Abundance of IgG4+ Plasma Cells in Isolated Reactive Lymphadenopathy Is No Indication of IgG4-Related Disease

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ABSTRACT

Objectives: IgG4-related disease is a recently recognized condition that can be associated with lymphadenopathy, with several histologic patterns and increased absolute number and ratio of immunoglobulin G4 (IgG4)-positive plasma cells. However, these findings are considered to be not exclusively specific for IgG4-related disease.

Methods: The occurrence of the histologic patterns reported in patients with isolated lymphadenopathy was studied and correlated with the clinical presentation to determine their predictive value for IgG4-related lymphadenopathy.

Results: We found cases meeting all histologic criteria for IgG4-related lymphadenopathy, without clinical signs of IgG4-related disease. The only pattern that was not seen in this series was an inflammatory pseudotumor-like picture.

Conclusion: Without a clinical suspicion of IgG4-related disease, these morphologic patterns and high numbers of IgG4-positive plasma cells should be interpreted with care to avoid an erroneous diagnosis of IgG4-related disease.

Immunoglobulin G4 (IgG4)-related disease is a recently described spectrum of systemic diseases that is characterized by mass-forming lesions in different organs with extensive lymphoplasmacytic infiltration, fibrosis or sclerosis, an elevated IgG4 serum level, an increase of IgG4+ plasma cells in the affected tissues, and a good response to steroid therapy. Concomitant, usually generalized, lymphadenopathy is a common feature in patients with an IgG4-related disease, seen in up to 80% of the cases. In most patients, the lymph nodes show a moderate enlargement because of various histologic patterns of hyperplasia that have been divided into five histologic subtypes: (1) multicentric Castleman disease–like, (2) reactive follicular hyperplasia, (3) interfollicular expansion and immunoblastosis, (4) progressively transformed germinal center–like, and (5) inflammatory pseudotumor-like. A unifying feature of all subtypes is an increase in IgG4+ plasma cells with an IgG4/IgG plasma cell ratio exceeding 0.4, combined with an absolute number of IgG4+ plasma cells of more than 50/high-power field (hpf).

These characteristics of lymphadenopathy were defined in patients with lymphadenopathy in the context of clinically confirmed IgG4-related disease. The diagnostic value of these criteria in patients with lymphadenopathy of unknown origin is, however, still unclear. Several authors have reported similar histologic features in autoimmune disease.

The aim of this study was to determine the incidence of the histologic patterns described in IgG4-related lymphadenopathy and the increase in IgG4+ plasma cells in solitary reactive lymphadenopathy without clinical signs of IgG4-related disease.
Materials and Methods

Cases

Consecutive solitary reactive lymph node biopsy specimens (n = 89) measuring at least 1 cm in diameter acquired between 2007 and 2012 were retrieved from the archive of the department of pathology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands. Cases with malignancies, lympholiferative disorders, specific infections, and autoimmune disease were not included. In addition to the reactive lymphadenopathy group, a group of 12 biopsy specimens of patients with known autoimmune disease (Sjögren syndrome, n = 3; systemic lupus erythematosus, n = 3; rheumatoid arthritis, n = 2) or immunodeficiency (common variable immunodeficiency, n = 2; autoimmune lymphoproliferative syndrome, n = 2) was selected.

Histology

Six histologic patterns were evaluated on H&E-stained routine sections and (if available) on Giemsa staining, CD79a, CD3, and BCL2 immunostaining. Follicular hyperplasia was recorded when at least 30 follicles were present and the secondary follicle/primary follicle ratio was more than 0.5. The numbers of progressively transformed and atrophic germinal centers were counted. Interfollicular expansion was graded 1 if the majority of the lymph node showed little or no expansion (×400 field fits between follicles), 2 in case of moderate expansion (×200 field fits between follicles), and 3 consistent with considerable expansion (×100 or lower magnification fits between follicles). Areas with dilated sinuses were not included in the measurements. Fibrosis was rated as follows: none (score 0), less than 10% of the lymph node occupied by fibrosis (score 1), 10% to 50% (score 2), and more than 50% (score 3). Eosinophils were considered to be increased at more than or equal to 5/hpf. If 5 or more eosinophils per high power field were seen, the number of eosinophils in 20 adjacent high power fields was counted and averaged. Because we were not able to determine a reproducible semiquantitative evaluation for vascular proliferation, this feature was discarded.

Immunohistochemistry

Additional immunostaining was performed on all slides for IgG (polyclonal; 1:12,000 dilution; Dako, Glostrup, Denmark) and IgG4 (monoclonal EP4420; 1:2,000 dilution; Abcam, Cambridge, England). The presence of interfollicular and intrafollicular plasma cells was determined on the IgG-stained slide. To determine the number of IgG4+ plasma cells, three high power fields were evaluated in the areas of highest density of IgG4+ cells in the IgG4-stained slide.

IgG4 Morphometry

To quantify the IgG4/IgG plasma cell ratio, 55 of the 89 cases and 10 of the 12 control cases were randomly selected for calculating the IgG4/IgG plasma cell ratio using digital image analysis. A Zeiss Axioshot microscope (Zeiss, Oberkochen, Germany) and a Zeiss AxioCam MRc camera (Zeiss) were used with KS400 image analysis software.
Examples of the scored histologic features: A, eosinophilia showing ≥ 5 eosinophils/high-power field (×40); B, follicular hyperplasia, when at least 30 follicles were present and the secondary follicle/primary follicle ratio was >0.5 (×2.5); C, atrophic germinal centers (×10); D, fibrosis score 3 (×2.5); E, interfollicular expansion score 2 (arrows, ×2.5); F, progressively transformed germinal centers (×2.5). (H&E)
(release 3.0, Zeiss). For this purpose the IgG- and IgG4-stained surface was used instead of the number of positive cells, because a large degree of nuclear overlap makes automated cell counts unreliable. For each case, the area was selected on the IgG-stained slide with the highest density of IgG+ plasma cells (×12.5 magnification). Within this area, 10 fields were measured at a magnification of ×200 on both the IgG- and IgG4-stained slides. Because of the reported preferential location of IgG4+ plasma cells within follicles in IgG4-related disease, if present, five intrafollicular fields were measured, and five complementary interfollicular fields were measured. In four cases the IgG4/IgG plasma cell ratio was heavily overestimated because the IgG4 staining of the plasma cells was more intense and the stained area appeared to be larger than that of the IgG-stained plasma cells. In these cases the positive cells were counted by hand to determine the correct IgG4/IgG plasma cell ratio Image 3I.

Results

Patients

The clinical, histologic, and immunohistochemical characteristics of all groups are summarized in Table 1 and Table 2I. The group with increased IgG4+ plasma cells showed a higher median age (54 years) compared with the whole reactive group (25 years). The groups were predominantly male (5:1 and 1.5:1, respectively) and the biopsy specimens were more often from the thorax or axilla than from the head or neck as in the group without increased IgG4.

Histology

Follicular hyperplasia was the most common histologic pattern in all groups, with the highest percentage in the group of patients with autoimmunity or immunodeficiency (92%). There was no difference in the presence of
follicular hyperplasia between the whole group of reactive lymphadenopathy and the patients with increased IgG4+ plasma cells (66% and 67%, respectively). Interfollicular expansion was also found in all groups, with the highest frequency of considerable expansion (grade 3) in the whole reactive group (39%). Progressively transformed germinal centers, on the other hand, were mainly found in the group with increased IgG4+ plasma cells (33%; median, 7; range, 6-8), as well as eosinophilic infiltration (33%; median, 11; range, 10-12). Atrophic germinal centers were solely found in cases without increased IgG4 (18%; median, 1 per section; range, 1-4). Slight fibrosis was infrequently seen in all groups, but score 3 (>50% fibrosis) was only seen in one biopsy specimen without increased IgG4.

**IgG4 Immunohistochemistry and Morphometry**

The majority of cases from the reactive lymphadenopathy group that was studied using morphometry (n = 55) had a very low IgG4/IgG plasma cell ratio between 0 and 0.2, with a median of 0.03. Only seven cases showed higher ratios, up to 0.89 in the whole lymph node, 0.85 in interfollicular areas, and 1.00 in the germinal centers of the follicles.

Seven of the 55 reactive lymph nodes showed an increased IgG4/IgG plasma cell ratio of >0.4, but only six showed more than 50 IgG4+ plasma cells/hpf, thus meeting the criteria described for IgG4-related lymphadenopathy (Table 2). IgG4+ plasma cell ratio (>0.40) was increased both in and outside the germinal centers in four cases. An

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**Image 3**

**A-C**, cases with a high background and nonspecific staining of dendritic cells and histiocytes with immunoglobulin (Ig) G4 immunohistochemistry (lower staining intensity compared with the plasma cells), increasing the measured positive area. These cases were manually counted. **D**, An example of an IgG4 staining that was evaluated automatically (×10).
isolated increase of IgG4+ plasma cells in the follicles was seen in one case, and in the interfollicular areas in two cases. All of these cases showed varying interfollicular expansion. No atrophy of germinal centers was seen, five of seven cases showed follicular hyperplasia, and only one case progressively transformed germinal centers. Only one of these cases showed fibrosis (<50%) combined with an IgG4/IgG plasma cell ratio of 0.85. The plasma cells were inhomogeneously distributed, but mainly formed small clusters around the sinuses.

Within the group of patients with autoimmunity or immunodeficiency, the highest IgG4/IgG ratio in the immunodeficiency group was 0.03, whereas the highest ratio in the autoimmune group was 0.44, both measured in the germinal centers of follicles. The latter finding was in a patient with rheumatoid arthritis with follicular hyperplasia and increased intrafollicular IgG4/IgG ratio and more than 50 IgG4+ plasma cells/hpf (see Table 2).

In the group of patients with autoimmunity or immunodeficiency, follicular hyperplasia was present in all cases with immunodeficiency and in most of the autoimmune cases (87.5%). Interfollicular expansion was slightly higher in the immunodeficiency cases (25% vs 13%). Only one case in this group had progressively transformed germinal centers—a patient who had been diagnosed with autoimmune lymphoproliferative syndrome.

### Clinical Features

The clinical presentation of the eight cases with an IgG4/IgG plasma cell ratio of more than 0.4 is summarized in Table 2. Most biopsies (4/7) were performed because of an unexpected enlarged local lymph node found at surgery. None of the patients developed a lymphoproliferative disease or IgG4-related disease during the follow-up that ranged from 3 to 5 years. Only patient 1 (Table 2) had several persistent enlarged lymph nodes during follow-up, but the IgG4 serum level was not elevated (1.12 g/L). After a thorough search, no clinical evidence was found for IgG4-related disease. An infection with Yersinia enterocolitica was treated with antibiotics, but the lymphadenopathy persisted despite antibiotics.

### Discussion

The histologic characteristics of the lymph nodes in IgG4-related disease show a spectrum that is not specific and may be associated with other generalized diseases, especially autoimmune diseases.\(^4\) Our study shows that most of the characteristics associated with an IgG4-related disease are also found in solitary reactive lymph nodes in patients without clinical signs of IgG4-related or autoimmune disease. In addition, six cases met the histologic criteria for the diagnosis of IgG4-related disease.\(^8\) This group was characterized by an older age, stronger male predominance, mainly thoracic or axillary lymph nodes, and a histologic pattern of follicular hyperplasia with frequent progressive transformation but no atrophic germinal centers, combined with moderate interfollicular expansion with the presence of eosinophils. Follicular hyperplasia was the most common finding in all groups, followed by interfollicular expansion. Grimm et al\(^6\) compared 29 lymph node biopsy specimens that met the criteria for IgG4-related lymphadenopathy with 18 reactive lymph node biopsy specimens without increased IgG4+ plasma cells. Although most clinical data were lacking, including IgG4 serum levels, the authors state “We would propose that on the basis of histopathological findings alone, our cases represent a manifestation of IgG4-related sclerosing disease, as the increase of IgG4-positive plasma cells is not seen in other types of lymphadenopathy analyzed in our series.” Comparing our results with those from that study, we can conclude that the histologic characteristics of our cases are not specific for IgG4-related lymphadenopathy and are seen frequently in reactive lymph nodes.
Table 2

<table>
<thead>
<tr>
<th>IgG4+/IgG+</th>
<th>IgG4+/IgG+ Inter</th>
<th>IgG4+/IgG+ Intra</th>
<th>&gt;50 IgG4+ PC/hpf</th>
<th>Clinics</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50</td>
<td>0.43</td>
<td>0.53</td>
<td>Y</td>
<td>Several enlarged lymph nodes, no elevated IgG4 level in serum (1.12 g/L), antibiotics for <em>Y enterocolitica</em></td>
</tr>
<tr>
<td>0.89</td>
<td>0.85</td>
<td>1.00</td>
<td>Y</td>
<td>Incidental finding CABG</td>
</tr>
<tr>
<td>0.50</td>
<td>0.54</td>
<td>0.18</td>
<td>Y</td>
<td>Incidental finding rehabilitation post-traumatic brain injury</td>
</tr>
<tr>
<td>0.74</td>
<td>0.74</td>
<td>0.74</td>
<td>Y</td>
<td>Incidental finding oesophageal atresia</td>
</tr>
<tr>
<td>0.42</td>
<td>0.44</td>
<td>0.00</td>
<td>Y</td>
<td>Incidental finding CABG</td>
</tr>
<tr>
<td>0.82</td>
<td>0.72</td>
<td>0.85</td>
<td>Y</td>
<td>Lymph node removed after nipple piercing</td>
</tr>
<tr>
<td>0.03</td>
<td>0.01</td>
<td>0.80</td>
<td>N</td>
<td>Incidental finding cochlear implant</td>
</tr>
<tr>
<td>0.26</td>
<td>0.19</td>
<td>0.44</td>
<td>Y</td>
<td>Rheumatoid arthritis, small lymph node, 1.5 y later prostate cancer</td>
</tr>
</tbody>
</table>

The histologic pattern of progressive transformation of germinal centers is considered to be rare in lymph nodes of adults and may be associated with IgG4-related lymphadenopathy. Our study showed that a slightly higher percentage of cases with increased IgG4 also had progressively transformed germinal centers. This finding suggests that progressively transformed germinal centers in adults may be associated with an increase of IgG4+ plasma cells. Indeed, Sato et al.\(^9\) described 40 cases with progressively transformed germinal centers with increased IgG4+ plasma and a significant eosinophilia in the interfollicular areas. Our study confirms this correlation of IgG4 and eosinophilia: 33% of the cases with increased IgG4+ plasma cells but only 13% overall showed eosinophilic infiltration.

Plasma cells in germinal centers are considered to be a rare finding in reactive lymph nodes.\(^6\) Our study shows, however, that in 91% of cases in the reactive lymphadenopathy group, plasma cells can be identified in the germinal centers with immunohistochemistry.

Atrophic germinal center is one of the characteristics of IgG-related lymphadenopathy pattern III. Cases with atrophic germinal centers were rarely seen in our study: 18% in the group with reactive lymphadenopathy, and none in the group of patients with autoimmunity or immunodeficiency.

In contrast to our study, 31% of the IgG4-related lymphadenopathy cases studied by Grimm et al.\(^6\) showed significant fibrosis. Fibrosis consisting of more than 50% of the lymph node surface was only seen in one case, possibly because reactive inguinal lymph nodes frequently show some degree of fibrosis. These data suggest that an unexplained significant fibrosis might be a reliable clue to IgG4-related lymphadenopathy, though several studies found no significant fibrosis in many cases.\(^6,12\)

The unifying feature of all cases of IgG4-related lymphadenopathy is an increase in IgG4+ plasma cells in the lymph nodes (IgG4/IgG plasma cell ratio >0.4 and absolute number of IgG+ plasma cells >50/hpf). Unexplained lymphadenopathy cases with increased IgG4+ plasma cells have been reported before, but were considered the primary lymph node manifestations of IgG4-related disease.\(^5\) IgG4+ plasma cells were increased in 4% of non–IgG4-related lymphadenopathy cases (1/26) in the study by Rollins-Raval et al.,\(^12\) and in 11% (6/55) of our cases. Based on these results, we can conclude that an increase in the absolute number and/or ratio of IgG4+ plasma cells is not specific for IgG4-related disease and can also be found in reactive lymphadenopathy. Previous reports of rheumatoid arthritis with increased IgG4+ plasma mimicking IgG4-related disease\(^14,20\) correspond to our patient with increased IgG4+ plasma cells in a lymph node in the groin (intrafollicular ratio of 0.44), combined with follicular hyperplasia without eosinophilic infiltration.

In conclusion, our study shows that increased IgG4+ plasma cells can be found in reactive lymphadenopathy and lymph nodes of patients with autoimmune disorders and are therefore not specific for IgG4-related lymphadenopathy. Diagnoses based on the overinterpretation of the increased number of IgG4+ plasma cells would put patients in a real risk of being registered with a systemic disease, resulting in an incriminating psychosocial situation and additional medical costs. Therefore awareness of the clinical context and the condition of the patient is essential in every suspected case. IgG4 staining in nongeneralized lymphadenopathy seems contraindicated, except for cases presenting with fibrosis or a progressively transformed germinal center–like pattern (pattern IV),\(^5\) as seen in follow-up studies, which showed that some of those patients may develop extranodal sclerosing lesions.\(^13\) Even in cases with pattern IV and increased IgG4+ plasma cells, a descriptive histologic diagnosis is recommended. Only when unexplained significant fibrosis is combined with increased IgG4+ plasma cells (pattern V), IgG4-related lymphadenopathy may be suspected and further diagnostic examinations should be performed.

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References