mice over-expressing VEGF-A164 in the epidermal basal layer were generated on FVB background by inserting an expression cassette containing the human K14 promoter and the gene encoding murine VEGF-A164 using the pronuclear microinjection technique [22]. The K14 promoter is expressed specifically in the basal layer of stratified squamous epithelium and controls expression of VEGF in the transgene construct [26,27]. Homozygous K14-VEGF mice were used in this study and wild type (WT) FVB mice were included as control groups. Female FVB/N JCL mice, 8 weeks of age, were purchased from CLEA Japan Inc. (Tokyo, Japan) and were maintained in specific pathogen-free conditions according to the guidelines of the Institute of Laboratory Animal Resources of Hamamatsu University School of Medicine. All animal experiments were approved by the Institution Animal Care and Using Committee.

2.2. Western blot analysis

We used skin samples from 8-week-old female mice for Western blotting. We pulverized skin in protein extraction buffer (100 mM phenylmethylsulfonyl fluoride, complete mini EDTA-free protease inhibitor cocktail 1 tablet) with Ultra-Turrax (IKA-Werke GmbH & Co. KG, Staufen, Germany). The homogenized tissues were disrupted by sonication. Cell lysates were centrifuged at 15,000 \times g, and the supernatants were collected into fresh tubes.

Then, 4 \times SDS buffer with 0.1 mol/L 2-mercaptoethanol was added to samples, which were boiled for 5 min at 95 $^{\circ}$ C. The extracts (30 μ g) were separated by 20% SDS-PAGE and electroblotted onto polyvinylidene difluoride (PVDF) membranes (Bio-Rad Laboratories, Hercules, CA). After electroblotting, PVDF membranes were incubated with rabbit polyclonal antibodies to anti-mouse IL-17 (1:2000; Abcam, Cambridge, MA), goat polyclonal antibodies to anti-mouse IL-23p19 (1:1000; R&D, Minneapolis, MN), or β -actin (1:1000; Cell Signaling Technology, Danvers, MA), and the reaction was detected with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:3000; Bio-Rad) or horseradish peroxidase-conjugated rabbit anti-goat IgG (1:1000; R&D). The signal was detected using an ECL Plus Western blot detection system (GE Healthcare, Piscataway, NJ).

2.3. Immunohistochemistry

Abdominal skin of mice was fixed in 4% paraformaldehyde for 10 min. Five μ m paraffin-embedded tissue sections were first deparaffinized and dehydrated. Endogenous peroxidase activity was quenched by incubating the slides in a solution of 700 μ l H₂O₂ (30%). The sections were incubated with primary antibody against mouse IL-17 (1:100; Abcam) and antibody against CCL20 (1:30; Abcam) at room temperature for 60 min. The slides were washed in Tris-buffered saline (TBS) and incubated with the peroxidase-conjugated affinity-pure goat anti-rabbit IgG (Jackson ImmunoResearch, West Grove, PA) at room temperature for 30 min. The slides were washed with TBS three times and incubated with diaminobenzidine-tetrahydrochloride (DAB) as substrate and counterstained with hematoxylin.

2.4. Immunofluorescence microscopy

Abdominal skin was embedded in OCT compound and snap-frozen. Cryostat sections were immunostained with anti-IL-17 (Abcam), anti-CCR6 (R&D), anti-SIGLEC H mAb (440c; Abcam), and anti-mouse CD11c mAb (Biolegend, San Diego, CA). The respective secondary antibodies were labeled with Alexa 488 or 594 (Molecular Probes, Eugene, OR). Nuclei were counterstained with 4',6'-diamidino-2-phenylindole (DAPI) (Molecular Probes). Sections were examined, and digital images were captured using the Olympus FluoView™ FV1000 confocal laser scanning microscope (Tokyo, Japan). For pDCs, an immunohistochemical staining for 440c, known as a pDC marker [28], was performed in sections.

2.5. Flow cytometry

The following monoclonal antibodies (mAbs) were employed: phycoerythrin (PE)-labeled anti-mouse IL-17A, CD4, TCR γ/δ and B220 mAbs (all from Biolegend), Alexa Fluor 488-conjugated anti-mouse VEGFR1 mAb (Flt-1; R&D), FITC (fluorescein isothiocyanate)-labeled anti-mouse VEGFR2 mAb (Flk-1; BD Pharmingen, San Jose, CA), and anti-neuropilin 1 (R&D), allophycocyanin (APC)-labeled anti-mouse PDCA-1 mAb (CD317; Biolegend), and PE-Cyanin7 (PE-Cy7)-labeled anti-mouse CD11c mAb (BD Pharmingen). All mAbs were used at 1–5 μ g/10⁶ cells, and incubation was performed for 30 min at 4 $^{\circ}$ C, followed by two washes in phosphate-buffered saline (PBS, pH 7.4) supplemented with 5% fetal calf serum (FCS) and 0.02% sodium azide. Non-specific stains were performed with the adequate same-class immunoglobulin for specific mAb. Fluorescent profiles were generated using FACSCanto II (BD). Cells were first incubated with anti-mouse Fc γ II/III receptor mAb for 10 min to prevent non-specific binding of the subsequent reagents to Fc receptors. 7-Amino-actinomycin D (7-AAD) was added to exclude dead cells.