Research sheds new light on how blood clots form

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Scripps Research Institute scientists have discovered new elements of the blood clot-formation process. The findings could lead to better drugs for preventing heart attacks and other clot-related conditions.

The work, which was published by the Journal of Clinical Investigation in an advance, online edition June 13, 2011, helps to establish a new model of clot formation.

According to the old model, an injury to the wall of blood vessels causes smooth muscle cells to expose a clot-organizing protein called tissue factor. "In the emerging new model, tissue factor exists on the surfaces of these smooth muscle cells, as well as on circulating immune cells, but in an inactive state," said Scripps Research Professor Wolfram Ruf. "In this study, we've shown that cell-surface receptor P2X7, which was known to promote inflammation when stimulated, also plays a major role in the clot-forming process by activating tissue factor."

An Intriguing Target

To better understand clot formation, Ruf and his colleagues performed a set of experiments on cultured mouse cells and transgenic mouse models. The team's investigation began with the P2X7 receptor, because of its known role in the inflammatory response that can lead to excessive clotting in sepsis, a severe illness in which the bloodstream is overwhelmed by bacteria.

Normally, when cells are damaged, they release large quantities of energy-storage molecules known as adenosine triphosphate (ATP). Previous research had hinted that when this freed ATP encounters passing immune cells, it serves as a damage signal, stimulating the immune cells' P2X7 receptors and causing the release of "microparticles" exposing the clot-promoting tissue factor. The new study showed that ATP can affect P2X7 receptors on both immune cells and smooth muscle cells.

To confirm the significance of the P2X7 receptor in the clot-forming process, the team bred transgenic mice that lacked functional P2X7 receptors, and found that these P2X7-knockout mice failed to form stable arterial blood clots when the vessel wall was exposed to a clot-inducing substance. Importantly, these mice did not suffer from uncontrollable bleeding. "This suggests that clot-preventing drugs targeting the P2X7 pathway might not have unacceptable side effects," said Ruf.

In the cell experiments, the team found that the cascade of molecular events following P2X7 stimulation alters the activity of a thiol-targeting enzyme known as protein disulfide isomerase (PDI), which Ruf's previous studies had implicated as a possible activator of tissue factor. In the new study, the scientists demonstrated the importance of PDI in this process by showing that they could block clot formation in normal mice with anti-PDI antibodies.

Targeting the top of the clot-formation pathway by blocking the P2X7 receptor might have even broader beneficial effects, since the activation of this receptor occurs in a number of inflammatory disorders.

"Cardiovascular disease and heart attacks are caused by chronic inflammation as well as clot formation," said Ruf, "so possibly P2X7 is a major explanation for the link between inflammation and thrombosis, as

well as a good target for preventing these conditions."

More information: P2X7 receptor signaling contributes to tissue factor–dependent thrombosis in mice, J Clin Invest. doi:10.1172/JCI46129

Abstract

Thrombosis is initiated by tissue factor (TF), a coagulation cofactor/receptor expressed in the vessel wall, on myeloid cells, and on microparticles (MPs) with variable procoagulant activity. However, the molecular pathways that generate prothrombotic TF in vivo are poorly defined. The oxidoreductase protein disulfide isomerase (PDI) is thought to be involved in the activation of TF. Here, we found that in mouse myeloid cells, ATP-triggered signaling through purinergic receptor P2X, ligand-gated ion channel, 7 (P2X7 receptor; encoded by P2rx7) induced activation (decryption) of TF procoagulant activity and promoted release of TF+ MPs from macrophages and SMCs. The generation of prothrombotic MPs required P2X7 receptor–dependent production of ROS leading to increased availability of solvent-accessible extracellular thiols. An antibody to PDI with antithrombotic activity in vivo attenuated the release of procoagulant MPs. In addition, P2rx7–/– mice were protected from TF-dependent FeCl3-induced carotid artery thrombosis. BM chimeras revealed that P2X7 receptor prothrombotic function was present in both hematopoietic and vessel wall compartments. In contrast, an alternative anti-PDI antibody showed activities consistent with cellular activation typically induced by P2X7 receptor signaling. This anti-PDI antibody restored TF-dependent thrombosis in P2rx7–/– mice. These data suggest that PDI regulates a critical P2X7 receptor–dependent signaling pathway that generates prothrombotic TF, defining a link between inflammation and thrombosis with potential implications for antithrombotic therapy.

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