Attenuation of Experimental Aortic Aneurysm Formation in P-Selectin Knockout Mice

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ABSTRACT: The aim of this study was to determine the role of P-selectin, an adhesion molecule found on the surface of activated platelets and endothelial cells during experimental aortic aneurysm formation. Infrarenal abdominal aortas of C57 black wild-type (WT) mice and P-selectin knockout (PKO) mice were measured *in situ* and then perfused with porcine pancreatic elastase (0.332 U/mL). Whole blood was drawn from the tail artery on day 2 pre-perfusion to determine total and differential white blood cell (WBC) counts. On day 14 postperfusion, aortic diameters (AD) of WT mice ($N = 19$) and PKO mice ($N = 9$) were measured. An aortic aneurysm was defined as a 100% or greater increase in AD from pre-perfusion measurement. Immunohistochemistry, including H&E, trichrome and von Gieson staining, was performed on harvested aortic tissue. Statistical analysis was performed by *t*-test and Fisher's exact test. There were no significant differences in peripheral leukocyte counts at baseline between the two groups. WT mice had significantly larger AD compared to PKO mice at day 14 postperfusion (116 % vs. 38 %, $P < 0.001$). Aortic aneurysm penetrance was 52% in WT mice, while 0% ($P = 0.01$) of PKO mice formed aneurysms. On histologic examination, WT mouse aortas were associated with a significant inflammatory response and degradation of elastin and collagen fibers, while PKO mouse aortas lacked signs of inflammation or vessel wall injury. P-selectin deficiency attenuates aneurysm formation in the elastase aortic perfusion model. This was associated with a blunting of the inflammatory response and preserved vessel wall intergrity following elastase perfusion in the P-selectin knockout mice. Further investigation to elucidate the independent contributions of endothelial cell and platelet P-selectin in experimental aortic aneurysm formation is required.

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BACKGROUND

Abdominal aortic aneurysms (AAAs) affect 3% to 9% of the United States population and are the 14th leading cause of death.[1] Currently, AAA formation is seen as a multifactorial process characterized by infiltration of inflammatory cells (e.g., neutrophils and monocytes) into the aortic wall followed by destruction of structural proteins such as elastin and collagen by extracellular matrix-degrading enzymes.[2]

The role of adhesion molecules during early inflammatory cell recruitment prior to AAA formation has not been well investigated. Recently, Hannawa *et al.* demonstrated the importance of a specific adhesion molecule, L-selectin, on the recruitment of neutrophils during the initial stages of AAA pathogenesis following elastase aortic perfusion in mice.[3] The objective of this study was to further investigate the role of another member of the selectin family, P-selectin, found on the surface of endothelial cells and platelets during AAA formation.

METHODS

Elastase Perfusion

C57Bl/6 wild-type (WT) mice and P-selectin KO (PKO) mice were anesthetized under 2% isofluorane inhalation on day 2 pre-perfusion and approximately 0.25 mL of blood was drawn from a prewarmed ventral tail artery by laceration and analyzed using a HEMAVET® 1500FS multispecies hematology instrument (CDC Technologies, Oxford, CT, USA). On the day of perfusion, baseline digital images of the aorta were obtained and aortic diameter (AD) was measured using Image Pro Express. Temporary control of the aorta was obtained and an aortotomy was made near the bifurcation using a 30-gauge needle. The aorta was then cannulated with PE-10 tubing and perfused with 0.332 units/mL of elastase in 1mL of saline for 5 min.[3] WT ($N = 19$) and PKO ($N = 9$) mouse aortas were measured and harvested at 14 days postperfusion. Aneurysmal aortas were defined as greater than a 100% increase from baseline.

Histology/Immunohistochemistry

Harvested aortas were fixed in fresh, cold 4% paraformaldehyde. Aortas were then paraffin embedded, cut into 4 mm sections, and mounted onto slides. Aortic sections were stained with hematoxlin/eosin, Masson's trichrome, and

Verhoff's von Gieson stain for qualitative analysis of aortic wall structure, collagen staining, and elastin staining, respectively.

RESULTS

There were no significant differences in circulating white blood cell (WBC) counts at baseline between WT and PKO mice (8.64×10^3 WBC/μL vs. 10.58×10^3 WBC/μL, $P = 0.078$, respectively). WT mice had significantly larger AD compared to PKO mice 14 days following elastase perfusion (116% vs. 38% increase from pre-perfusion diameter, $P < 0.001$, FIG. 1). In addition, 52% of WT mice formed an AAA, while none of the PKO mice formed AAAs ($P = 0.01$).

Representative abdominal aortic tissue sections of WT mice documented increased inflammatory response and destruction of aortic wall structure. (FIG. 2A) More specifically, trichrome (FIG. 2B) and von Gieson staining (FIG. 2C) demonstrated medial and adventitial collagen degradation (*arrows*) and loss of medial elastin (*arrows*), respectively. In contrast, aortic sections from PKO mice demonstrated preserved aortic wall structure (FIG. 2D) as well as intact collagen (FIG. 2E, *arrows*) and elastin fibers (FIG. 2F, *arrows*).

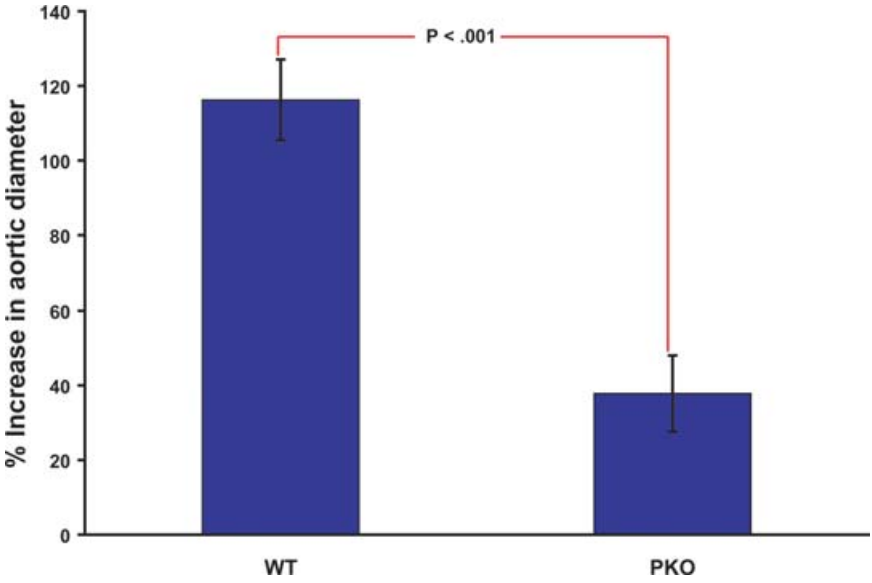

FIGURE 1. WT mice had significantly larger AD compared to PKO mice at day 14 postelastase perfusion (116% vs. 38%, $P < 0.001$). In addition, more WT mice (52%) than PKO mice (0%; $P = 0.01$) had aneurysms. An aortic aneurysm was defined as a 100% or greater increase in AD from pre-perfusion measurement (*dashed line*).

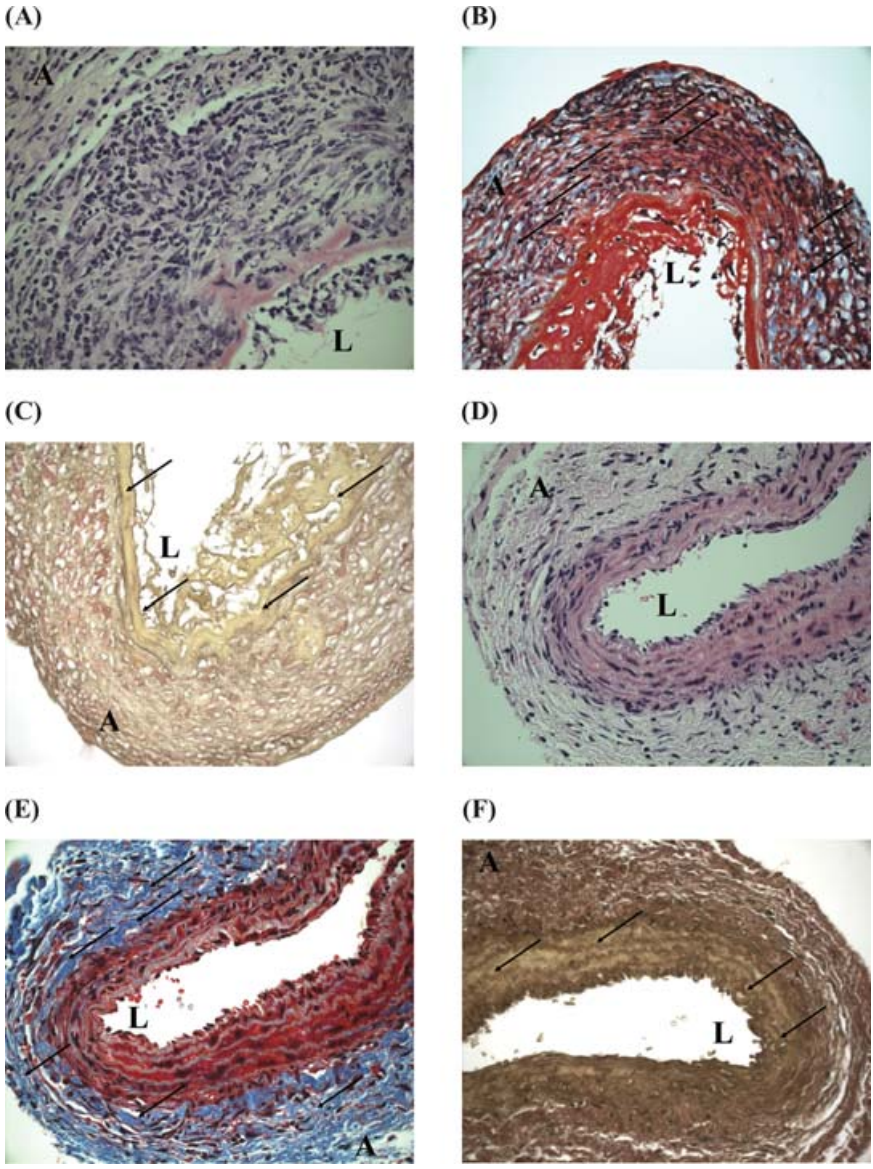

FIGURE 2. Representative abdominal aortic tissue sections (40× magnification) at 14 days postelastase perfusion of aortas in WT and PKO mice. Aortic sections from WT mice (**A**) show an increased inflammatory response and destruction of aortic wall structure. More specifically, trichrome staining (**B**) and von Gieson's stain (**C**) demonstrate adventitial collagen degradation (*arrows*) and loss of medial elastin (*arrows*), respectively. In contrast, PKO mouse aortic sections demonstrated preserved aortic wall structure (**D**), collagen staining (**E,** *arrows*), and elastin fibers (**F,** *arrows*). **A** = adventitia; **L** = lumen.

DISCUSSION

In this study, AAA formation in PKO mice was attenuated suggesting a critical role for P-selectin. This inhibition of AAA development in PKO mice was associated with diminished aortic wall degradation and preserved elastin and collagen.

AAA pathogenesis is a multifactorial process[2,4] that may be initiated by some unknown injury to the aortic wall. Following this event, which serves as a catalyst for the inflammatory response, enhanced chemokine and cytokine production further promotes leukocyte recruitment into the aortic wall. Ultimately, secretion of proteolytic enzymes such as matrix metalloproteinases by leukocytes and smooth muscle cells results in aortic wall extracellular matrix destruction and aneurysm formation.

A better understanding of the initial events associated with recruitment of inflammatory cells into the aortic wall is needed as most studies of AAA disease have focused on understanding the enzymology associated with vessel wall degradation and repair. A study by Ricci *et al.*[5] focusing on events initiating inflammatory cell recruitment demonstrated that inhibiting leukocyte recruitment diminished AAA formation in an experimental model. Using antibody blockade of CD18, a subunit of the integrin adhesion molecule found on leukocytes, which promotes firm adhesion to the endothelial surface, AAA size and macrophage recruitment were inhibited following rodent aortic elastase perfusion.

This study sought to clarify the role of the selectins, specifically P-selectin, during AAA formation. The selectins are a family of three adhesion molecules: E-selectin on the surface of endothelial cells, P-selectin on the surface of endothelial cells and activated platelets, and L-selectin that is constitutively expressed on the surface of most leukocytes.[6,7] The primary function of selectins is to promote leukocyte capture to sites of inflammation. Without selectins, inflammatory cell recruitment, an early and critical event during AAA formation, is significantly diminished.[8–12] While the role of P-selectin during AAA formation prior to this study has not been investigated, a study by Hannawa and colleagues documented the critical nature of L-selectin, found only on leukocytes, during AAA formation.[3] After documenting early upregulation of L-selectin in the rat elastase AAA model, L-selectin knockout mice were studied and found to have attenuated AAA formation associated with diminished aortic wall neutrophils and macrophages.

P-selectin in particular appears to be an attractive target for pharmacologic therapy to inhibit leukocytes in the inflammatory influx. Myers and others, in a model of deep venous thrombosis, using an oral, novel P-selectin inhibitor documented decrease in thrombus weights associated with increased vessel wall leukocytes.[13] In this same model, Thanaporn and colleagues documented that a P-selectin receptor antagonist was associated with decreased perithrombotic

inflammation and increased thrombus dissolution.[14] These strategies may also be attractive for inhibition of AAAs in the elastase AAA model.

Limitations of this study include the observation that the cell of origin was not determined. To differentiate whether endothelial cell P-selectin, platelet P-selectin, or both are necessary for aneurysm formation, a set of experiments involving either bone marrow transplant or aortic transplant between WT and PKO mice should be undertaken. Despite this limitation, we have identified another critical component, P-selectin, of the inflammatory cascade that contributes to the formation of experimental AAAs.

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