Recent Highlights of ATVB

Aneurysms

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Aortic diseases are common in many populations and are receiving increasing research focus. There are a broad spectrum of aortic diseases that occur in specific regions and appear to have different causes. For example, abdominal aortic aneurysms (AAAs) are most common in aged men. In contrast, many forms of thoracic aortic aneurysms (TAAs) occur early in life with a strong genetic basis and no sex discrimination. Both aortic aneurysms are amenable to surgical repair. Although surgical approaches have become increasingly sophisticated and less invasive, there remains an urgent need to determine factors that predispose to susceptibility and to divert treatment from surgical to medical approaches. This switch to medical treatment will require an increased knowledge of the mechanisms for several facets of aneurysms that cover the span of initiation, progression, and rupture. In this regard, many recent publications in ATVB have provided further insight into established pathways contributing to aneurysm development such as proteolysis, inflammation, and attenuation of the medial smooth muscle cell population, and a few publications have raised the possibility of new pathways such as adipokines and mineralocorticoid signaling. This article highlights these recent publications within a brief context of the literature.

Risk Factors

Cigarette smoking remains the major risk factor for development and progression of AAAs. Several experimental studies have demonstrated that smoke exposure augments AAA induced in mice by either subcutaneous angiotensin II (AngII) infusion or intra-aortic elastase perfusion. However, it is unclear whether cessation of smoking impacts the development of AAAs. The study of Jin et al demonstrated that cessation of cigarette smoking exposure did not immediately decrease the augmentation of AAAs. This sustained effect was attributable to regulation of leukocytic metabolism. Also of note is that cigarette smoking–induced augmentation of AAAs was unaffected by deficiency of matrix metalloproteinase-9 (MMP-9), MMP-12, cathepsin-S, and neutrophil elastase. AAA formation was also not reduced by doxycycline that is considered to be an MMP inhibitor of broad specificity. Doxycycline is currently under evaluation for efficacy on human AAAs (NCT01756833).

Leukocyte-Related Mechanisms

Human AAAs have many populations of infiltrated leukocytes. Macrophages are a major cell type infiltrating AAAs, although there have been surprisingly few studies that have determined the specific function of this cell type. Boytard et al demonstrated the presence of proinflammatory macrophages, with the subtype that lacks mannose receptor expression, in the adventitia and intraluminal thrombus. The study noted the increased expression of peroxiredoxin-1 as a potential target for regulating AAA development. Another macrophage-derived molecule implicated in AAA was angiopoietin-like protein 2. This protein has an increased abundance in AAA tissues harvested from humans and calcium chloride–induced disease in mice.

T lymphocytes accumulate in large numbers in aneurysmal tissue, but the function of these cells in the disease process has not been defined. There is a diversity of T lymphocytes accumulating in AAA tissue, including natural regulatory T lymphocytes. Deletion of natural regulatory T lymphocytes, either by CD80+/−×CD86+/− or CD28−/−, augmented AngII-induced AAAs. This was assumed to be attributable to reduction of interleukin (IL)-10, with IL-10−/− mice also being susceptible to AngII-induced AAAs. Mice with signal transducer and activator of transcription 3-defective T lymphocytes also had augmented AAAs in a model induced by AngII infusion and coadministration of a transforming growth factor-β neutralizing antibody. Consistent with this observation, Ju et al demonstrated that inhibition of signal transducer and activator of transcription 3 downregulated IL-6 secretion, resulting in a decrease of AngII-induced aortic dissections in mice. This decrease was attributed to decreased recruitment of IL-17 secreting T-helper lymphocytes. Contrary to the beneficial role of natural regulatory T lymphocytes, other T lymphocytes have detrimental effects on AngII-induced AAAs as demonstrated by enhanced CD4+ cells infiltrating augmented AAAs in AngII-infused syndecan-1–deficient mice.

Leukocytic infiltration is modulated by a large number of chemokines in different inflammatory conditions. Chemokine (C-C motif) ligand 5 is one of several chemokines that has an inferred role in AAAs. Iida et al demonstrated that a peptide inhibitor (MKEY) of the interaction of chemokine (C-C motif) ligand 5 with its receptor, chemokine (C-X-C motif) ligand 4, inhibited elastase-induced AAAs in mice. This study was an example of one of the few to determine the effect of an intervention on established AAAs. Although MKEY administration did not promote AAA regression, it attenuated aortic...
enlargement. IL-1β has also been proposed as an inducer of leukocyte infiltration into AAAs. Antibody-based neutralization of IL-1β effects is currently under study in a large clinical trial to determine effects on atherosclerotic diseases and a smaller trial to assess its efficacy in limiting the growth rate of rapidly growing small AAAs (NCT02007252). The same antibody was also used to determine effects on elastase-induced AAAs. This antibody, anakinra, was effective in reducing AAAs. These data were complimented by reduced AAAs in mice deficient in IL-1β or IL-1 receptors. Another cytokine that has attracted attention in aneurysms is chemokine (C-C motif) receptor 2. Moran et al demonstrated that the current approved drug as a rapamycin inhibitor, everolimus, decreased chemokine (C-C motif) receptor 2 expression and reduced AngII-induced AAAs. In addition, another immunosuppressive drug, azathioprine, was shown by Marinkovic et al to decrease AngII-induced AAAs. Azathioprine reduced expression of several chemokines implicated in AAA, including chemokine (C-C motif) ligand 2 and chemokine (C-C motif) ligand 5. Reduced secretion of these chemokines was via inhibition of Rac1, a c-Jun-terminal-N-kinase, which has previously attracted considerable attention as pathways that promoted AAA regression.

The Notch signaling encompasses several receptors and ligands and is required for a wide range of activities during development. Notch1 signaling is critical for development and functions of macrophages and lymphocytes. Hans et al demonstrated that haploinsufficiency of Notch1 reduced AngII-induced AAAs in mice through a macrophage-based mechanism.

Smooth Muscle Cell–Related Mechanisms

Aneurysms are characterized by pathology in the medial layer in which smooth muscle cells predominate. Smooth muscle cell–specific deletion of low density lipoprotein receptor–related protein 1 (LRP1) has previously been demonstrated to promote aortic aneurysms. Muratoglu et al have demonstrated that LRP1 deficiency in smooth muscle cells increases tissue content of a molecule, high temperature requirement factor A1, that regulates extracellular matrix integrity. High temperature requirement factor A1 was identified as one of the many ligands for LRP1 and hence its extracellular accumulation in the lack of an removal system.

Leeper et al also focused on the role of smooth muscle cells in mechanisms by which the genome-wide association study identified CDKN2B tumor suppressor gene participated in aneurysms formation. They found that CDKN2B was not related to inflammatory components, but instead reduced expression of the gene led to smooth muscle apoptosis via a p53-dependent mechanism. Smooth muscle cell apoptosis was also inferred in AAA formation through increases in protein kinase C-δ. Mice deficient in protein kinase C-δ were resistant to calcium chloride–induced AAAs, and local repopulation of the protein in deficient mice restored the susceptibility to disease. Of greater translational potential, attenuation of protein kinase C-δ activity in established AAAs reduced further aortic expansion.

Many intracellular signaling pathways have been inferred in development of aneurysms. This has included the diverse pathways regulated by oxidation. Catalase is an important regulator of hydrogen peroxide in determining physiological and pathological responses in smooth muscle cells. Parastatidis et al demonstrated that administration of a stabilized form of catalase decreased calcium chloride–induced AAAs in mice. The smooth muscle cell specificity of the effect was demonstrated using mice in which overexpression was restricted to this cell type by expressing the protein under the control of the myosin heavy chain-α promoter. A subsequent study by Sutliff et al complemented this finding by demonstrating that deficiency of polymerase δ-interacting protein 2, which also regulated hydrogen peroxide availability, decreased calcium chloride–induced AAAs in mice.

Proteolysis

Permanent aortic dilation is a defining characteristic of aortic aneurysms. For dilation to occur in any region of the aorta, the integrity of the extracellular matrix, specifically elastin and collagen, must be compromised by proteolysis. The most commonly considered classes of extracellular proteases are MMPs and cathepsins. Upregulation of protease activity is likely to involve multiple mechanisms. A potentially major regulator was identified as osteoprotegerin that was detected in a large number of human AAA biopsies and determined to correlate with tissue content of several cathepsins and MMPs. Further evidence of the involvement of cathepsins was provided by the recent determination that deficiency of cathepsin K attenuated AAAs provoked by intra-aortic perfusion of elastase via a coordinated action on facilitating the proteolytic activity of other enzymes. As noted above, a role of LRPI in aortic pathology in mice has indicated that this protein regulates protease activity, particularly the serine protease Htr-1 (a novel ligand for LRPI) in aneurysmal tissue. The contribution of proteolysis was also determined by overexpression of serine protease inhibitors, protease nexin-1 and plasminogen activator inhibitor-1, respectively. Overexpression of these 2 serpins in smooth muscle cells or tissue extracts harvested from thoracic aortas resulted in protection of cellular integrity through a Sma- and Mad-related protein 2 (Smad2)-dependent mechanism. Conversely, many studies have demonstrated that MMP inhibition reduced aortic aneurysm initiation.

Other Mediators

Hypercholesterolemia is a modest risk factor for human AAAs with relatively small literature of positive and negative associations for high low-density lipoprotein cholesterol and low high-density lipoprotein cholesterol, respectively. An active role of high-density lipoprotein in AAA formation was revealed by reductions of AngII-induced AAAs in apolipoprotein E–deficient mice or calcium chloride–induced AAAs in C57BL/6 mice by injection of native or recombinant high-density lipoprotein. This effect was attributed to regulation...
of extracellular signal–regulated kinase signaling, which is an example of the growing complexity of high-density lipoprotein function.\textsuperscript{45}

The role of carbohydrate metabolism in aneurysm formation is unclear. Although aberrant carbohydrate metabolism in diabetics is assumed to augment atherosclerosis, it confers protection to AAA development.\textsuperscript{46} Glucose metabolism was enhanced in AAAs as determined by positron emission tomography using \textsuperscript{18}F-fluorodeoxyglucose as a tracer.\textsuperscript{47} The direct role of enhanced glucose metabolism on AAA formation was demonstrated by inhibition of glycolysis attenuating AAAs in both calcium chloride and AngII models of the disease. In another study, advanced glycation end products present in the skin were positively correlated with AAAs.\textsuperscript{48}

AngII has been implicated in both AAAs and TAAs with antagonism of AT1 receptors as the focus of several clinical trials.\textsuperscript{49} Further experimental proof was provided for benefit in TAAs in the demonstration by Kuang et al.,\textsuperscript{50} who demonstrated that ascending aortic remodeling promoted by constriction of the aortic arch was reduced by angiotensin II type 1 receptor antagonism.

\textbf{Novel Pathways}

Two articles from \textit{ATVB} raise the role of additional pathways in aortic aneurysm formation. The first study reported that leptin was present in human AAAs, and this adipokine, which applied to periaortic fat of apolipoprotein E-deficient mice, led to medial degeneration and later aneurysm formation.\textsuperscript{51} Periaortic fat is associated with increased aortic diameter, and the role of periaortic fat and its adipokines merits further investigation. The second was the study reported by Liu et al.,\textsuperscript{52,53} reporting that mineralocorticoid receptor agonists induce aneurysm formation in mice. This study noted the aneurysm-provoking effects of aldosterone that were independent of mechanisms involving AngII.

\textbf{Summary}

Development of a validated medical therapy for treatment of aneurysms remains a major unmet medical need.\textsuperscript{4,5} In response to this need, there has been an expansion in research into mechanisms of aneurysmal formation, propagation, and rupture. Currently, there are several clinical trials ongoing that primarily focus on determining the benefits of inhibiting proteolysis or the renin–angiotensin system in AAAs and TAAs.\textsuperscript{49} Initial reports provide hope that medical therapies will be validated soon in demonstrating effectiveness in reducing expansion of TAAs.\textsuperscript{54}

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