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EDITORIAL

Seventy Years Later: Systemic and Local Properdin in Atherosclerosis

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The study of properdin by Louwe et al.[1] in the current issue of the *Journal of the American Heart Association* (*JAHA*) uses 2 large well-characterized clinical cohorts to rigorously document changes in properdin and its relation to carotid atherosclerosis and adverse outcomes associated with plaque vulnerability. The measures of properdin used are validated procedures and importantly examine circulating and local properdin. Overall, circulating properdin is higher in a cohort with atherosclerosis compared with control. However, closer examination of atherosclerotic individuals below the median circulating properdin demonstrated they had increased cardiovascular death versus those with properdin greater than the median. Importantly, the study demonstrates that properdin location is critical. Low circulating properdin is associated with increased cardiac events and high properdin within the plaques is associated with plaque vulnerability. This study will help focus future clinical studies of properdin and atherosclerosis to assess its utility as a predictor of risk of adverse cardiovascular events, its potential as a therapeutic target, and its role in the pathophysiology of the disease.

See Article by Louwe et al.

The discovery of properdin was reported some 70 years ago, in 1954 by immunologist Dr Louis Pillemer, who touted its ability to positively regulate complement and the effects it could have in a complement-activating pathway independent of antibody.[2] As with any new paradigm-shifting discovery, the techniques were scrutinized, and doubt was cast on the validity and interpretation of his experiments. In particular, a paper by Nelson[3] suggested that the results could be explained by natural antibody in the samples and insensitivity of the methods. The controversy around properdin continued for decades as the biochemistry of the complement system was unraveled and is an excellent example of the role that personalities and scientific controversy play in the acceptance of new ideas.[4,5] Debate continued but studies of properdin lulled. In 1968, Lepow published the purification of properdin, but the importance of the properdin system was not really recognized until the evidence continued to mount that there was an alternative pathway of complement activation. Gewurz et al.[6] demonstrated that incubation of guinea pig serum with endotoxin or zymosan activated C3 to C9 without affecting C1, C4, or C2. With this discovery and application of more modern biochemical techniques, research with properdin began again in earnest in the 1970s. Finally, the demonstration of interactions of properdin, Factor B, and C3 were

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elucidated and the importance of the alternative complement pathway as an antibody-independent pathway was defined. Properdin was not central to the alternative pathway as originally projected but stabilized the alternative pathway C3 convertase, C3bBb. Properdin is now clearly recognized as the only positive regulator of the complement system with identification of a preponderance of negative regulators in both the fluid phase and membrane bound. With properdin stabilization, alternative pathway complement activation continues, held in check by negative regulators on site. In the total absence (deficiency or knockout) or partial absence of properdin (partial deficiency or inhibition), alternative pathway activation still occurs but is not exacerbated or sustained and may be more influenced by the negative regulators.

In contrast to many complement products produced primarily in the liver, properdin is secreted by leukocytes, including monocytes/macrophages, mast cells, and T cells.[7] Importantly, inflammatory neutrophils also release significant quantities of properdin from secondary granules into serum and tissues including atherosclerotic plaques.[7] Recent studies also demonstrate that shear stress and turbulent blood flow such as that around a plaque can induce endothelial cells to release properdin.[7] Thus, local production of properdin is likely more influential than general circulating concentrations of this complement regulator in predicting its role in the pathophysiology of atherosclerosis and the consequences of destabilization of plaques.

Atherosclerosis and complement have been studied extensively, particularly in animal models, and as in so many arenas, results indicate that complement activation can be harmful and helpful. All 3 complement pathways are initiated in atherogenesis[8] and a noncanonical role for complement is also being identified.[9] A role for complement in atherosclerosis dates back to 1977 where studies in C6-deficient rabbits fed a cholesterol-rich diet demonstrated fewer atherosclerotic lesions compared with complement-sufficient rabbits,[10] suggesting that activation of the terminal pathway of complement contributed to development of atherosclerotic lesions. In general, in experimental models, the classical complement pathway tends to have a protective effect on atherosclerosis through C3 activation, whereas the terminal pathway tends to accelerate atherogenesis.[10] Thus, one would expect properdin working to stabilize an alternative pathway C3 convertase would be detrimental if it led to continued terminal pathway activation or could be protective if it allowed continued C3 activation in the absence of continued downstream C5 activation. The Kiss study supports this latter possibility, showing that Factor H-deficient macrophages with C3 overactivation did not culminate in downstream C5 activation and ultimately decreased plaque necrosis in a mouse model of atherosclerosis.[11]

In studies with global properdin knockout mice, atherosclerosis was greater in mildly atherosclerotic mice on a low-fat diet suggesting properdin was protective and complement activation at C3 helped control atherosclerosis.[12] However, the effect was not significant in mice who had more extensive atherosclerosis due to a high-fat diet. In contrast, in the human study by Louwe et al. in this issue,[1] circulating properdin was high in individuals with carotid atherosclerosis suggesting a stabilized alternative pathway convertase and increased complement activation contributed to atherosclerosis. However, with continued examination of the data, Louwe et al.[1] found that individuals with carotid atherosclerosis and with circulating properdin below the median of the atherosclerotic patients had the highest risk for atherosclerosis-induced cardiovascular events. This suggests that in patients with properdin above the median with higher levels of circulating properdin, complement activation was stabilized and protected against atherosclerosis-induced cardiovascular events. However, this stabilization in the blood tells only part of the story, because they also evaluated the amount of properdin in the atherosclerotic plaque in relation to plaque vulnerability. That is, the more properdin in the plaque, the more vulnerable the plaque was to cause a cardiovascular event. This indicates that properdin must be measured in both the circulation and within the plaque to assess the importance of properdin in cardiovascular events. In addition, the use of properdin knockout mice with total properdin loss does not mimic the atherosclerotic clinical situation and cannot mimic the increased properdin locally in the plaque. Thus, experimental models must be chosen carefully to mimic the situation in atherosclerosis and cardiovascular mortality in humans.

Defining properdin as harmful or helpful because of its location has also been investigated in other hypoxic or ischemic events. For example, within the confined area of brain hypoxia, properdin stabilized the alternative pathway leading to microglial cell death as determined by TUNEL staining for apoptosis and histology.[13] Also, systemic properdin knockout attenuated damage in early intestinal ischemia reperfusion[14] and properdin inhibitors decreased liver ischemia injury without inhibiting liver recovery after 70% hepatectomy.[15] In contrast, systemic loss of properdin increases glomerular damage in C3 glomerulopathy.[16,17] Together these data suggest that the effect of properdin on the complement cascade depends on location of the injury and whether the enhanced alternative pathway activation translates to activation of C5 and the terminal pathway.

The other opposing force to properdin, as a stabilizer of the alternative pathway convertase C3bBb, is the negative regulation of the alternative pathway by Factor H. Importantly, properdin itself decreases Factor H activity.[7] Presence of Factor H will decrease

C3 activation, and macrophages that produce both C3 and Factor H are present in both mouse and human atherosclerotic lesions.[8] A study by Kiss et al. using Factor H-deficient macrophages demonstrated that in the absence of Factor H in the macrophage, increased alternative pathway activation decreased the necrotic core of the plaque in the atherosclerotic lesion in mice.[11] In the study by Louwe et al.,[1] circulating properdin was increased overall in the atherosclerotic cohort, but circulating Factor H was unchanged so whether Factor H affects the risk of atherosclerotic lesions in humans is not tested by this study. The data suggest that atherosclerotic plaque destabilization occurs when properdin stabilizes the alternative pathway convertase locally and is even further destabilized when properdin decreases Factor H-mediated degradation of the convertase.

Although we are making strides in understanding the harmful and helpful roles of properdin, additional work is needed to make assays more widespread, available, and comparable across laboratories for detecting functional properdin. Serum properdin is readily measured by ELISA but functional properdin and tissue properdin within plaques is more difficult to evaluate. Modeled after the functional classical pathway assay, the alternative pathway hemolytic assay, AP50, uses hemolysis of sensitized rabbit erythrocytes to determine the dilution of serum that lyses 50% of the erythrocytes via the alternative pathway.[18] Clinically, the assay continues to be used today despite a readout of percent lysis of each specific lot of sensitized cells. This creates problems for researchers and for clinical comparisons across laboratories and over time. Genetics are expensive and time consuming and cannot determine the amount of residual function in a partial deficiency. ELISA quantitates serum properdin well but measurement of tissue-deposited properdin depends on the method of sample homogenization, which adds variability to the results and can increase laboratory-to-laboratory variation. Properdin specificity of an assay can be demonstrated with either properdin-deficient sera or properdin-dependent and -independent antibodies.[19] However, properdin bound to atherosclerotic plaques and in the tissue microenvironment would not be accurately detected. New assays are needed for both clinical and research studies. As always, the difficulty with complement research is the lack of cross-reactivity of human and rodent antibodies against complement proteins, so being able to measure specific molecules in a rodent model does not translate to clinical utility and vice versa. One new product for human properdin combines 2 ELISAs to determine total serum concentration and functional properdin activity.[20] The first ELISA determines total properdin and the second ELISA binds properdin in the sample before providing a properdin-deficient serum containing complement components for C3 cleavage, allowing C3b binding to the surface for detection. These newer functional assays provide simpler and more comparable assays but still require a homogenous solution, which is not ideal for measurement of properdin in tissue or plaques, indicating more work to be done.

This study of Louwe et al.[1] importantly uses clinical cohorts and measurements in humans with atherosclerosis to address the harmful or helpful effects of properdin and contributes a very important study investigating human atherosclerosis and complement where studies are urgently needed. Clearly, the inability to easily and accurately measure tissue properdin with comparability across laboratories has thwarted progress in this field. In addition, the complexity of the regulation of the complement pathway by numerous soluble and membrane-bound regulators adds to the uncertainty regarding the activation status of the pathway systemically and locally. The importance of local tissue (plaque) properdin versus circulating properdin must be underscored. Thus, understanding the critical location of properdin and complement activation will assist in understanding when and where it is helpful versus harmful.

ARTICLE INFORMATION

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Disclosures

None.

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