the life-prolonging benefit of ruxolitinib in MF. By inhibiting inflammatory cytokines and controlling the signs and symptoms of MF, the patient's body condition improves as the disease is kept under good control for a prolonged period of time, preventing "disease progression" (see figure).[10]

There are several prerequisites related to the optimal management of patients on ruxolitinib therapy in order to provide long-term benefit and potentially extend life expectancy.[2,10] Guidelines for the starting dose of ruxolitinib are well established and should be followed closely: most dose adjustments happen within the first 3 months of therapy. This is a period where most benefits are also observed. Due to its short half-life, ruxolitinib should be used in a twice daily (BID) schedule; daily dosing was reported overall to be ineffective. Proactive dose adjustments are recommended to maintain patients on therapy with an effective dose and without interruptions. The higher the dose of ruxolitinib, the better is the spleen response. This appears to be important for survival benefit: 2 studies so far reported a correlation between the degree of spleen reduction and survival. However, 10 mg of ruxolitinib BID is equally as effective in controlling constitutional symptoms as higher doses (maximum dose is 25 mg BID). If starting with a low dose (eg, 5 mg BID in patients with low platelets), dose increases should be made monthly, if safe; later increases provide less benefit. Anemia has been identified as the most common side effect of ruxolitinib. The development of significant anemia on ruxolitinib therapy does not diminish its benefits: patients with or without ruxolitinib-related anemia experienced the same level of improvements in spleen and quality of life. In addition, with proper dose adjustments, there is usually a rebound in hemoglobin to near baseline levels in patients on therapy. In general, interruption of ruxolitinib therapy leads to the return of constitutional symptoms to baseline within 7 to 10 days, while regrowth of spleen usually happens at a slower rate. In a case of significant myelosuppression, 5 mg ruxolitinib BID can be used, but doses of 10 mg BID or higher have been shown to be good maintenance therapy.

How to further optimize therapy with ruxolitinib is a goal of many ongoing clinical studies, where new investigational agents are being combined with ruxolitinib to further increase its benefits, decrease its side effects (eg, improve platelets or red blood cell count), or bring additional benefits (eg, antifibrotic agents). These efforts may make MF even more indolent and prolong life further.

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● ● ● PHAGOCYTES, GRANULOCYTES, & MYELOPOIESIS

Comment on Weckbach et al, page 1887

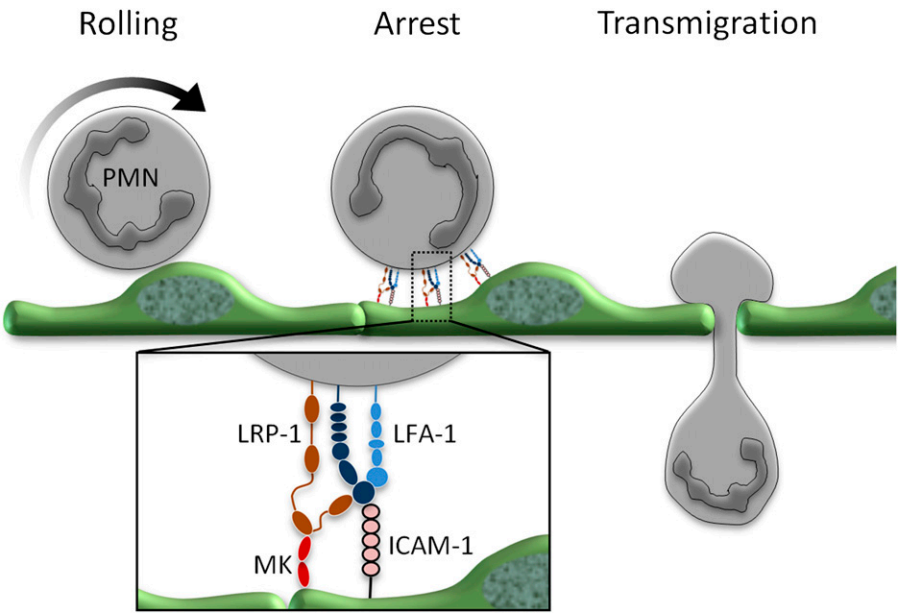
Midkine, a middle manager of β_2 integrins

Jan M. Herter[1] and Tanya N. Mayadas[1] [1]HARVARD MEDICAL SCHOOL

In this issue of *Blood*, Weckbach et al demonstrate that midkine (MK), a described regulator of inflammation, supports neutrophil recruitment by promoting the high-affinity conformation of the β_2 integrin lymphocyte function-associated antigen 1 (LFA-1), a step required for neutrophil arrest on the activated endothelium.[1]

The authors report that the heparin-binding growth factor MK (also referred to as neurite growth-promoting factor 2) is essential for neutrophil arrest at the site of inflammation.[1] Mice deficient for MK had markedly reduced numbers of adherent neutrophils in response to tumor necrosis factor (TNF), as assessed by intravital microscopy. This correlated with an impressive decrease in extravasated neutrophils and amelioration of tissue damage in a model of limb ischemia.[1] These findings are in line with previous studies showing that MK-deficient mice are protected from organ damage in a variety of sterile inflammatory models, including rheumatoid arthritis, renal ischemia/reperfusion injury, and inflammatory bowel disease.[2] Furthermore, others have used anti-sense oligoDNA and an RNA aptamer to inhibit MK functions and have shown a reduction in leukocyte infiltration in models of nephritis and autoimmune encephalitis (for an overview, see Muramatsu[3]). Thus, several lines of evidence suggest that MK plays a critical role in leukocyte recruitment.

Weckbach et al now identify a molecular mechanism that can explain the observed



Midkine (MK) in leukocyte recruitment. Following rolling, neutrophils arrest on the inflamed endothelium and then transmigrate. The conversion from rolling to arrest is regulated by MK binding to LRP-1 and the subsequent conformational change of LFA-1 to its high-affinity state, a step required to allow firm integrin binding to ICAM-1 on the activated endothelium.

MK-dependent leukocyte accumulation. The authors show that MK is required specifically for the induction of the high-affinity conformation of the LFA-1 adhesion molecule on neutrophils.[1] This β_2 integrin possesses at least 3 distinct molecular configurations: low affinity (inactive); intermediate affinity, as induced by selectins during slow leukocyte rolling; and high affinity, as triggered by chemokine activation. Neutrophil arrest critically depends on the activation of LFA-1 to a high-affinity conformation.[4] Interestingly, the authors found that only the induction of the high-affinity conformation required MK, as assessed by reporter antibodies, while MK was dispensable for the intermediate-affinity state, as implied by the lack of effect of MK deficiency on leukocyte slow rolling (see figure). Adhesion strengthening, a process involving clustering of high-affinity LFA-1 at the neutrophil-endothelial interface and cytoskeletal rearrangements following chemokine activation[4] was independent of MK even though MK was necessary for inducing the integrin conformation required for this step. It is worth mentioning that β_2 integrins are also required for leukocyte recruitment steps after arrest, such as intravascular crawling, transmigration, and detachment.[4] Although the authors' results can be explained by MK's requirement for the adhesion step alone, it would be interesting to explore whether consecutive steps are affected in a similar way in neutrophils as well as mononuclear populations. The latter could be particularly interesting because monocytes and T cells rely on both β_2 integrins and the β_1 integrin VLA-4 for arrest[4] and might therefore not have an absolute requirement for MK for this step. However, all leukocyte populations seem to require LFA-1 for transmigration,[4] which may predict that transmigration of these cells is equally affected by MK independently of the requirement for MK in the arrest step. Although differences in the recruitment of other cells to the TNF-inflamed cremaster muscle was not observed, this model is not optimal for evaluating mononuclear cell accumulation.

Several interesting characteristics of MK were revealed in this study. It does not behave like a canonical cytokine, since it had no effect on neutrophil activation per se and did not induce endothelial cell activation. Reminiscent of chemokines such as stromal cell-derived factor 1α (SDF-1α), immobilization of MK was required to induce polymorphonuclear neutrophil (PMN) adhesion and transition of the β_2 integrin to the high-affinity state. Thus, in vivo, the observed rescue of adhesion in $MK^{-/-}$ mice by delivery of soluble MK may reflect the activity of MK bound to proteoglycans presented on the endothelium together with intercellular adhesion molecule 1 (ICAM-1). In line with this, the sulfated glycosaminoglycan Heparin, which can potentially interfere with this binding has been shown to inhibit many MK functions.[3]

Multiple receptors for MK have been identified, including the anaplastic lymphoma kinase receptor (CD246) and receptor-like protein tyrosine phosphatase ζ.[3] Relevant to the current study, low-density lipoprotein receptor-related protein 1 (LRP-1) has previously been described as being associated with the I-domain of the α-chain of the β_2 integrin macrophage antigen-1 (Mac-1)[5] and being required for integrin clustering and adhesion of the monocytic cell line U937.[6] Using pharmacologic inhibition, the authors provide evidence that LRP-1 is the receptor for MK on neutrophils because blockade of this receptor inhibited the MK-mediated LFA-1 high-affinity conformation and neutrophil adhesion in vitro.

MK is produced by immune cells and also by endothelial cells.[3] In vitro, PMN-derived MK does not appear to be required for adhesion, suggesting that in vivo, MK produced by the endothelium possibly serves as a local regulator, with concentrations of this molecule potentially playing a key role in modulating leukocyte recruitment. In line with this concept, hypoxia, activation of the nuclear factor κB (NF-κB) pathway as well as cytokines like TNF-α and interleukin-1β (IL-1β) upregulate MK expression.[7] Moreover, MK levels may be downregulated by its internalization by the endocytic receptor LRP-1.[8]

This study raises several questions for fruitful future investigation. For example, given the significant effects on transmigration, does MK influence ICAM-1 signaling, which affects junctional reorganization associated with transmigration? How does LRP-1 mechanistically regulate β_2 integrin activation? Is MK association with LRP-1 and subsequent modulation of LFA-1 modulated by the priming of neutrophils?

In summary, Weckbach et al have provided a molecular mechanism for the observed requirement for MK in leukocyte extravasation in many inflammation models. The specific function of MK for a discrete process in leukocyte recruitment and its extracellular accessibility make it an ideal target for pharmacologic intervention.

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Comment on Colucci et al, page 1905

Desmopressin and super platelets

Alessandro Pecci[1] and Carlo L. Balduini[1] [1]ISTITUTO DI RICOVERO E CURA A CARATTERE SCIENTIFICO POLICLINICO SAN MATTEO FOUNDATION

In this issue of *Blood*, Colucci et al provide experimental data supporting the hypothesis that desmopressin (DDAVP) favors the hemostatic process not only by inducing the release of von Willebrand factor (VWF) from endothelial cells, but also by enhancing the procoagulant activity of platelets.[1]

DDAVP is an analog of the antidiuretic hormone that interacts with type 2 vasopressin receptors of endothelial cells and induces secretion of ultralarge VWF multimers, resulting in sustained rise in plasma levels of VWF and associated factor VIII (FVIII). After demonstration in 1977 that the release of VWF occurs and is hemostatically effective in subjects with mild hemophilia and von Willebrand type 1 diseases,[2] DDAVP has become a mainstay of treatment of these conditions. The excitement about this important achievement encouraged studies to test this drug, even in hemorrhagic conditions that, based on their pathogenesis, were not expected to benefit from increased levels of VWF or FVIII. The results dashed hopes that DDAVP could be a sort of panacea—good for all purposes—since negative or conflicting results have been obtained in most cases.[3] However, some findings supported the efficacy of DDAVP in platelet disorders. In particular, clinical studies with surrogate end points showed that this drug was effective in improving hemostasis in subjects with thrombocytopenia associated with bone marrow failure,[4] in platelet dysfunction due to antiaggregant agents,[5] and in some inherited thrombocytopenias and inherited defects of platelet function.[6,7] Moreover, a few case reports suggested that DDAVP was effective in halting or preventing bleeding in specific forms of inherited platelet disorders.[8] Despite these appealing premises, the interest of researchers in this topic progressively diminished over time, partly because of the lack of plausible mechanisms explaining the possible efficacy of DDAVP in platelet disorders. Because of the shortage of sound clinical evidence on relevant end points, recent guidelines on management of patients with platelet defects recommended using this drug only as a last resort to stop bleeding after all other treatments failed.[8]

The proposal in this issue of *Blood* of a novel mechanism by which DDAVP improves hemostasis by a platelet-mediated effect is therefore important news that could bring the topic back to the attention of researchers.

When platelets are costimulated in vitro with collagen and thrombin, a portion of them expose on their surfaces negatively charged aminophospholipids together with α-granule proteins, including fibrinogen, VWF, thrombospondin, fibronectin, alpha2-antiplasmin, and factor V (collagen- and thrombin-activated [COAT] platelets).[9] It is expected that COAT platelets are formed in vivo under circumstances of extreme hemostatic need, as immobilization of platelets on the collagen surface of a damaged vessel in the presence of thrombin generation. COAT platelets therefore represent a unique component of hemostasis, since their coating with adhesive and procoagulant proteins is potentially able to boost the hemostatic process at the sites of vascular injury.

Colucci et al administered DDAVP intravenously to 78 patients with mild primary platelet secretion disorders and investigated the percentage of COAT platelets generated in their blood samples by the combined action of thrombin and convulxin, a rattlesnake venom that activates platelets by mimicking the action of collagen. They found that generation of COAT platelets in blood samples taken after DDAVP was significantly increased with respect to baseline. Moreover, they also observed that DDAVP enhanced platelet-dependent thrombin generation.

The main hemostatic role of platelets is to localize the coagulation cascade at the site of a vascular injury (see figure). Circulating platelets immediately recognize exposed subendothelium and interact with specific molecules that include collagen. Then, platelets undergo a series of changes that support blood clotting and result in a mesh-like fibrin deposition that stops bleeding. COAT platelets are expected to do this job better because of their increased adhesiveness and enhanced procoagulant activity. It therefore conceivable that the improved hemostatic activity of platelets induced by DDAVP can compensate for quantitative or qualitative defects of these cells.

Colucci et al investigated patients with mild defects of platelet secretion who usually do not present with spontaneous bleeding. It would be interesting to know whether DDAVP also promotes COAT platelet formation in patients with Bernard-Soulier syndrome or Glanzmann thrombasthenia, who have a much more severe tendency toward bleeding and could benefit most from an enhanced platelet function. Obtaining this information is important because it has also been suggested that DDAVP has little effect on hemostasis in Glanzmann thrombasthenia while it is effective in Bernard-Soulier syndrome.[7] The demonstration that DDAVP-induced formation of COAT platelets differs between these two conditions would be an indirect confirmation of the hemostatic role of this platelet subpopulation.

Acquiring the ability to improve the hemostatic activity of platelets in patients with functional platelet defects is clinically relevant, especially in Bernard-Soulier syndrome and