

Increased Immunoglobulin (Ig) G4-Positive Plasma Cell Density and IgG4/IgG Ratio Are Not Specific for IgG4-Related Disease in the Skin

Julia S. Lehman, MD,^{1,2} Thomas C. Smyrk, MD,¹ and Mark R. Pittelkow, MD²

From the ¹Department of Laboratory Medicine and Pathology and ²Department of Dermatology, Mayo Clinic, Rochester, MN.

Key Words: Dermatopathology; Basic science; Anatomic pathology

DOI: 10.1309/AJCPTMWTCN04GSJH

ABSTRACT

Objectives: Immunoglobulin (Ig) G4-related disease (IgG4-RD), a fibroinflammatory condition that can affect multiple organs, is suggested by lymphoplasmacytic inflammation, fibrosis, phlebitis, and increased IgG4+ plasma cell (PC) tissue density. In patients with suspected IgG4-RD and skin changes, skin biopsy may serve as a diagnostic screen or to supplement nondiagnostic visceral biopsy specimens. We aimed to determine whether increased cutaneous IgG4+ PCs or IgG4/IgG ratio is specific for IgG4-RD.

Methods: We examined 50 mucocutaneous specimens representing seven PC-rich dermatoses and reactive PC-rich infiltrates with IgG and IgG4 immunohistochemical stains.

Results: IgG4+ density exceeded 10 cells per high-power field in 22 (44%) of 50 specimens, representing six of seven diagnoses and reactive infiltrates. In five specimens (10%), the IgG4/IgG ratio exceeded 0.40.

Conclusions: Moderately elevated IgG4+ PC density or IgG4/IgG ratio is a nonspecific finding in the skin. In cutaneous biopsy specimens showing increased IgG4+ PCs, careful consideration should be given to clinical, serologic, and other histopathologic features before attributing clinical changes to IgG4-RD.

Upon completion of this activity you will be able to:

- list skin conditions other than immunoglobulin (Ig) G4-related skin disease that can be associated with increased tissue density of IgG4-positive plasma cells.
- outline the current histologic criteria for the diagnosis of IgG4-related disease.
- recognize that the presence of IgG4-positive plasma cells in the skin is not specific to IgG4-related skin disease.

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The authors of this article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose. Questions appear on p 294. Exam is located at www.ascp.org/ajcpccme.

Immunoglobulin (Ig) G4-related disease (IgG4-RD) is a fibroinflammatory process first described in the context of autoimmune (sclerosing) pancreatitis.¹ It has since been found to affect virtually every organ, including the gallbladder (chronic cholecystitis), lung (interstitial pneumonia), and salivary gland (chronic sclerosing sialadenitis).²⁻⁴ Patients may present with mass lesions of the involved organ(s); often have elevated serum IgG, IgG4, and IgE; and generally improve with systemic corticosteroid treatment.^{2,4,5}

A recent consensus statement addressed the pathology of IgG4-RD.⁶ After acknowledging that certain organs do not usually become fibrotic (such as the lacrimal gland, lymph node, salivary gland, lung, and kidney), the authors summarized the characteristic histopathology as lymphoplasmacytic inflammation, fibrosis that is often storiform in nature, and obliterative phlebitis. They proposed that increased tissue

IgG4+ plasma cells be part of the diagnostic criteria and outlined various cutoff points for the different organs that can be affected, ranging from 10 per high-power field (hpf) in a bile duct biopsy specimen to 100 per hpf in a resected salivary gland. Depending on the organ suspected of being involved, biopsies may be difficult to obtain and falsely negative based on patchy disease distribution.⁷ When suspicion for pancreatic involvement by IgG4-RD remains despite unremarkable findings on a pancreatic biopsy specimen, for example, some have proposed that evidence of IgG4+ plasma cell infiltration in other organs could be used to confirm the diagnosis.^{1,6,8-12}

Although cutaneous manifestations of IgG4-RD appear to be rare, they may produce nonspecific erythematous papules, nodules, and plaques.¹³⁻¹⁷ Therefore, it would follow that evidence for IgG4-RD in the skin (an organ readily accessible for biopsy) may be of diagnostic utility, particularly in patients with nondiagnostic biopsy specimens from visceral organs. Investigators have defined cutoff values for IgG4+ tissue densities for the diagnosis of IgG4-RD for extracutaneous organs,¹⁸ and a recent pathology consensus statement recommended that the IgG4 density for skin should exceed 200 cells per hpf⁶ on the basis of anecdotal evidence.^{13,19} Although elevated IgG4+ plasma cell density and IgG4/IgG ratio have been shown to be a nonspecific finding in extracutaneous tissues,^{18,20} the specificity of these changes in the skin has been unknown.

In this study, we sought to determine the mean absolute and relative density of IgG4+ plasma cells in mucocutaneous biopsy specimens from patients with plasma cell-rich dermatoses distinct from IgG4-RD and plasma cell-rich reactive inflammatory infiltrates.

Materials and Methods

After obtaining institutional review board approval from the Mayo Clinic, Rochester, MN, we searched the Mayo Clinic tissue registry database for the following dermatoses associated with elevated levels of dermal and/or subcutaneous plasma cells: pemphigus vulgaris, lupus panniculitis, necrobiotic xanthogranuloma (NXG), morphea, cutaneous plasmacytosis, plasmacytoma, plasma cell (Zoon) mucositis, and Rosai-Dorfman disease. We also selected specimens from the head and neck with squamous atypia (actinic keratosis or squamous cell carcinoma) that were also noted to contain many plasma cells.

We examined the H&E-stained slides for plasma cell density and included specimens that contained at least five plasma cells per hpf ($\times 40$ objective lens) in any part of the tissue specimen. Specimens with tissue blocks containing insufficient tissue for additional immunostaining were excluded from the study.

Cases selected for inclusion underwent immunohistochemical staining for IgG and IgG4 on the formalin-fixed, paraffin-embedded tissue using appropriate positive and negative controls and methods described previously from our institution.²¹ Specifically, mucocutaneous tissue was fixed in 10% buffered formalin before being embedded in paraffin. Using standard immunohistochemistry techniques, monoclonal anti-human IgG4 antibody (Zymed, San Francisco, CA) was applied to 4-mm-thick sections and exposed to diaminobenzidine solution containing 0.05% H₂O₂. Following counterstaining with hematoxylin, the tissue sections were dehydrated, cleared, and mounted on glass slides. Fields with the highest subjective density of IgG4-positive cells were photographed using a $\times 40$ objective lens, as were the corresponding regions on IgG-stained sections. We counted cells with plasmacytoid morphology and cytoplasmic immunostaining. Individual cells were included in counts if greater than 50% was present in the field of view. The number of IgG4+ and IgG+ plasma cells per hpf in areas of highest IgG4+ plasma cell density was recorded for each specimen. Mean IgG4+ plasma cell density from three fields was calculated. As proposed previously,⁷ tissue with fewer than five positive cells per hpf was scored as few; five to 10 cells per hpf, slight; 11 to 30 cells per hpf, moderate; and more than 30 positive cells per hpf, marked/severe. The mean IgG4/IgG ratio from the three highest IgG4+ plasma cell density fields (or two, in cases with limited tissue size) was also calculated.

Results

We included 50 cases representing seven dermatoses, as well as reactive inflammatory infiltrates associated with squamous atypia: pemphigus vulgaris (n = 8), lupus panniculitis (n = 7), NXG (n = 8), morphea (n = 5), plasmacytoma (n = 3), plasma cell (Zoon) mucositis (n = 11), Rosai-Dorfman disease (n = 2), and reactive inflammatory infiltrates (n = 6). Our search did not identify any patients with cutaneous plasmacytosis.

Mean IgG4 density and IgG4/IgG ratio are reported by diagnosis in **Table 1**. A moderate density of IgG4+ plasma cells was noted in at least one field in 22 (44%) of 50 specimens, which represented NXG (n = 3), pemphigus vulgaris (n = 5), plasmacytoma (n = 1), plasma cell mucositis (n = 7) **Image 1**, lupus panniculitis (n = 2), Rosai-Dorfman disease (n = 1), and reactive inflammatory infiltrates (n = 3). Ten (20%) of 50 specimens displayed a marked increase in density of IgG4+ plasma cells in at least one field, representing NXG (n = 2), pemphigus vulgaris (n = 3), plasmacytoma (n = 1), plasma cell mucositis (n = 2), and reactive infiltrates (n = 2). The highest IgG4+ plasma cell density in any given case was 97 per hpf (plasma cell mucositis). The mean density of

■Table 1■

IgG4+ Plasma Cell Density in Each Tissue Section and Mean IgG4+ Plasma Cell Density by Case and Category^a

Diagnosis	Case No.	Field 1: IgG4 Cells/hpf	Field 2: IgG4 Cells/hpf	Field 3: IgG4 Cells/hpf	Mean per Case, IgG4 Cells/hpf	Mean per Case, IgG4/IgG Ratio	Mean per Category, IgG4 Cells/hpf	Mean per Category, IgG4/IgG Ratio
Necrobiotic xanthogranuloma	1	7	13	18	12.67	0.17	8.2	0.07
	2	41	55	6	34.00	0.17		
	3	1	0	0	0.33	0.02		
	4	39	2	8	16.33	0.14		
	5	1	2	1	1.33	0.03		
	6	0	0	0	0.00	0.00		
	7	0	2	0	0.67	0.03		
Pemphigus vulgaris	8	1	0	0	0.33	0.03	15.6	0.44
	1	17	10	4	10.33	0.37		
	2	5	6	3	4.67	0.18		
	3	10	21	x	15.50	0.22		
	4	44	35	19	32.67	0.92		
	5	32	14	10	18.67	0.67		
	6	4	0	0	1.33	0.11		
	7	7	7	9	7.67	0.39		
Plasmacytoma	8	17	39	47	34.33	0.67	19.5	0.24
	1	28	94	54	58.67	0.71		
	2	0	0	0	0.00	0.00		
Plasma cell mucositis	3	0	0	0	0.00	0.00	16.7	0.18
	1	11	11	12	11.33	0.27		
	2	97	87	95	93.00	0.67		
	3	35	18	14	22.33	0.39		
	4	12	9	12	11.00	0.10		
	5	14	6	9	9.67	0.10		
	6	11	3	0	4.67	0.14		
	7	16	9	8	11.00	0.08		
	8	7	0	x	3.50	0.01		
	9	4	7	8	6.33	0.13		
	10	3	2	6	3.67	0.08		
Lupus panniculitis	11	2	3	3	2.67	0.05	6.4	0.12
	1	5	11	32	16.00	0.33		
	2	14	21	4	13.00	0.12		
	3	3	2	1	2.00	0.16		
	4	1	2	1	1.33	0.05		
	5	6	7	5	6.00	0.15		
	6	0	NA	NA	3.00	0.00		
Morphea	7	3	2	2	2.33	0.06	0.2	0.02
	1	1	0	0	0.33	0.03		
	2	1	0	0	0.33	0.03		
	3	0	0	0	0.00	0.00		
	4	0	0	0	0.00	0.00		
Rosai-Dorfman disease	5	1	0	0	0.33	0.02	8.4	0.15
	1	5	7	4	5.33	0.04		
	2	10	16	NA	13.00	0.26		
Reactive inflammatory infiltrates	1	5	7	4	5.33	0.08	15.4	0.21
	2	4	11	10	8.33	0.20		
	3	9	7	NA	8.00	0.16		
	4	35	17	13	21.67	0.43		
	5	1	1	0	0.67	0.06		
	6	55	48	33	45.33	0.33		

hpf, high-power field; IgG, immunoglobulin G; IgG4, immunoglobulin G4; NA, not enough tissue was available to evaluate an additional high-power field; x, a third hpf was not available for review due to limited tissue size.

^a Mean IgG4/IgG ratio by case and category. Bolded IgG4+ plasma cell densities are those displaying more than 10 cells/high-power field. IgG4/IgG ratios are greater than 0.40.

IgG4+ plasma cells was moderately elevated (ie, >10 cells per hpf) in three of the seven diagnoses tested (ie, pemphigus vulgaris, plasmacytoma, and plasma cell mucositis), as well as in reactive inflammatory infiltrates (Table 1).

The mean IgG4/IgG ratio by diagnosis was greater than 0.40 in pemphigus vulgaris only. The IgG4/IgG ratio, averaging more than 3 hpf from each case, exceeded 0.40 in five

cases (pemphigus vulgaris, n = 3; plasmacytoma, n = 1; and plasma cell mucositis, n = 1).

Discussion

IgG4, a unique subclass of immunoglobulin that activates regulatory T cells when induced by a Th2 immune

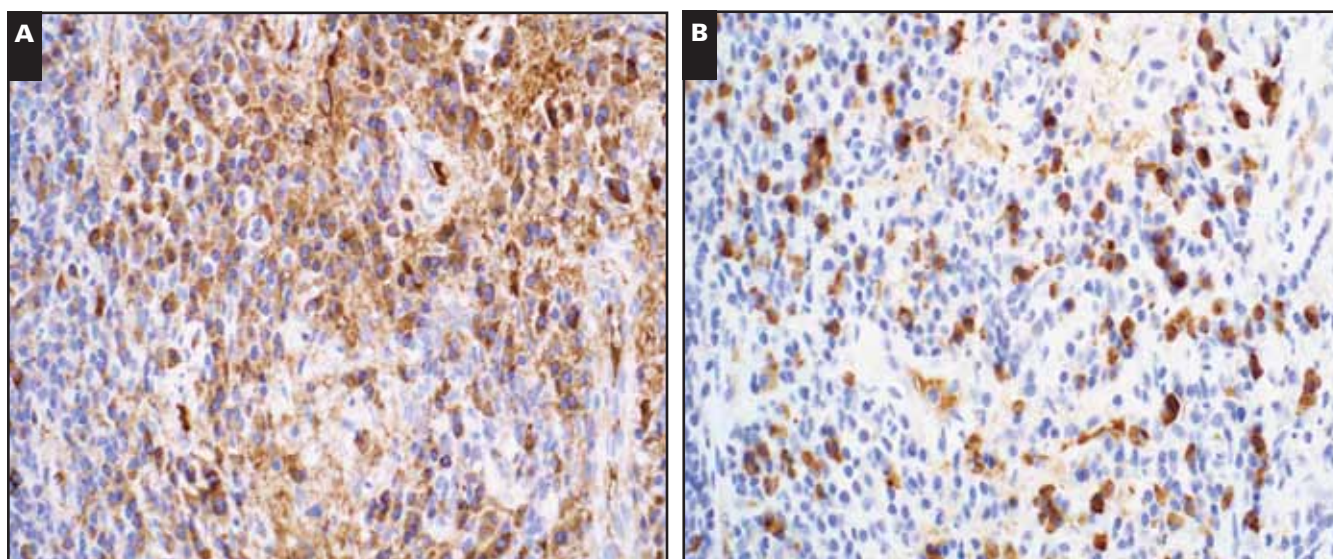


Image 1 Plasma cell mucositis, showing an elevated immunoglobulin (Ig) G4 (IgG4)+ plasma cell density and an IgG4/IgG ratio. **A**, IgG stain. **B**, IgG4 stain (×40).

response,^{22,23} is implicated in the development of pemphigus.²⁴⁻²⁶ Although the pathogenesis of IgG4-RD is not entirely understood, contributing factors include genetic predisposition, bacterial infection with molecular mimicry, and Th2-related autoimmunity.²² In IgG4-RD, elevated IgG4 may function in tissue destruction or, more likely, may arise in response to an undefined inflammatory trigger.²²

The largest study of patients with IgG4-related skin disease to date (n = 10) reported an IgG4+ plasma cell density of 49 to 396 cells per hpf, with an IgG4/IgG ratio of 0.62 to 0.92.¹⁴ Another study of five patients with cutaneous manifestations of IgG4-RD reported an IgG4+ density of 23 to 128.6 per hpf.¹⁵ Our data indicate that moderately elevated IgG4+ plasma cell density and IgG4/IgG ratio are not specific to IgG4-RD. This finding has considerable practical implications when evaluating skin biopsy specimens in patients with equivocal systemic evidence for IgG4-RD. Although no case in the present study contained IgG4+ plasma cell levels approaching the upper limit of those reported previously in cutaneous IgG4-RD¹⁴ or the threshold of 200 IgG4+ cells per hpf recommended in the recent consensus statement,⁶ caution should be used when interpreting the finding of elevated dermal and submucosal IgG4+ plasma cells before rendering a diagnosis of IgG4-RD.

An IgG4/IgG ratio exceeding 0.40 is widely cited as being supportive of the diagnosis.^{6,12} We concur with the recent consensus statement that an elevated IgG4/IgG ratio is insufficient for a diagnosis of IgG4-RD since, in this study, IgG4/IgG ratios surpassing 0.40 were observed in at least one hpf in cases of pemphigus vulgaris, plasmacytoma, and plasma cell mucositis. In the present study, pemphigus vulgaris, a

disease not believed to be part of the IgG4-RD spectrum, had the highest mean IgG4/IgG ratio of all conditions studied. The finding of frequent IgG4+ plasma cells in pemphigus vulgaris confirms previous observations²⁷ and may be related to the central role of IgG4+ autoantibodies in this condition.

Authors have previously proposed that cutaneous involvement by NXG and Rosai-Dorfman disease, two plasma cell-rich conditions, be classified as part of the IgG4-RD disease spectrum.^{28,29} In the present study, however, NXG had moderately elevated levels of IgG4+ plasma cells but did not differ substantially from other dermatoses tested. It is conceivable that a previously reported patient with orbital NXG, an elevated tissue IgG4/IgG ratio, and systemic signs of IgG4-RD actually may have had the xanthogranulomatous variant of IgG4-RD.³⁰ In our cases of Rosai-Dorfman disease, we were unable to substantiate increased IgG4+ plasma cell density or the IgG4/IgG ratio. However, we acknowledge that the number of cases we tested was low due to the rarity of this condition. Cutaneous plasmacytosis has also been linked to IgG4-RD.²⁷ We could not identify any cases of cutaneous plasmacytosis to study.

A prior study found that only 3 of 16 cases of scleroderma tested, 1 of 2 cases of morphea tested, and no cases of other scleroderma-spectrum conditions tested contained IgG4+ plasma cells, thereby essentially excluding scleroderma-spectrum conditions from the IgG4-RD spectrum.³¹ Our finding that IgG4+ plasma cells were sparse to absent in most cases of morphea confirmed these findings. Interestingly, the authors of the prior study also discovered that no IgG4+ plasma cells were identified in any of the five cases of normal skin studied.³¹

We agree with the assertion that the mere presence of elevated absolute and relative numbers of tissue IgG4+ plasma cells and/or elevated serum IgG4 levels is insufficient for inclusion into the IgG4-RD disease category.^{6,12} As is recommended when diagnosing IgG4-RD in visceral organs, we advocate careful correlation among clinical features, serologic findings, and histopathologic evidence before including mucocutaneous disease entities in the category of IgG4-RD.

Address reprint requests to Dr Lehman: Dept of Laboratory Medicine and Pathology, Mayo Clinic, 200 First St SW, Rochester, MN 55905; Lehman.julia@mayo.edu.

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