Original Article

Inflammatory aortic aneurysm: possible manifestation of IgG4-related sclerosing disease

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Abstract: In this study, we investigate the hypothesis that IgG4-related autoimmune reaction is involved in the formation of inflammatory aortic aneurysms (IAA). We obtained 23 cases of IAA and 11 cases of atherosclerotic aortic aneurysms (AAA) as control group. We evaluated the expression of IgG4 in both IAA study cases and AAA control cases. In addition, immunohistochemical expression of C-Kit, CD21, CD34, S-100 protein, SMA, vimentin, p53, beta-catenin, and ALK-1, and EBV-LMP1 expression by in situ hybridization were performed only in IAA cases. Of the 23 patients, 20 were males and 3 were females (M: F ratio 6.7:1); age ranged from 43 to 81 years (average 64.3 years). Histologically, all 23 cases of IAA formed a mass that displayed inflammatory myofibroblastic tumor-like features. All lesions stained strongly and diffusely for vimentin and SMA (100%); 17 stained strongly and focally for CD34 (74%); and all were negative for C-Kit, CD21, S-100 protein, p53, beta-catenin, EBV-LMP1, and ALK-1. The numbers of infiltrating IgG4-positive plasma cells in IAA cases exceed that of AAA cases. Score 3 (>50 plasma cells/one 40X field) of IgG4-positive plasma cells was only seen in IAA cases (13/23, 57%), whereas none of the 11 cases of AAA showed score 3 IgG4-positive plasma cells (P=0.0018, Fischer’s exact test). Our findings suggest that IAA may be an aortic manifestation of the IgG4-related sclerosing disease. The high number of positive plasma cells, >50 plasma cells/one 40X field is more specific for the IAA than for AAA; however, lesser number can be seen in both IAA and AAA patients.

Keywords: Inflammatory aortic aneurysms, IgG4 related sclerosing disease, immunohistochemistry

Introduction

Abdominal aortic aneurysm (AA) occurs in 4% to 10% of people older than 60 years and is more common in men than in women. Inflammatory aortic aneurysm (IAA) variant accounts for 5% to 10% of all the cases of abdominal AA [1]. In contrast to the atherosclerotic variant (AAA), IAA occurs more often in symptomatic younger patients. Usually, IAA patients present with either abdominal pain and/or an abdominal mass. The pathogenesis appears to involve an immune response localized to the vessel wall, but the etiology of the inflammatory reaction is still unknown. In this study, we investigate the hypothesis that IgG4-related autoimmune reaction is involved in the formation of IAA, like various other idiopathic sclerosing lesions such as those involving the pancreas and retroperitoneum. We also made an attempt to study the immunohistochemical profile of IAAs and to clarify the role of myofibroblasts in the development of IAA.

Materials and methods

Paraffin blocks of formalin fixed tissue were retrieved from the surgical pathology files of the Department of Pathology and Genomic Medicine at The Methodist Hospital in Houston, Texas from 1995 to 2007. A single block from each case was selected for the construction of the tissue microarray and one area representative of the lesion was selected by two pathologists. Tissues from 23 cases of IAA were included in the study. 4-µm-thick tissue microarray sections (size 2mm) were stained with hematoxylin-eosin and Verhoeff Van Gieson (VVG) elastic stains. In addition, we evaluated the expression of C-Kit, CD21, CD34, S-100 protein, smooth muscle actin (SMA), vimentin, p53, beta-catenin, and ALK-1 by immunohistochemi-
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**Table 1. Immunohistochemical Profile of Inflammatory Aortic Aneurysms**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Dilution</th>
<th>Manufacturer</th>
<th>Localization</th>
<th>Antigen Retrieval</th>
<th>IAA No. (% +)</th>
<th>IMT (literature) % +</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-kit</td>
<td>1:100</td>
<td>Dako</td>
<td>Cytoplasm</td>
<td>Steam</td>
<td>0 (0)</td>
<td>0-15%</td>
</tr>
<tr>
<td>Vimentin</td>
<td>1:500</td>
<td>Dako</td>
<td>Cytoplasm</td>
<td>Steam</td>
<td>23 (100)</td>
<td>98%</td>
</tr>
<tr>
<td>CD21</td>
<td>1:400</td>
<td>Dako</td>
<td>Cytoplasm</td>
<td>Steam</td>
<td>0 (0)</td>
<td>0%</td>
</tr>
<tr>
<td>CD34</td>
<td>1:50</td>
<td>Dako</td>
<td>Cytoplasm</td>
<td>Steam</td>
<td>17 (74)</td>
<td>0-35%</td>
</tr>
<tr>
<td>S100</td>
<td>1:1000</td>
<td>Dako</td>
<td>Cyto/Nuclear</td>
<td>Steam</td>
<td>0 (0)</td>
<td>0%</td>
</tr>
<tr>
<td>SMA</td>
<td>1:2000</td>
<td>Dako</td>
<td>Cytoplasm</td>
<td>Steam</td>
<td>23 (100)</td>
<td>82-92%</td>
</tr>
<tr>
<td>β-catenin</td>
<td>1:250</td>
<td>Dako</td>
<td>Cyto/Nuclear</td>
<td>Steam/EDTA</td>
<td>0 (0)</td>
<td>0%</td>
</tr>
<tr>
<td>p53</td>
<td>1:200</td>
<td>Dako</td>
<td>Nuclear</td>
<td>Steam</td>
<td>0 (0)</td>
<td>30-77%</td>
</tr>
<tr>
<td>ALK-1</td>
<td>1:25</td>
<td>Dako</td>
<td>Cyto/Nuclear</td>
<td>Citrate buffer</td>
<td>0 (0)</td>
<td>36-60%</td>
</tr>
<tr>
<td>EBV-LMP1</td>
<td>-</td>
<td>Dako</td>
<td>Cytoplasm</td>
<td>-</td>
<td>0 (0)</td>
<td>0-60%</td>
</tr>
<tr>
<td>IgG4</td>
<td>1:200</td>
<td>Invitrogen</td>
<td>Cytoplasm</td>
<td>CCI/EDTA</td>
<td>23(100)</td>
<td>3%</td>
</tr>
</tbody>
</table>

IAA: Inflammatory Aortic Aneurysm; IMT: Inflammatory Myofibroblastic Tumor.

The grade and the distribution of the signal were scored semiquantitatively from absent (-) to strong (++++) and from focal to diffuse. We also evaluated the expression of IgG4 in both IAA study cases and 11 cases of AAA as control group. The number of IgG4 positive plasma cells were scored semi-quantitatively per a single 40X field as follows: 0 (no positive plasma cells), 1(≤20 cells), 2(21 to ≤50 cells), and 3(>50 cells). Clinical information was obtained from the patient’s records.

**Results**

IAA occurred in patients between the ages of 43 and 81 years (median, 51.3 years). These lesions were more common in males (20 cases) than females (3 cases) with a male to female ratio of 6.7:1. All patients with IAA had history of smoking and arterial hypertension or coronary artery disease. Clinically, an abdominal mass was detected in 19 patients (82.5%) and abdominal pain was present in 4 patients (17.5%) at presentation. None had a serum measurement of IgG4. None of these patients showed other manifestations of IgG4-related disease. Histologically, all 23 cases formed an inflammatory mass with spindle fibroblastic cell proliferation and collagen admixed with a moderate to severe inflammatory infiltrate, predominantly composed of diffuse clusters of plasma cells and lymphocytes. Reactive lymphoid follicles, eosinophils, and neutrophils were occasionally seen (Figure 1). None of the cases showed an obliterative phlebitis. No granulomas, medial degeneration, or areas of necrosis were identified. As a complement for H&E stain, VVG stain revealed duplication and disruption of the external elastic lamina at the point of aneurysm formation. Increased fibrous proliferation of the intima and atheromatous plaque formation were seen in both the IAA and AAA groups. Thickening of the wall was more prominent in the cases of IAA than those of AAA.

All IAA lesions stained strongly (+++) and diffusely for vimentin and SMA (100%); 17 stained strongly (+++) and focally for CD34 (74%). All were negative for C-Kit, CD21, S-100 protein, p53, beta-catenin, EBV-LMP1, and ALK-1 (Figure 2). Immunohistochemically, the number of infiltrating IgG4-positive plasma cells in IAA cases exceeded that of AAA cases. Score of 0 was seen in 2/23 of IAA and 3/11 of AAA patients; score of 1 was seen in 7/23 of IAA and 5/11 of AAA patients; score of 2 was seen in 1/23 of IAA and 3/11 of AAA and score of 3 was seen in 13/23 of IAA and 0/11 of AAA patients (Figure 3 and 4). Thus, 57% of IAA cases showed score 3 of IgG4-positive plasma cells, whereas none of the 11 cases of AAA showed score 3 IgG4-positive plasma cells (P=0.0018, Fischer’s exact test).

**Discussion**

IAA has been known to be a special variant of abdominal AA and accounts for 5% to 10% of all cases of abdominal AA [1]. IAA may rarely involve the thoracic aorta with few reported cases [2]. This entity was first described by Walker et al in 1972 [3]. The patients usually present with abdominal or back pain and weight loss. They often have elevated erythrocyte sedimentation rate (ESR) and C-reactive protein.
Male sex and smoking are the main risk factors for both AAA as well as IAA [1].

Histologically, there is expansion of the adventitia by a marked inflammatory reaction, which includes plasma cells, lymphocytes (mostly B cells and smaller numbers of CD4+ T cells), and macrophages. Occasionally eosinophils and rare neutrophils can also be seen. The intima reveals atherosclerosis, and the media shows atrophy and loss of elastic tissue. On radiology, these patients often have mantle sign with the thickening of the anterior and lateral wall of the abdominal aorta by CT scan with contrast [4].

Figure 1. Aortic samples from patients diagnosed with inflammatory aortic aneurysms (IAA) show spindle fibroblastic-type cell proliferation in the adventitia with a collagenized background (a) admixed with moderate to severe inflammatory infiltrate (b) with diffuse clusters of plasma cells and lymphocytes (c) (Hematoxylin and Eosin stain 40X, 100X and 400X respectively).

Figure 2. IAA lesions stained strongly (+++) and diffusely for smooth muscle actin (a, SMA); and focally for CD34 (b). (200X magnification).
The etiology of the IAAs is not clearly understood. There are different hypotheses including transformation of the adventitial fibroblasts into myofibroblasts [5], drug induction by methysergide, analgesics or antihypertensives, lymphatic obstruction or aortic trauma, infection or perhaps an autoimmune disease [6, 7]. IAA, perianeurysmal fibrosis and idiopathic retroperitoneal fibrosis are considered as a manifestation of one disease entity, so-called peri-aortitis [8]. Nine of 14 consecutively studied patients with retroperitoneal fibrosis had either proven atherosclerosis of the abdominal aorta or multiple vascular risk factors leading to atherosclerosis [9].

Inflammatory myofibroblastic tumors (IMT) can arise in the lung, mesentery, retroperitoneum or other locations, as a solitary mass or multicentric masses. These lesions display a proliferation of spindle fibroblastic and myofibroblastic cells accompanied by an inflammatory infiltrate including plasma cells with variable numbers of lymphocytes and eosinophils, features similar to those seen in IAAs. ALK immunostaining has been reported positive in 36% to 60% of the IMT patients [10]. Since both IAA and IMT can present as an abdominal mass and both share some histologic features, one of the purposes of our study is to define whether these represent a single entity or they are two separate entities with an overlapping spectrum of behavior, histological and immunohistochemical appearances. As described in IMT, all cases of IAA showed strong and diffuse positivity for SMA and vimentin. S-100, C-kit, and CD21 were negative in the spindle cells, but positive in scattered reticular histiocytes, mast cells, and follicular dendritic cells, respectively that was present within the inflammatory infiltrate in IAAs. P53, beta-catenin, EBV-LMP1, and ALK-1 were negative. Myofibroblasts are known to play an important role in granulation tissue formation and wound healing. The abundance of myofibroblastic population in IMT reflects their role in this process. In our study, 17 out of 23 cases (74%) of IAA showed strong and focal staining for CD34. These cells are an important component for the pathogenesis of IAA and IMT, perhaps regulating periaortic fibrosis and cell proliferation.

As in IAA, various pathogenetic factors have been proposed for IMT (i.e. reactive, infectious, autoimmune, and neoplastic). Few cases of splenic and hepatic IMT have been related to Epstein Barr virus genome [11]. In our study, all cases of IAA were negative for EBV-LMP-1 by in situ hybridization. The relatively high frequency of ALK staining in IMT (36-60%) and the demonstration of ALK rearrangements suggest that ALK dysregulation is an important mechanism of tumorigenesis of IMT. In our study, all cases of IAA showed negative staining for ALK-1, similar to a subset of ALK-1 negative IMPs, which are thought to be reparative or reactive in nature and some studies reported different therapeutic and prognostic implications when compared with their ALK-1 positive neoplastic counterpart [10]. The potential for aggressive growth, recurrence, and malignant transformation in IMT is often correlated with a number of features including oncogenic protein overexpression, such as ALK-1 and p53 [12]. In our study, all cases of IAA were negative for ALK-1 and p53, a feature that supports the reactive nature of this lesion. Beta-catenin expression is related to proliferative activity in high grade sarcomas. Dysregulation of beta-catenin is important in desmoid-type fibromatosis, as well as in synovial sarcoma, but no expression of beta-catenin has been reported in IMT in the literature [13]. Neither cytoplasmic nor nuclear staining was observed in all cases of our IAA.

IgG4 related sclerosing disease is characterized by a fibroproliferative process with lymphocytic and plasma cell infiltration, including numerous IgG4 positive plasma cells and obliterative phlebitis. This disease can occur in various organs such as the pancreas, bile duct, salivary glands, mediastinum, lacrimal glands,
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Multiple lesions can develop synchronously or metachronously in different organs in a patient. These patients often show elevated levels of IgG4 in tissue and serum. IgG4-related sclerosing disease may cause vascular lesions including abdominal and thoracic aortas and coronary artery aneurysms or a periarterial mass lesion [14-17]. IgG4-related disease involving the retroperitoneum and aorta can be classified into: retroperitoneal fibrosis, inflammatory abdominal aortic aneurysm, a combination of retroperitoneal and aortic involvement, and thoracic aortitis. “IgG4-related retroperitoneal fibrosis” and “IgG4-related aortitis” are now the terms of choice for describing these variations of the disease [18]. Although the majority of reports are from Japan, our study supports that this condition can occur in other populations as well and can present as aneurysmal rupture.

The pathogenesis of this entity still remains unclear; hypothesis includes an allergic reaction and T helper (Th2) cell response with potential role of interleukins. The inflammatory process can disrupt the elastic lamellar fibers in the media, which could be a critical event leading to aneurysmal transformation in the aorta [14].

Our patients with IAAs showed increase lymphoid follicles composed of CD20 positive B cells surrounded by CD3 positive T cells as compared to AAAs. IAA patients also showed a significant increase in the infiltration of IgG4-positive plasma cells. Fifty-seven percent of our IAA cases showed score 3 of IgG4-positive plasma cells, whereas none of the 11 cases of AAA showed score 3 IgG4-positive plasma cells ($P=0.0018$, Fischer’s exact test). A recent study showed that all cases of IgG4-related IAA were characterized by more significant thickening of the adventitia and more numerous IgG4-positive plasma cell infiltrations [19]. Several recent studies have also shown that IgG4-related IAA may be the aortic and periaortic lesions of an IgG4-related sclerosing disease [20-22].

Figure 4. Lesions showing IgG4-positive plasma cells with score 1 (a), score 2 (b) and score 3 (c) in patients with IAA. (400x magnification).
The number of patients presenting with ruptured IAAs has doubled, but the mortality rate has been shown to be decreased, which may be attributable to improved preoperative, perioperative, and postoperative management [23]. Corticosteroids or immunosuppressive therapies such as Rituximab may also have a role in the management of these patients apart from the need of surgical intervention in certain cases [24]. However, clinical studies are needed to determine the effectiveness of steroid therapy in the management of these patients, as steroids can lead to thinning of the arterial wall and eventual rupture. Because this study was a retrospective study, serum IgG4 measurement was not performed. The other limiting factor of our study is the follow up of the patients, as majority of our patients were lost to follow-up.

Conclusion

In conclusion, we describe the immunohistochemical profile of IAA, where the proliferation of the adventitia in IAA shows strong positivity for SMA and vimentin in spindle cells, indicating myofibroblasts being predominant cells, similar to IMT. The expression of CD34 in IAA, suggests that dendritic-type interstitial fibroblastic cell proliferation, in addition to myofibroblasts, is an important cellular component for the pathogenesis of IMT as well as IAA. Our findings indicate that IAA lacks ALK-1 and p53 which have been reported positive in the neoplastic form of IMT. Thus, IAA may represent to be a reactive form of IMT. The high number of positive plasma cells, >50 plasma cells/one 40X field is more specific for the IAA than for AAA; however, lesser number can be seen in both IAA and AAA patients. Thus our findings also suggest that IAA is an aortic manifestation of the IgG4-related sclerosing disease, and not a simple inflammatory aneurysm. This finding may be important for both histogenesis and treatment modality of IAA, and further studies including serum IgG4 measurement and clinical follow up are required to prove our findings.

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