Factors Contributing to Individual Propensity for Arterial Thrombosis

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Objective—Occurrence of arterial thrombosis secondary to vascular disease in an individual is not easily predicted. After establishing that this poor predictability arises at least in part from an intrinsic thrombosis propensity of the individual, we sought to determine whether the propensity for arterial thrombosis is governed by blood or arterial wall factors.

Methods and Results—To evaluate the variability arising from the blood, autologous 111In-labeled platelet deposition was measured after high-shear perfusion of compressed aortic strips, prepared from a single pig, with heparinized blood from 25 pigs. To evaluate the variability arising from the vessel wall, aortic strips from 8 pigs were superfused with blood from a single animal. Blood samples from 25 animals superfused over aortic substrate from a single source yielded a 24-fold range of platelet deposition. In contrast, when aortic substrates from 8 different animals were superfused with blood from a single animal, platelet deposition spanned a 3-fold range. Platelet deposition was significantly correlated with whole-blood lymphocyte counts and with platelet counts.

Conclusions—Individual propensity for arterial thrombosis in pigs is more greatly influenced by blood components than by elements within the arterial wall. (Arterioscler Thromb Vasc Biol. 2002;22:887-894.)

Key Words: thrombosis ▪ lymphocytes ▪ platelets

The clinical presentation of atherosclerosis varies considerably among individuals with the disease. On one end of the spectrum are young patients who suffer from acute arterial thrombosis on a substrate of minimal arterial stenosis. On the other end are elderly patients, seemingly resistant to arterial thrombosis on a substrate of minimal arterial stenosis. Indeed, patients with chronic stable angina have more extensive coronary atherosclerosis than do those individuals with acute events.1 This disparity between atherosclerosis and acute thrombosis could occur randomly or arise from differences in atherosclerotic plaque composition, geometry, or stability (rate of plaque rupture). Alternatively, an individual propensity for thrombosis independent of vascular pathology may dictate the outcome of the disease. Several lines of evidence suggest the latter to be the case. Although a platelet-rich thrombus can form at the site of a ruptured atheromatous plaque, not all ruptured plaques result in thrombosis.2–5 Many coronary thrombi occur without demonstrable plaque disruption, and 20% to 30% of events occur in “angiographically normal” coronary arteries.6 Tools capable of determining an individual predisposition for arterial thrombosis would be beneficial in directing the use of prophylactic antithrombotic agents. To develop such tools, factors contributing to this broad range of thrombotic response must first be identified. The first step in this search is to determine whether this predisposition is more greatly affected by vascular or blood variables.

Using a porcine model of carotid crush injury, we have previously found evidence of a basal predisposition to arterial thrombosis, independent of shear stress and several hemostatic variables.7 Within a group of healthy young female pigs of uniform age and size, platelet deposition spanned a broad range among individual pigs but was remarkably constant when one carotid artery was compared with the other within an individual pig. Furthermore, this broad range of response is independent of the artery injured, with considerable variability of thrombus mass observed after crush injury to either carotid and femoral arteries.8 We have recently developed a method of arterial substrate preparation for perfusion-chamber experimentation that provides a uniform injury and exposure of all layers of the arterial wall to flowing blood.8,9 By use of this assay, various components of thrombus formation can be isolated to determine which variables influence the thrombotic propensity. To determine whether blood or vessel contributes more to the thrombotic propensity, the range of platelet deposition in blood from multiple animals perfused over an aortic substrate prepared from a single pig was compared with platelet deposition in blood from a single animal perfused over substrate prepared from...
multiple aortas. After determining that this predisposition arises from circulating blood components rather than elements within the arterial wall, cellular and plasmatic constituents were explored for their relative contributions.

Methods

Animals

Four-month-old, preestrus, female pigs of the Babcock 4-way cross stock (a mixture of Landrace, Yorkshire, Hampshire, and Duroc breeds) were purchased through the section of Veterinary Medicine, Mayo Clinic, from 2 local farmers and housed at the Mayo Institute Hills Facility. The present study was approved by the Mayo Clinic Animal Care and Use Committee and conformed to guidelines of the National Institutes of Health and US Department of Agriculture.

Platelet Labeling

One day before experimentation, autologous venous blood (42 mL) was collected into 1/7 vol of anticoagulant citrate dextrose (ACD, Baxter Healthcare Corp). Platelet-rich plasma was prepared by centrifugation of whole blood at 700g for 10 minutes. Platelets were then isolated from plasma by recentrifugation at 2800g for 10 minutes, washed once with 0.9% NaCl, and labeled with 500 µCi of 111In-tropolone for 20 minutes at room temperature. After 1 additional wash, labeled platelets were resuspended in autologous plasma (6 mL) and injected into the animal.

Flow Chamber

Perfusion chamber experiments using a novel preparation of arterial substrate were performed as previously described. Normal porcine abdominal aortas were surgically harvested, immersed in 2-methylbutane, frozen in liquid nitrogen, and stored at −70°C until use. To provide a consistent injury and equally expose each layer of the arterial wall to flowing blood, thawed aortas were cut into longitudinal segments (2.5 cm×0.5 cm), sandwiched between 2 sheets of polytetrafluoroethylene (Teflon), and serially compressed (1 ton/cm2 for 10 seconds, 2 tons/cm2 for 10 seconds, and 3 tons/cm2 for 30 seconds) with the use of a hydraulic platen press (Carver Inc). Compressed arterial segments were placed inside a methacrylate Badimon perfusion chamber and were perfused for 10 minutes at a flow rate of 5 mL/min (shear rate of 845 s−1) with HEPES- (3 U/mL) porcine whole blood containing 111In-labeled platelets drawn by a peristaltic pump (Drake-Willock). Aortic strips and duplicate EDTA-anticoagulated autologous whole-blood samples (5 mL) were then assayed for 111In content in a scintillation spectrometer (IsoData 500 series, Global Medical Instrumentation, Inc). To determine 111In content per platelet, whole-blood platelet counts were measured. Platelet deposition was then calculated by comparing 111In content of the perfused aortic strips (per cm2) with that of whole-blood samples.

Laboratory Assessment

Platelet and leukocyte counts with a 3-part leukocyte differential were obtained from EDTA-anticoagulated whole blood and measured with a Coulter Model S-plus IV. Fibrinogen was measured by light-scattering photometry with a centrifugal analyzer (ACL System). Plasma von Willebrand factor antigen was measured with a microtiter plate immunoassay (STA-Liatest vWF, Diagnostica Stago).

Results

Interindividual predisposition for arterial thrombosis and reproducibility of the perfusion chamber assay were assessed by perfusing duplicate blood samples from 25 pigs over compressed arterial substrate strips taken from a single aorta (Figure IA and IB, multiple pigs). There was a strong intraindividual correlation comparing platelet deposition on the first and second aortic strips (R²=0.56 and 0.86 with and without the outlier included, respectively). Within this cohort of female animals of similar age, platelet deposition from the lowest to the highest responders spanned a 24-fold range despite uniformity of source and injury to the arterial substrate and shear stress of the perfusate (coefficient of variation 1.05). To determine whether this interindividual variability of platelet deposition was best attributed to whole blood or vascular wall components, aortic substrate obtained from 8 pigs was perfused in duplicate with whole blood from a single animal (Figure 1B, multiple aortas). In contrast to the 24-fold distribution observed in the first set of experiments, platelet deposition in the second experiment spanned only a 3-fold range (coefficient of variation 0.39). ANOVA revealed a significant difference in platelet deposition when blood from 25 animals was perfused on a single aortic substrate source (P=0.0001). In contrast, no significant difference was noted when aortic strips from 8 animals were perfused with blood.
from a single animal \((P=0.75)\). The day-to-day intraindividual variability of platelet deposition was determined by perfusing daily blood samples in triplicate from a single animal sequentially over a 1-week period (Figure 1B; 1 pig, 1 week). Autologous platelets were labeled once with \(^{111}\text{In} \) before the first blood sampling. Daily blood samples for perfusion experiments were obtained until label decay prevented accurate scintillation detection (day 6). Over this time period, platelet deposition spanned a 2.3-fold range. The within-day variability depicted numerically compared favorably with the day-to-day variability of platelet deposition.

Hematological factors known to participate in arterial thrombosis were analyzed for their contribution to platelet deposition on perfused aortic substrate. The most striking association with aortic substrate platelet deposition was the whole-blood lymphocyte count (Figure 2A). Although none of these animals appeared ill to either the investigators or our institutional veterinary staff, the lymphocyte count spanned a range from 6 to \(13\times10^9/L\). By linear regression analysis, the absolute lymphocyte count correlated directly with substrate platelet deposition \((P=0.001)\). In contrast, platelet deposition did not correlate with either the granulocyte or monocyte count (data not shown). In pigs with lymphocyte counts exceeding \(9\times10^9/L\), platelet deposition was significantly greater compared with animals with lower lymphocyte counts \((P=0.005, \text{Figure 2B})\).

By linear regression analysis, whole-blood platelet counts \((P=0.02)\) were significantly correlated with substrate platelet deposition (Figure 3A). Platelet deposition was not statistically different when whole-blood platelet counts exceeded \(300\times10^9/L\) compared with counts below this value \((519\pm411 \text{ versus } 205\pm90 \text{ [mean±SD]}, \text{respectively}; P=0.06)\). However, platelet deposition was significantly increased in animals with an elevated platelet count combined with an elevated plasma fibrinogen concentration \((>450 \text{ mg/dL})\) compared with animals with lesser values \((P<0.02, \text{Figure 3B})\). In isolation, neither von Willebrand factor antigen nor fibrinogen content was correlated with platelet deposition (data not shown).

Pigs were purchased from 2 unrelated local farmers and housed at our institutional veterinary facility for \(3 \text{ to } 4 \text{ days}\) before experimentation. To determine the contribution of differences in animal husbandry to thrombotic response, platelet deposition was compared between animals obtained from these 2 vendors (Figure 4). The lymphocyte counts were significantly higher in animals from vendor A compared with vendor B \((P=0.003)\), and a trend is apparent \((P=0.07)\) in corresponding platelet deposition.

**Figure 2.** Platelet deposition and lymphocyte counts. A, Platelet deposition was significantly correlated with whole-blood lymphocyte counts \((P=0.001 \text{ by linear regression analysis})\). B, Platelet deposition was significantly increased when lymphocyte counts exceeded 9 compared with lesser values \((P=0.005 \text{ by unpaired 2-tailed Student } t \text{ test})\).

**Figure 3.** Platelet deposition and platelet counts. A, Platelet deposition was significantly correlated with whole-blood platelet counts \((P=0.02 \text{ by linear regression analysis})\). B, Platelet deposition was significantly increased when an elevated platelet count was combined with elevated fibrinogen \((P=0.02 \text{ by unpaired 2-tailed Student } t \text{ test})\).
Discrimination,

Platelet-rich thrombosis varies substantially among individual pigs irrespective of the artery injured or whether the measurement is performed in vivo or in vitro.\textsuperscript{7,8} Within the cohort of young preestrus female animals free of gross atherosclerosis, platelet deposition spanned a 24-fold range in response to a reproducible injury to a uniform substrate in a perfusion chamber assay with the use of heparinized whole blood (3 U/mL), yet platelet deposition was remarkably constant when duplicate samples were compared. In contrast, when blood from 1 animal was perfused over substrate samples from multiple aortas, the range of platelet deposition spanned only a 3-fold range. This latter range is comparable to that observed when blood from 1 animal was sampled serially over a 1-week time course and may reflect the inherent variability of the assay. These findings support the concept of an individual propensity for arterial thrombosis and provide an in vitro counterpart to previous in vivo findings from our laboratory. Factors governing this predisposition appear to arise from circulating blood components rather than from elements within the arterial wall. The lymphocyte count stands out among contributing factors.

Although the association between leukocytosis and coronary artery disease has long been recognized, the extent to which this association is atherogenic versus thrombogenic is unclear.\textsuperscript{15–20} Leukocytes are actively recruited into growing arterial thrombi by platelet P-selectin–mediated cell-cell interactions and can support the thrombin generation.\textsuperscript{21–25} Although lymphocytes do not appear to accumulate in thrombi, they were the only leukocyte subtype that was significantly correlated with substrate platelet deposition.\textsuperscript{26}

Lymphocytes have been shown to influence monocyte tissue factor expression in humans. Serneri et al.\textsuperscript{27} found that monocytes isolated from the blood of patients with unstable angina express little tissue factor activity. However, the addition of lymphocytes from these same patients to monocytes isolated from normal control subjects or patients with stable angina stimulated a significant increase in procoagulant activity. Direct lymphocyte-monocyte contact was required, and the effect was no longer evident 8 to 12 weeks after recovery. In any case, the finding of a large difference in lymphocyte count and associated difference in propensity for thrombosis raises the intriguing hypothesis of a viral connection. The number of observations is too small to be definitive, but the vendor supplying the pigs with the high lymphocyte counts is a large-scale commercial confinement facility, whereas the other is a modest family farm.

Plasmatic factors have been extensively studied for their contribution to arterial thrombosis.\textsuperscript{29–33} Fibrinogen has been consistently, independently, and strongly related to coronary disease and acute coronary syndromes. Regarding the leukocyte data, the causal relationship between fibrinogen concentration and either atherosclerosis or arterial thrombosis has not been established. In this and previous studies from our laboratory, we have been unable to show a correlation between either fibrinogen or von Willebrand factor content and platelet deposition. However, when combined with an elevated platelet count, an elevated fibrinogen level was significantly associated with increased platelet deposition. These data provide evidence of a plausible relationship between platelet-rich thrombus formation and fibrinogen levels independent of the atherosclerotic process.

Although it was not assessed in these experiments, circulating tissue factor is an additional variable of interest that may contribute to the individual propensity for thrombosis. Giesen, Rauch, and colleagues\textsuperscript{34,35} have provided convincing evidence supporting the role of circulating tissue factor in platelet thrombus formation. Although vascular tissue factor, known to be rich in atheromatous plaque, may initiate thrombosis after plaque disruption, deposition of circulating tissue factor on platelets at the blood-thrombus interface may be important for thrombus propagation. The source and regulation of blood-borne tissue factor and its contribution to an individual propensity for arterial thrombosis remain important questions.

In conclusion, these data support the concept of an individual propensity for arterial thrombosis. This predisposition appears to be more greatly influenced by circulating cellular components than by elements within the arterial wall. Although the whole-blood lymphocyte count was found to be significantly correlated with platelet deposition, the mechanism underlying this correlation and the contribution of the lymphocyte to arterial thrombosis in humans require further study. The shear conditions (845 s\textsuperscript{-1}) chosen for these experiments approximate the carotid circulation; therefore, these results may not be generalized to other vascular beds.\textsuperscript{8}
References


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