Immunohistochemical Studies on Annexin I and II in Takayasu Arteritis

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Annexin I and II are classical types of the annexin family and known for their physiological functions in inflammatory processes and thrombus formation. Takayasu arteritis, on the other hand, is a vasculitis of unknown etiology characterized by the inflammatory involvement of the internal vessel wall and thrombus formation. It causes the stenotic and/or obstructive changes of the arterial wall leading to the characteristic clinical feature of pulselessness.

Expression of annexin I and II in vessel walls obtained from nine patients with Takayasu arteritis was studied immunohistochemically to investigate their roles in this morbid condition. Expression of annexin I was recognized in foamy cells in the thickened intima and/or medial layer and in inflammatory cells in vessel walls, but neither in endothelial cells nor in normal or proliferated smooth muscle cells. Strong expression of annexin II was present in foamy cells as well as in endothelial cells both in thickened intima and in proliferated smooth muscle cells in the intima, but not in smooth muscle cells in the media. The stronger expression of annexin II was seen in the endothelial cells in the vasa vasorum of both thickened and normal adventitia. These results suggest the participation of annexin I and II in the process of this vasculitis, and an essential role of vasa vasorum in the pathogenesis of Takayasu arteritis.

Key words: Annexin I, Annexin II, Takayasu arteritis

I. Introduction

Annexins are a family of Ca2+-dependent phospholipid binding proteins. Annexins consist of a COOH-terminal domain that confers Ca2+-dependent binding to membranes and a NH2-terminal domain. Annexins are widely distributed among species and tissues. A variety of roles for annexin have been recognized such as mediation of membrane trafficking and membrane cytoskeleton interactions, regulation of phospholipase activity and eicosanoid release, receptor signal transduction, modulation or formation of Ca2+ channels, and control of cellular proliferation and differentiation [4–6, 8, 18–20, 27–29]. Thirteen annexins have been purified and characterized, and these proteins are present in all mammalian cells except erythrocytes.

Annexin I is a steroid-regulated protein that controls the synthesis of pro-inflammatory eicosanoids through inhibition of phospholipase A2 and thereby inhibits acute inflammation. It’s anti-inflammatory action is due to inhibition of leucocyte migration. Annexin I is expressed in peripheral blood leucocytes and monocytes [2, 9, 26].

Annexin II is a Ca2+ and phosphate-binding protein that can exist as a monomer (36 KDa), as a heterodimer or as a heterotetramer. Annexin II tetramer is composed of two heavy chains and two 11 KDa light chains. The roles suggested for annexin II include exocytosis, endocytosis, cell-cell adhesion, inhibition of phospholipase A2, inhibition of blood coagulation, transduction of signals for differentiation, mitogenesis, regulation of cell-matrix interaction and regulation of cell-cell adhesion [4–6, 8, 17–20, 27–30, 32]. Annexin II was initially identified as a plasminogen/t-PA coreceptor expressed on the endothelial cells. Likewise, annexin II appears on the surface of the endothelial cells of the vessels. Contact of plasminogen and tissue plasminogen activator in blood with the endothelial cell surface leads to the transformation of plasminogen into plasmin, thereby activating fibrinolysis [10–16]. The activity of annexin II is known to be influenced by various substances. For instance, Lp(a)
is known to interfere with the interaction of annexin II and plasminogen. Although many of their important physiological functions have already been elucidated in recent years, there are few reports demonstrating their activities in clinical contexts. Dreiser et al., for example, recently demonstrated the presence of annexin I, II and IV in a broad range of human tissues using immunohistochemical techniques [7]. They stressed the strong expression of annexin I in leukocytes, tissue macrophages, T-lymphocytes and in certain epithelial cells and of annexin II in endothelial cells and myoepithelial cells.

Takayasu arteritis is a chronic vasculitis involving mainly the aorta, its main branches, the coronary and the pulmonary arteries, causing clinically various ischemic symptoms due to stenotic lesions and/or obstruction by thrombus formation [23–25] (Fig. 1A). Pulselessness resulted from obstruction of upper extremity vessels by thrombus formation and has led to the eponym “pulseless disease” in western countries (Fig. 1A, B) [21, 22]. Histologically, this disease displays involvement of inflammation of all three layers of the vessels, characterized by intimal thickening, medial fibrosis and/or necrosis and fibrous thickening of the adventitia (Fig. 1C). The etiology of this disease is still not fully understood. However, recent progress in vascular biology has focused on the role of inflammatory cellular infiltration in this disorder.

In this report we describe our recently conducted immunohistochemical studies in order to elucidate the pathetiological condition in Takayasu arteritis and the putative roles of annexin I and/or II in the arterial walls of patients with Takayasu arteritis.

II. Materials and Methods

We examined one hundred arterial sections including specimens from aorta, subclavical artery, common artery,
Fig. 2. Thoracic aorta of a 68-year-old female patient with Takayasu arteritis (×100). Positive reaction to annexin II was recognized (A) compared with control (B). Enlarged picture stained by the azan mallory method (×100).
cervical artery, renal artery, left anterior and descending artery obtained from 9 postmortem cases (53±20 years of age) with Takayasu arteritis. The duration of the disease in these nine cases was 10 years or longer. The sections were fixed in 10% phosphate-buffered formalin. Paraffin sections of 4 μm thickness were prepared for immunohistochemistry by deparaffinization in xylene and hydration in alcohol. After preincubation for 30 min at room temperature with methanolic H₂O₂ (0.3%), followed by rinsing for 2 min in tap water and then in fresh deionized water, all sections were blocked in blocking buffer containing horse serum diluted (0.15:10) in PBS for 20 min and incubated with the primary antibodies. Primary antibodies were (1) 100 μl of monoclonal mouse IgG1 antibody (10 μg/ml) directed against annexin I (Transduction Laboratories, Lexington, KY); or (2) 100 μl of monoclonal mouse IgG1 antibody (10 μg/ml) directed against annexin II (p36 monomer) (Transduction Laboratories, Lexington, KY). To confirm the characteristics of foamy cells and infiltrated cells, we used monoclonal mouse IgG antibody to human macrophage antigen (HAM65 obtained from Tsukada [31]) and monoclonal mouse IgG antibody to CD45RO antigen (isotype: IgG2a) (Nichirei Corp. Chuo-ku, Tokyo). One hundred μl of isotype-matched non-immunized mouse IgG (10 μg/ml) was used as control (Nichirei Corp. Chuo-ku, Tokyo).

Primary antibodies were labeled by avidin-biotinFig. 3. Subclavicular artery of a 48-year-old female patient with Takayasu arteritis (×400). Positive reaction to annexin II in normal appearing intima was recognized (A). B is a control.
complex method (Vector Laboratories, Inc. Burlingame, CA) and visualized with peroxidase-coupled anti-mouse antibody using diaminobenzidine (DAB) (Vector Laboratories, Inc. Burlingame, CA). The sections were counterstained with methylgreen (Nichirei Corp. Chuo-ku, Tokyo).

III. Results

Histologically, all specimens presented similar characteristic features of Takayasu arteritis, namely a fibrous and thickened intima, accompanied by characteristic atherosclerotic changes, destructed medial layer by elastolysis, calcification and fibrosis; and a thickened adventitia (Fig. 1C). In these specimens positive reaction for annexin II was detect-
ed on the surface of the thickened intima, in macrophages and foamy cells found in the intima, and in the medial layers (Fig. 2A). Histological examination revealed that these cells are endothelial cells and foamy cells originating from macrophages (Figs. 2C, 4B). Smooth muscle cells in the medial layer and proliferated smooth muscle cells in the intima were both annexin II-negative (Fig. 3A). Positive reaction was clearly demonstrated in the endothelial cells where vessel walls appear to be not yet involved (Fig. 3). Similarly, positive reactions to annexin II were also obtained for both foamy cells and endothelial cells lining the thickened intima (Fig. 4A). Positive reaction with anti-macrophage antibody suggests that these foamy cells are derived from macrophages (Fig. 4B).

Another characteristic feature obtained in this study was the strong positive reaction for annexin II frequently found in the endothelial cells of the vasa vasorum in adventitia (Fig. 5A). Magnified image demonstrated positive reactions not only in the vasa arteriarum but also in the vasa venarum. Positive reactions were also found in organized thrombi (Fig. 5B).

Annexin I-positive reaction was observed in macrophage-derived foamy cells in the intima (Fig. 6A, B). Magnified images reveal that these positively reacting cells are macrophages and/or foamy cells. Positive reaction to annexin I was also recognized in spindle-shaped cells in the intima and the outer part of the media (Fig. 7A). Magnified views demonstrated that these positively reacting cells in the intima were myointimal cells which were no longer viable being entirely, surrounded by their own produced fibrous tissue (Fig. 7B). Neither the endothelial cells nor the smooth muscle cells reacted with anti-annexin I. Fig. 8A shows the infiltrated cells on the arterial wall, which are CD45 positive as well as annexin I-positive (Fig. 8B, C).

IV. Discussion

Since the discovery of annexin by Crumpton and Dedman in 1990 [3], attention was focused on annexin as one of the key substances controlling physiological and pathophysiological events. Thirteen annexins have so far been detected and they are collectively called the annexin family. Annexin I and II are the classical ones, both of which exert important physiological actions through their N-terminal region which is identical in annexin I and II. They play important roles in inflammatory processes of the circulatory system. Annexin I shows the anti-inflammatory effect through its inhibition of phosphodiesterase A2 and leucocyte diapedesis by binding to the specific surface receptor of granulocytes and macrophages [2, 9]. Annexin II is also implicated in membrane aggregation, membrane fusion and membrane organization, as well as in exocytotic and endocytotic processes [1]. Recently its function as an extracellular endothelial cell surface receptor for tenasin C or plasminogen activator was recog-
Annexin I & II in Vessel Wall of Takayasu Arteritis

itized suggesting that it plays an important role in the inflammation process and in anti-thrombotic activity [10–14].

Though their significant physiological functions have already been elucidated in recent years, there are few reports demonstrating their activities in clinical contexts. As described in the introduction, Takayasu arteritis is a chronic vasculitis characterized by easy thrombus formation as one of its serious clinical features. From this point of view, we studied the expression of annexin I and II immunohistochemically in the vessel wall of the patients with Takayasu arteritis. Positive expression of annexin II was observed in endothelial cells and macrophage-derived foamy cells in the thickened intima. These data suggest essential roles of the annexins in the pathophysiology of thrombus formation. Furthermore, it is very noteworthy that strong expression of annexin II was found in endothelial cells of vasa vasorum, which lends support to an important role of the vasa vasorum in the etiology of Takayasu arteritis. Studying genetic factors in the pathogenesis of this vasculitis, Numano confirmed the close relationship between HLA types and Takayasu arteritis, speculating that subjects carrying a special type of HLA are prone to induce autoimmune reaction triggered by some agents such as viral infection, to induce an inflammatory process [21, 22, 24], and that vasa vasorum could well be the place where these initial inflammatory changes begin to take place. In fact, Takayasu arteritis is

Fig. 6. The left cervical artery of a 68-year-old female patient with Takayasu arteritis (x100). Annexin I-positive reaction was observed in macrophage-derived foamy cells in the intima (A, B). Annexin I staining of foamy cells (A), HAM 65 staining of foamy cells (B).
Ohkawara and Numano

characterized by a thickened adventitia. We also confirmed positive reaction to annexin I in macrophages, myointimal cells and inflammatory cells in the thickened intima. Although smooth muscle cells are generally negative for annexin I, myointimal smooth muscle cells that were confirmed to be imbedded in the fibrous tissue exhibited an interestingly positive reaction which merits further investigation. In addition, some inflammatory cells in the vessel wall exhibited a positive reaction and these were confirmed immunohistochemically as T cells. T cells are now the leading candidate cells to promote inflammatory changes in vasculitis. Our next study will focus on these autoimmune-associated inflammatory processes in vasculitis, specifically in association with annexin expressions and T lymphocytes.

Immunohistochemical expression of annexin I & II was studied in vessel walls of Takayasu arteritis. Annexin I was mainly expressed in myointimal smooth muscle cells, T cells and foamy cells in involved vessels. Strong expression of annexin II was confirmed in endothelial cells of thickened intima as well as in endothelial cells of as yet apparently not thickened intima in Takayasu arteritis patients. Strong expression of annexin II was also detected in endothelial cells of vasa vasorum and T cells. These data suggest an important role of vasa vasorum in the pathogenesis of Takayasu arteritis.

Fig. 7. The left renal artery of a 68-year-old female patient with Takayasu arteritis. Positive reaction to annexin I was recognized in spindle-shaped cells in the intima and media (A: ×25). Higher magnification demonstrated these cells are myointimal cells (B: ×100).
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VI. References


