Buerger’s disease (thromboangiitis obliterans) is considered to be a nonatherosclerotic, inflammatory, and vasoocclusive disease that most commonly affects small and medium arteries and veins of the limbs.1 The etiology of this disease has remained unknown ever since Leo Buerger gave the first pathological description in 1908.2 Buerger described a disease of young individuals under the age of 40 years, in whom there were symptoms and signs of progressive and recurrent vascular insufficiency. The disease may result in gangrene and major amputation. Buerger named this disease “thromboangiitis obliterans” and distinguished it from atherosclerosis.3 He considered acute inflammation and occlusive thrombosis of both arteries and veins to be the characteristic lesions of the clinically pathological entity.3,4

In 1878, Winiwarter had reported a case of a 57-year-old man who underwent a below-the-knee amputation because of right foot gangrene with rheumatic-like pain.5 In Tokyo, Japan, Haga reported clinical features of 10 cases and pathological findings of 13 specimens in 1889.6 and 9 years later, he contributed a paper with additional cases of spontaneous gangrene to Virchow’s Archive, and Virchow commented on the significance of syphilitic lesion in the vessel.7

Even though the etiology of the disease remains unknown, there are important specific features of Buerger’s disease that distinguish it from atherosclerotic obliterans (ASO) and other types of vasculitides. Here, we review the clinical and pathological aspects of Buerger’s disease.

Clinical Features

Currently, Buerger’s disease is recognized as being a specific vascular disease based on typical clinical picture, natural history, and histopathology.8-11 The diagnosis of Buerger’s disease, however, still remains controversial and has not been settled because of conflicting criteria used by many researchers all over the world.8-13

Imprecise and conflicting clinical and pathologic criteria have caused much confusion and ambiguity in the diagnosis and treatment of the disease during this long period of time. We think that the reason for this situation is that tissue specimens seldom include the acute-stage lesion, which Buerger considered as essential for diagnosis.3,4,14

At our institute, the clinical criteria of Shionoya are used for the diagnosis: (1) smoking history, (2) onset before the age of 50 years, (3) infrapopliteal arterial occlusion, (4) either upper limb involvement or phlebitis migrans, and (5) absence of atherosclerotic risk factors other than smoking. Definitive presentation of Buerger’s disease is considered to occur when all 5 criteria are met. Patients meeting all but the 4th criterion were diagnosed as probably suffering from Buerger’s disease.9,15

Despite significant evidence that inflammation and autoimmunity play a central role, the precise pathogenetic mechanism underlying Buerger’s disease have not been solved. The inciting antigen in Buerger’s disease still remains unknown.9 Smoking, infection, nutritional deficits, or general autoimmunity may be responsible for the antigen in Buerger’s disease.16,17

Smoking is known to be the strongest risk factor. Most of the patients are people who started smoking at a young age and...
the intima of the arteries and veins. The original description by Leo Buerger was followed by considerable debate and controversy, and histopathological findings have been accepted as being of little importance and indefinite.\[^{9,21,22}\]

**Phases of the Disease**

Histologically, the disease is subdivided into 3 stages: acute, subacute, and chronic stages.\[^{9,13,21}\]

A typical acute-stage lesion is shown in Figure 1. The lumen is occluded by fresh thrombi and intimal thickness with remarkable leukocyte infiltration. Multinucleated giant cells are seen in the thrombi, but necrotizing inflammation or granulomatous lesions are not observed.

In the subacute stage, the lumen is occluded by fresh and organized thrombus with partial recanalization. Multinucleated giant cells are diminished in number in thrombi or vessel walls.

In the chronic stage, the occlusive thrombi are organized and recanalized extensively, different from the acute or subacute stage lesions. Mild cell infiltration is seen in the intima, media, and adventitia.

**Features of Buerger’s Disease**

Regardless of its pathologic stage, there are 2 pathologic findings. One is that inflammatory infiltrating cells are well recognized, mainly in the intimal layer. The other is that the elastic lamina and all 3 layers of the vessel wall are well preserved.

As Leo Buerger stated in his report,\[^{2}\] histological findings reveal that the general architecture and elastic laminae remain

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**Pathophysiological Characteristics**

Regarding the histopathological features, Buerger described the lesions as being acute and chronic, segmental, with intense inflammatory infiltrate. He believed that the original pathologic process was thromboarteritis or a thrombophlebitic process, rather than a proliferative or obliterating process derived from the intima of the arteries and veins. The original description by Leo Buerger was followed by considerable debate and controversy, and histopathological findings have been accepted as being of little importance and indefinite.\[^{9,21,22}\]

**Figure 1.** Photographs of acute (A, B: popliteal artery) and chronic stage (C: anterior tibial artery) lesions. In the acute-stage lesion, fresh thrombi and organized thrombi are seen. Remarkable inflammatory cell infiltration is observed in the thrombus and intima. In the chronic stage, the lumen is occluded by organized thrombi with recanalized vessels. Inflammatory cell infiltration can scarcely be seen. Note that in the acute stage, the internal elastic lamina (arrows and arrowheads) is almost intact. Whereas, in cases of atherosclerosis (D, E: superficial femoral artery), fibro-intimal proliferation and hyaline degeneration are seen and cell infiltration is barely observed in any of the 3 layers. The internal elastic lamina and elastic fibers are severely disrupted and fragmented (A, B, D: H&E stain, C, E: elastica-van Gieson stain). A, adventitia; M, media; I, intima; T, thrombus.
Endarteritis Obliterans in Buerger’s Disease

Large and medium-sized muscular arteries. To the best of our knowledge, there are few published papers discussing this characteristic preservation of the vessel wall structure in Buerger’s disease, which is different from other vascular diseases.

We hypothesize that the differences might be related to the degree of extracellular matrix (ECM) degradation, which is caused by the plasminogen activator system and matrix metalloproteinases (MMPs). According to some reports, MMPs, in particular MMP-1, -3, and -9, are activated by both urokinase-type plasminogen activator (uPA) and plasmin.

That is to say, uPA activates the conversion of plasminogen to plasmin, which subsequently activates MMPs directly and degrades the essentially intact and that cell infiltration is observed predominantly in the thrombi and intima. No calcification or hyaline degeneration is found in Buerger’s disease.

In atherosclerosis, the general architecture and elastic lamina are destroyed and degenerated and inflammatory infiltrating cells are scarcely recognized in any of the 3 layers.

These histological features of Buerger’s disease are not common among other vasculitides. For instance, in giant cell arteritis, the characteristic lesion is the destruction and fragmentation of the internal elastic lamina by a granulomatous infiltrate and affected arteries are medium-sized in older individuals in polyarteritis nodosa (classified as a necrotizing vasculitis), fibrinoid necrosis occurs in the vessel wall, mainly affecting large and medium-sized muscular arteries.

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Figure 4. Immunostaining of the internal elastic lamina. (A) IgG, (B) IgM, (C) C3d. Adapted with permission from Kobayashi M et al. 11

Figure 5. (A,D) Immunostaining for plasminogen activator inhibitor-1 (PAI-1), (B,E) urokinase-type plasminogen activator (uPA), and (C,F) matrix metalloproteinase (MMP)-3 in Buerger's disease and atherosclerotic obliterans (ASO). A, adventitia; M, media; I, intima.
Endarteritis Obliterans in Buerger’s Disease

Inflammatory Infiltrating Cell-Related Immune Reaction

In Buerger’s disease, more infiltrating cells are recognized in acute and subacute lesions than in chronic ones. Most of the infiltrating cells are seen in the intima, where CD3+ T cells, which are equivalent to pan-T lymphocytes, outnumber CD20+ B cells, and are recognized in all B lymphocytes in acute lesions (Figure 2). In the chronic stage, CD20+ B cells are scarcer than CD3+ T cells. The CD3+ T cells are seen mainly in the intima; however, some are seen dotted through the media and adventitia. Regarded as a T lymphocyte subset, CD8+ T cells, which are known as the suppressor/cytotoxic subset of T lymphocytes, in the acute stage, dissimilar to their normal ratio in blood. The number of CD8+ cells, on the other hand, predominates slightly over that of CD4+ T cells in the chronic stage.

CD68+ cells, which define macrophages, are observed particularly in thrombi and intima during the acute and subacute phases (Figure 3A). After the CD3+ cells, the population of CD68+ cells is second in abundance. S-100-positive cells, which are recognized as dendritic cells, are readily detected in the intimal layer and thrombi (Figure 3B), but very few are detected in the media or adventitia in acute or subacute lesions. Particularly, S-100-positive dendritic cells in the thrombi and intima show well-developed processes. More than 95% of S-100-positive cells simultaneously show the CD68+ antigen. Very few S-100-positive dendritic cells are recognized in chronic stage lesions.

CD57+ natural-killer cells are observed to a slight extent in the thrombi and intima and media, but not at all in the adventitia. In all stages, especially the acute and subacute lesions, HLA-DR-positive macrophages and dendritic cells are observed in the thrombi and intima (Figure 3C). Interestingly, giant cells in acute stage lesions express HLA-DR and CD68+ simultaneously, but never express S-100 in the cell.

Immunohistochemical Findings of Affected Arteries in Buerger’s Disease

Immunoglobulins, including IgG, IgA, and IgM, are found deposited in a linear manner along the inner aspect of the internal elastic lamina (Figures 4A, B). Complement factors, C3d and C4c, are found deposited in a similar manner (Figure 4C). Other unique and important observations in Buerger’s disease are the infiltration of HLA-DR-positive cells and CD68+ cells, and deposition of immunoglobulins and complement factors linearly arranged along the elastic lamina in an undisturbed internal elastic lamina.

In atherosclerosis, leukocyte infiltration occurs in all 3 layers, but numbers are scarce compared with Buerger’s disease (Figures 1D, E). CD3+ T cells predominate, and among T lymphocytes, CD4+ T cells outnumber CD8+ T cells. A linear distribution of immunoglobulins and complement factors along the elastic lamina is never recognized. In the intima, HLA-DR-positive cells are observed much less often than in Buerger’s disease. In the control group, as expected, there are no inflammatory infiltrating cells detected in any of the 3 layers.

The expression of PAI-1 is scarce in all 3 layers (Figure 5A). On the other hand, uPA and MMP-3 were abundant in the media (Figures 5B, C). In cases of Buerger’s disease, the expression of PAI-1 is very well recognized in the media, particularly around the internal elastic lamina (Figure 5D); however, MMP-3...
It is also involved in vascular development and angiogenesis. In ECs, Notch is downstream of vascular endothelial growth factor (VEGF), and signaling of the Notch receptor through Delta-like 4 regulates the expression of VEGF receptor-1 and -2 (VEGFR-1 and -2) to control EC function and sprouting.

and uPA are scarce in the intima (Figures 5E, F).

Involvement of Notch Signaling Pathway
The Notch signaling pathway plays a role in cell differentiation, survival, and proliferation through diverse mechanisms.31,32

![Figure 7](image1.png)

**Figure 7.** (A–D) Immunohistochemical staining for Jagged-1 in Buerger’s disease and atherosclerotic obliterans (ASO). Adapted with permission from Tamai H et al.40

![Figure 8](image2.png)

**Figure 8.** (A–D) Immunohistochemical staining for Hes-1 in Buerger’s disease and atherosclerotic obliterans (ASO). Adapted with permission from Tamai H et al.40
angiogenesis. 33–37

Recent reports have shown the important role of the Notch signaling pathway in vascular development, including proliferation and migration of ECs, smooth muscle differentiation, and arterial-venous differentiation. 38,39 However, little information has been available about the relationship between Buerger’s disease and the Notch signaling pathway. Recently, we studied the expression of Notch in the cells infiltrating the intima of arteries affected by Buerger’s disease and we discovered that Notch signaling could be related to the arterial occlusive mechanism of Buerger’s disease. 40 In our results, all of Notch-1, Jagged-1 and Hes-1 were expressed in smooth muscle cells in the media and in ECs. In the infiltrating cells in the intima, Notch-1, Jagged-1 and Hes-1 were more expressed in Buerger’s patients than in ASO patients (Figures 6–8).

Our findings demonstrate that, in Buerger’s disease, the Notch signaling pathway is expressed in the inflammatory cells infiltrating the intima and thrombus. Therefore, it is possible that the Notch signaling pathway interacts with the mechanism responsible for the formation of a thrombus and for the inflammation in Buerger’s disease.

Furthermore, the Notch signaling pathway is expressed in the activated angiogenesis in the intima along the internal elastic lamina and the thrombus. Therefore, we speculate that the Notch signaling pathway may contribute to the underlying mechanism of endarteritis, angiogenesis, and recanalization of the arteries in patients suffering from Buerger’s disease.

Hypothetical Etiology of Buerger’s Disease

Many researchers have studied the specific pathologic mechanisms in Buerger’s disease. Historically, Buerger emphasized that thrombotic occlusion, periarthritis, and arteriosclerosis are characteristic findings of the affected arteries, but he took his interest in the periarthritis as evidence that justified his inflammation theory. 3,4

There have been investigators who studied the acute phase. Olin and Shih 41 and Lie 42 observed panvasculitis within small and medium-sized arteries and veins. The intense inflammatory infiltration and cellular proliferation are very specific to the acute-phase lesion. Decreased fibrinolytic activity of the intima may also contribute to formation of thrombus. 43 Inflammatory infiltrating cells such as CD3+ pan-T cells, CD4+ helper-inducer cells, and CD20+ pan-B cells respond to the elastic lamina of the affected vessels in the subacute phase. 44,45 The chronic phase is characterized by organization of occlusive thrombosis with well-developed recanalization, prominent vascularization of the media, and perivascular fibrosis. 41,46

Regardless of its stage, the elastic lamina and the architecture of the vascular walls are well preserved in Buerger’s disease, in contrast with atherosclerosis and other type of vasculitis. 1,4,42,46 Furthermore, inflammatory cellular infiltration is found predominantly in the thrombi and the intima. 43 Kurata et al suggested that the histologic findings, including the onion-shaped recurrent formation of the occluded arteries, adventitial fibrosis without medial fibrosis, swelling of the endothelium of the vasa vasorum, and edema beneath the elastic lamina, are distinctive in Buerger’s disease. 47 These pathologic findings are helpful in the diagnosis and they speculate that occlusion of the vasa vasorum may play an important role in the development of Buerger’s disease. 47 Some suggest that Buerger’s disease could be a form of endarteritis from the viewpoint of endothelial function. ECs play an important role in the initiation and perpetuation of the inflammatory response. Increased expression of adhesive molecules, such as VCAM-1, ICAM-1 and selectin, on the surface of ECs from patients with Buerger’s disease has been described. 44,49,50 but further study is necessary in this regard.

On the basis of our pathologic and histopathologic observations, we believe that Buerger’s disease is an endarteritis induced by some antigen that has not yet been detected, in the intimal layer and/or thrombus. 50 In the acute phase particularly, microvascious are observed, including acute inflammation in the intima, which likely induces thrombus formation. Later, in the healing stage, various immunological phenomena appear in the thrombus site.

CD4+ T lymphocytes are inducers of antibody production and regulators of cell-mediated immune responses. CD8+ T lymphocytes show most of the cytotoxic activity against antigens and act as a suppressor of the production of immunoglobulins. CD8+ T cells may be involved in the initiation of the lesion, but CD4+ T cells are at least as evident as CD8+ T cells during progression of the lesions.

CD68+ macrophages and S-100-positive dendritic cells, which are thought to play an important role in the immune reaction, are readily observed in the intima. Both HLA-DR-expressing macrophages and dendritic cells, as antigen-presenting cells, are also demonstrated mainly in the intima. In the acute and subacute stages, macrophages and dendritic cells in the intimal layer appear to have been activated by some antigen. In Buerger’s disease, such HLA-DR-positive macrophages and dendritic cells may present the antigen from the intimal layer to the T lymphocytes.

Both immunoglobulins and complement factors are deposited linearly along the elastic lamina in Buerger’s disease, which is specific and characteristic of the acute or subacute phase. Although some have reported that atherosclerotic plaques contain immunoglobulin deposits, 52,53 linear deposits similar to those of Buerger’s disease are never found in atherosclerotic arteries. Additionally, in polyarteritis nodosa, deposits of immunoglobulins and complement are not consistently found within lesions, although circulating immune complexes are common. 54 These findings suggest that humoral immunity, which is induced by B cell activation, also plays an important role at the site of Buerger’s disease. Usually, tissue damage caused by an immunoreaction, such as vasculitis or glomerulonephritis, shows deposition of not only immunoglobulins, but also complements.

Conclusions

From our observations, no responsible or possible antigen can be identified. Nevertheless, it seems very likely that in Buerger’s disease antigen-presenting cells are activated by an as-yet-unknown antigen in the blood, resulting in an immunoreactive to activate, for example, the Notch signaling pathway, in the intima. Restriction of the immune reaction (cellular as well as humoral) to the arterial intima defines Buerger’s disease as an endarteritis. Furthermore, in Buerger’s disease, expression of PAI-1 in media may inhibit the function of uPA and MMP-3, which disrupt and degenerate ECMs. This can be related to preservation of the wall architecture in vessels affected with Buerger’s disease.

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