it is clear that allogeneic T cells in the transplant and in donor lymphocyte infusions can bring about a graft-versus-leukemia effect. Evidence for the importance of an autologous T-cell response against AML has come from the tumor vaccination field. Vaccination against the leukemia-associated antigens[2] Wilms tumor protein 1 (WT1),[3-6] PR1 (derived from proteinase 3),[4] and receptor for hyaluronic acid–mediated motility (RHAMM)[7] can bring about clinical antileukemic effects in AML. The clinical response was generally correlated with the T-cell responses elicited.[3,4,6] Loss of clinical response has been reported to be associated with decrease or loss of specific T-cell immunity.

But can an antileukemic immune response be elicited in patients not receiving immunotherapy? The answer comes from a vast body of work, demonstrating that, contrary to general belief, certain chemotherapeutic agents can augment immune responses against tumors.[8] Chemotherapy thus not only has direct cytotoxic effects on cancerous cells, but can also boost the immunity against them by different mechanisms, including stimulating tumor antigen presentation by dendritic cells to cytotoxic T lymphocytes. This is particularly true of anthracyclines, still the mainstay of treatment of AML, which have been demonstrated to be a prototype of immunogenic chemotherapy.[9] It was already known for a while that the antitumoral effect of doxorubicin in certain animal models was strongly reduced if the immune system was not functioning properly.

In the case of *NPM1*^{mut} AML, especially if it is also *FLT3-ITD*^{neg}, the autologous T-cell response induced by the mutated NPM1 could bring about a significant antileukemic effect directly after chemotherapy (figure panels A and C). At this stage, the number of leukemic cells would significantly be reduced, the immune response could be strengthened, and the stimulated anti-NPM1^{mut} cytotoxic T lymphocytes could mount a final attack against the remaining leukemic cells. This could account for the cures seen with chemotherapy alone in *NPM1*^{mut} AML. But not all patients with *NPM1*^{mut}*FLT3-ITD*^{neg} AML are cured by chemotherapy alone. The findings by Greiner et al theoretically suggest the possibility that postremission immunotherapy directed against NPM1^{mut} could induce cures and/or longer-lasting remissions in this type of AML and maybe even in *NPM1*^{mut}*FLT3-ITD*^{pos} AML, especially if there is molecular evidence of residual disease.

An additional potential advantage of the T-cell immune response directed against certain leukemia antigens is that it may also be directed against the leukemic stem cells.[2] Leukemic stem cells are relatively resistant to chemotherapy,[10] accounting at least in part for the (minimal) residual disease persisting after cytotoxic treatment in a majority of AML cases (figure panel B). The chemotherapy resistance of minimal residual disease has led to the development of another type of postremission treatment, that is, immunotherapy, to try to definitively cure AML patients (figure panel D). NPM1^{mut}, a leukemia-specific antigen,[2] is expressed in leukemic stem cells,[11] making those cells vulnerable to immune eradication, as discussed above.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● THROMBOSIS & HEMOSTASIS

Comment on Fuchs et al, page 1157

A second hit for TMA

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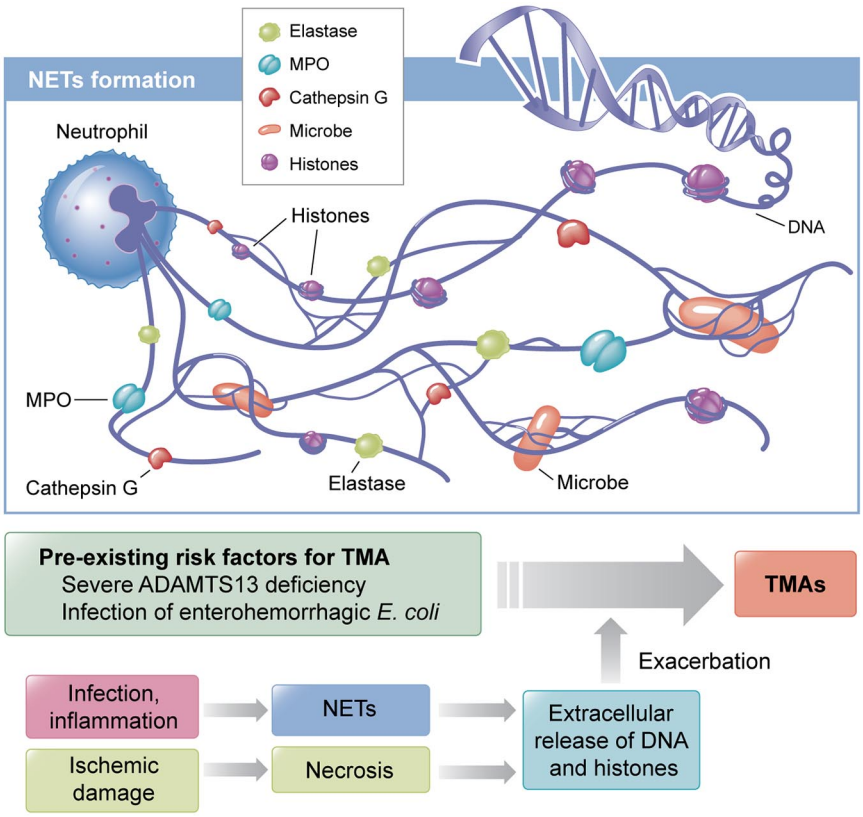
In this issue of *Blood*, Fuchs and colleagues provide evidence that circulating DNA and histones, presumably released from neutrophils, would be the second hit for development of thrombotic microangiopathies (TMAs), a group of life-threatening disorders characterized by thrombi in the microvasculature resulting in thrombocytopenia, microangiopathic hemolysis, and organ dysfunction.[1]

TMA includes thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS). TTP is caused by a severe deficiency of von Willebrand factor cleaving protease, ADAMTS13, because of autoantibodies or genetic mutations. HUS is caused by infection with Shiga-toxin–producing *Escherichia coli* and is typically associated with bloody diarrhea. Atypical HUS, which has a link with defective complement regulation, is also present. Other conditions such as cancer, bone marrow transplantations, and lupus can present with features of TMA. Although patients with congenital TTP show severe ADAMTS13 deficiency, some patients may remain asymptomatic for many years.[2] An infection often precedes acute TMA.[3]

The innate immune response plays a crucial role for defense against invading microbes. Neutrophils, the most abundant leukocytes, are early responding cells that migrate in large numbers to sites of infection and release



Neutrophils release nucleosomes, a complex of DNA and histones, in response to infection or inflammatory stimuli. Neutrophil extracellular traps (NETs) are composed of nucleosomes decorated with granular components including myeloperoxidase (MPO), neutrophil elastase, and cathepsin G.[4] NETs bind and kill microbes.[4] NETs also immobilize platelets[10] and erythrocytes. Histones are known to stimulate thrombosis and to cause cytotoxicity in mice.[5-7] Once patients with pre-existing risk factors for thrombotic microangiopathy (TMA) are infected, DNA and histones, in conjunction with granular proteins, are released and acute TMAs would be induced. Another possible origin of extracellular DNA and histones is necrotic tissue released after ischemic damage. Professional illustration by Kenneth X. Probst.

nuclear chromatin associated with nuclear histones and granular antimicrobial proteins after cell death to form neutrophil extracellular traps (NETs; see figure).[4] Microbes bind to NETs and are subsequently killed by antimicrobial proteins. Extracellular DNA and histones have recently been shown to have prothrombotic characteristics.[5,6] Histones cause thrombocytopenia, promote thrombosis, and contribute to organ damage and death.[7,8] In most cases, in vivo studies demonstrating the prothrombotic characteristics of histones have been performed in the mouse or baboon model.

At Bern University Hospital and the University of Bern, plasma samples from TMA patients of different clinical categories have been collected and stored more than 10 years. Retrospectively, Fuchs and colleagues used these samples to investigate the possible risk factors to develop TMAs.[1] First, they found elevated plasma levels of DNA, nucleosomes, lactate dehydrogenase (LDH), myeloperoxidase (MPO), and S100A8/A9 in acute TMA patients. LDH, a cytoplasmic enzyme, is a marker of tissue damage. MPO is abundantly stored in granules of neutrophils. S100A8/A9, present in the cytosol of neutrophils and monocytes, is a marker of inflammation. In clinical remission, plasma levels of DNA, LDH, MPO, and S100A8/A9 were decreased. Importantly, the great reduction of plasma levels of DNA and MPO was concomitant with the increase in platelet counts and plasma ADAMTS13 levels in acute TTP patients with remission, indicating correlation of DNA and MPO levels in disease state. Severe ADAMTS13 deficiency per se does not lead to an increase in plasma DNA or MPO levels, while DNA and MPO are elevated only during disease flare-up. These findings indicated that extracellular DNA and histone levels during acute TMA could increase the risk for developing TMAs and provide a second hit that triggers acute disease in patients at risk for TMA. Disease pathogenesis sometimes involves additional unknown genetic factors and/or environmental triggers. For example, mice lacking the ADAMTS13 gene are predisposed to acute TMA, but Shiga-toxin is needed to induce the acute disease.[9]

What is the triggering event for extracellular DNA and histones in TMA patients? Recent studies showed infection as the most commonly identified etiologic factor for TMA,[3] and LPS can induce NET formation through platelet TLR4.[10] Therefore, it is conceivable that release of DNA and histones from neutrophils is caused by a preceding infection. What is the origin of extracellular DNA and histones? Beside NETs, necrotic tissue after ischemic damage could be a source of these 2 compounds (see figure). What are the natures of DNA and histones? Circulating DNA is likely fragmented by endogenous DNases[4] and histones are cleaved by activated protein C[7] and/or other proteases. Therefore, tools that degrade and inactivate prothrombotic DNA and histones would seemingly be promising candidates for preventing TMAs and other thrombotic complications. Finally, this is a retrospective study; thus, whether nucleosomes, DNA, or histones contribute directly to the clinical manifestation of acute TMAs remains to be determined. Nevertheless, evidence that extracellular DNA and histones are elevated in patients with acute TMA and decreased in remission concomitant with the increase in platelet counts advances the understanding of the pathogenesis of acute TMAs.

Conflict-of-interest disclosure: T.M. is an inventor of the ADAMTS13 assay method, which is related to its patent. X.P.F. declares no competing financial interests. ■

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VASCULAR BIOLOGY

Comment on Cloutier et al, page 1334

The functional dissonance of platelets

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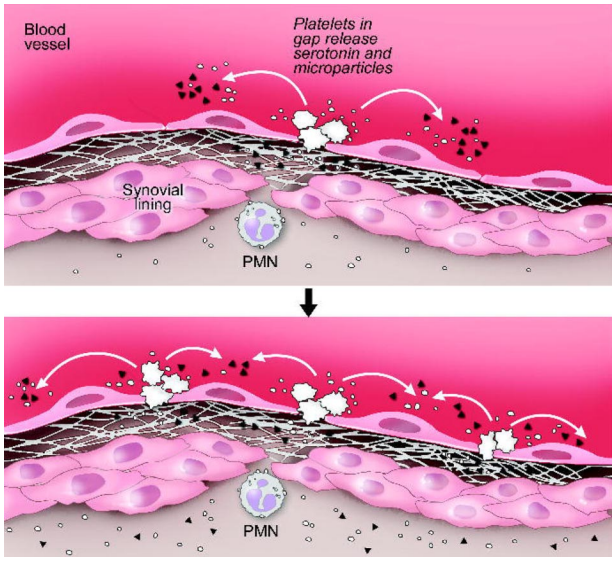
In this issue of *Blood*, Cloutier et al answer a long-standing but unappreciated question about the influence of platelets on vascular permeability.[1]



Proposed pathway for the formation of gaps and amplification of the vasculature permeability by platelets during arthritis. (Top panel) Gaps between endothelial cells in arthritic joints are formed. The GPVI-expressing platelets are activated by the leakage during disease. Note that the precise anatomic location of platelet activation, and the route by which microparticles enter the joint remains speculative. Δ indicates serotonin and ○, platelet microparticle. Professional illustration by Steve Moskowitz, Advanced Medical Graphics. See Figure 7 in the article by Cloutier et al that begins on page 1334.

The role of platelets in responding to vascular injury and the prevention blood loss is very well understood. Cloutier and colleagues have now used fluorescent microspheres, 2-photon microscopy, and genetically modified mice to convincingly demonstrate that in addition to their role in hemostasis, platelets can also promote vascular leakage. More importantly, using a model of rheumatoid arthritis, they present data that uncoupled vascular permeability from inflammation, and their data suggest that platelets can increase vascular permeability directly, through the release of serotonin (see figure).

The concept of platelet-induced vascular permeability is not new; more than 40 years ago Nachman et al reported that platelet granule extracts were capable of inducing vascular permeability.[2] However, despite efforts to identify the molecular components responsible for this activity,[3] the molecular mechanism whereby platelets promote vascular permeability have remained elusive. Nevertheless, over the years circumstantial evidence has supported the notion that platelets can induce vascular leakage.[4,5] Here, Cloutier et al present convincing evidence that endothelial gap formation in arthritic vessels depends on the presence of platelets, and that this activity is independent of the inflammation normally seen in rheumatoid arthritis. Specifically, animals treated with platelet-depleting antibodies showed significantly less vascular leakage than controls after the onset of arthritis, as demonstrated by the direct injection of fluorescent microspheres. In addition, inducing inflammation with injections of IL-1β did not change the outcome of platelet-depleting antibody treatment on vascular permeability, suggesting that platelets and not inflammation were the cause of increased vascular permeability in rheumatoid arthritis.

Interestingly, the size of microspheres that gained access to arthritic joints in this study appeared to be limited to 0.45 μm to 0.84 μm, a range that is very similar to serotonin-induced endothelial gaps seen in vessels in the cremaster muscle identified by electron microscopy (0.1-0.8 μm).[6] Because platelet-dense granules are known to contain high concentrations of serotonin,[7] Cloutier et al investigated whether serotonin in platelets was associated with vascular permeability in rheumatoid arthritis. These studies demonstrate that unlike patients with osteoarthritis, patients with rheumatoid arthritis have both more platelet microparticles and serotonin in their synovial fluid. They also found that direct injection of serotonin into healthy mice induced vascular leakage reminiscent of arthritic animals. These data suggest that platelet-derived serotonin was important in endothelial gap formation. To test this hypothesis, Cloutier et al took advantage of mice deficient in the serotonin transporter (SERT), which enables platelets to take up and store serotonin.[8] Using the SERT-deficient mice, Cloutier et al convincingly demonstrated that mice with low levels of serotonin in their platelets had significantly reduced fluorescent microspheres accumulation in their joints during arthritis development. Finally, Fluoxetine, a psychiatric drug that inhibits the uptake of serotonin, significantly reduced vascular leak in their rheumatoid arthritis mouse model. This observation is consistent with a previous report by Sacre et al on the efficacious effect of Fluoxetine in rheumatoid arthritis but may offer a different mechanism.[9]

Overall, this novel finding that platelet-induced vascular permeability is mediated via serotonin in rheumatoid arthritis signifies a change in the view about how platelets can affect vascular integrity. More importantly, understanding this new pathway may generate new treatment options for diseases such as rheumatoid arthritis.