The efficacy of a B-cell–depleting therapy, despite the presence of autoreactive T cells, led Stasi and colleagues in this issue to demonstrate that the therapeutic efficacy of rituximab is actually due to normalizing abnormal autoreactive T-cell responses in patients with ITP.

The authors studied 30 adult patients with chronic ITP that were treated with the standard rituximab dose of 375 mg/m^2 weekly for 4 weeks, and employed a variety of sophisticated techniques to analyze several T-cell parameters prior to treatment and at 3, 6, and 12 months after treatment. The results were correlated with the patients' responder status. What they found was not only convincing, but quite astonishing. The pretreatment T-cell abnormalities, including elevated Th1/Th2 (and Tc1/Tc2) cytokine ratios, elevated CD4$^+$ T-cell–associated Bcl-2/Bax mRNA levels, and oligoclonal T-cell expansion, were completely reversed by 3 months after treatment. This was observed only in those patients who responded to rituximab therapy; these normalization changes persisted for as long as 6 to 12 months after therapy. These results suggest the intriguing hypothesis that only when T-cell subsets can be modulated is rituximab therapy effective. The reasons for these results are not clear, but may relate to how B-cell populations can be important in maintaining T-cell activation patterns by interactions such as CD40/CD40L ligation.

The results are also consistent with the recently reported efficacy of rituximab in patients with refractory chronic graft-versus-host disease, which is known to be a T-cell–mediated disorder.[4] Significantly decreasing the total mass of CD20$^+$ B cells by rituximab therapy in ITP may cause a collapse of autoreactive T-cell stimulation and normalization of the T-cell repertoire even as the B cells begin to return months after the therapy. Clearly, more research is warranted to further understand this most interesting phenomenon. This important work truly lends credence to the notion that attacking T cells in ITP, even if via the slaughter of B cells, is perhaps the real way to design successful therapies for this disorder.

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

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● ● ● HEMOSTASIS

Comment on Mateo et al, page 1895

Does angiotensin-II link arteries and monocytes?

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Mateo and colleagues demonstrate the ability of angiotensin-II to induce selective recruitment of mononuclear leukocytes to the arterial vasculature in both animals and simple but relevant human systems, a process that might underlie the earliest events of atherosclerosis.

Atherosclerosis is a disease of large arteries in which macrophage recruitment to the vascular wall plays an integral role. The prevailing view is that for these cells to be recruited to sites of lesion development, they must adhere to the endothelial lining of large arteries, an environment of high shear forces and low adhesion molecule expression. Mediators like TNF or Toll-like receptor (TLR) ligands, which induce neutrophil adhesion to venular endothelium, induce very subtle or no adhesion on the arterial side of the circulation.[1-3] This raises the question of whether alternative mediators exist with a unique ability to induce recruitment in arteries.

Important previous work from Alvarez et al has demonstrated that angiotensin-II (Ang-II), a molecule implicated in numerous cardiovascular diseases, induces significant leukocyte adhesion in both venules and arterioles.[4] However, the arterial response to Ang-II was strikingly different from the venous response, in that mononuclear cells adhered to arterial endothelium whereas neutrophils adhered to venular endothelium. Now, Mateo and colleagues provide further detail on the mechanism underlying the differential effect of Ang-II on arterial endothelial cells. Their data show that Ang-II–mediated mononuclear leukocyte recruitment in arterioles occurs in part via release of TNF, which drives CC chemokine expression, as well as via IL-4. In sharp contrast, neither of these cytokines contributes to Ang-II–induced neutrophil recruitment in adjacent postcapillary venules (summarized in the figure). These results are explained by observations that arterioles, but not venules, (1) constitutively express IL-4 and (2) express TNF in response to Ang-II, and that TNF in combination with IL-4 is necessary for the arterial response. The latter point is not trivial, as TNF or IL-4 alone are poor inducers of arterial adhesion.[1,5] Human aortic endothelial cells as well as monocytes are also shown to respond to Ang-II by expressing TNF mRNA and releasing chemokines such as MCP-1 in a TNF-dependent fashion.

Importantly, these findings demonstrate that Ang-II has the ability to induce unique responses from arterial endothelial cells, which favor selective recruitment of mononuclear leukocytes. These responses were observed in arterioles; however, there is potential for differential responses between macrovascular and microvascular endothelium, as well as between endothelium from different organs. Since atherosclerosis is a disease of large arteries, it begs the question of whether Ang-II can also mediate these effects on endothelial cells in large arteries. Studies using in vivo or ex vivo imaging of atheroma-affected arteries could be used to address this issue.[3,6] Often forgotten is that large arteries have their own microvasculature (the vasa vasorum), and it remains unclear how much of the mononuclear cell recruitment occurs via this microvasculature.[7] As a final note, infection and the related activation of TLRs have recently received attention as inciting agents in atherosclerosis.[8] It will be interesting to determine what relationship, if any, exists between Ang-II and TLRs.

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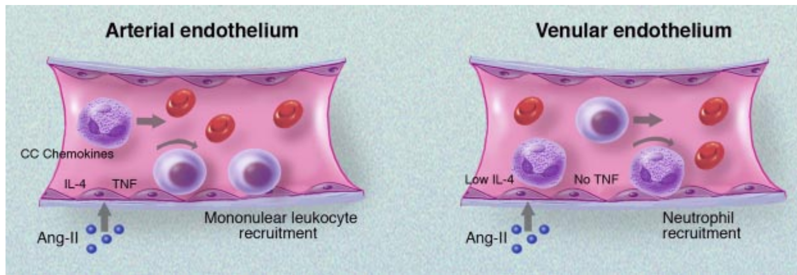

Comparison of angiotensin-II (Ang-II)–mediated leukocyte recruitment in arterioles and venules. In arterioles, high constitutive expression of IL-4 and Ang-II–induced TNF induces expression of CC chemokines such as MCP-1 and RANTES over a time course of several hours. These chemokines mediate selective arrest of mononuclear leukocytes in arterioles. In contrast, in postcapillary venules, Ang-II induces rolling and arrest within 60 minutes, predominantly recruiting neutrophils to the endothelial surface. This process does not require either IL-4 or TNF.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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● ● ● HEMOSTASIS

Comment on Shi et al, page 2899

Endostatin finds a new partner: nucleolin

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In this issue of *Blood*, Shi and colleagues examine the endostatin binding of nucleolin on angiogenic endothelial cells, as well as the transport of nucleolin to the nucleus, where it prevents proliferation—thus revealing a novel mechanism for endostatin's antiangiogenic activity.

During the past 3 years, 8 new drugs with antiangiogenic activity have received approval from the Food and Drug Administration of the United States for the treatment of cancer and age-related macular degeneration, and have also been approved in more than 30 other countries. These are mainly antibodies, aptamers, or synthetic molecules. They block at least 1 to 3 proangiogenic proteins or their receptors.[1] This new class of drugs has been prescribed for more than 1.2 million patients. More than 50 drugs with antiangiogenic activity are in phase 2 or 3 clinical trials.[2] Since 1980, 28 endogenous angiogenesis inhibitors, including platelet factor 4, angiostatin, endostatin, thrombospondin-1, tumstatin, and canstatin, have been discovered in blood or tissues.[3,4] At this writing, more than 1000 reports on endostatin have been published since its discovery in 1997, and reveal that endostatin has the broadest antitumor spectrum of the endogenous angiogenesis inhibitors. It is also the first endogenous inhibitor to receive approval for anticancer therapy, under the trade name "Endostar" in China.

The integrin $\alpha_5\beta_1$ has been proposed as a receptor for endostatin, and endostatin has been shown to regulate an entire program of antiangiogenic gene expression in human microvascular endothelial cells stimulated by VEGF or bFGF.[5] Nevertheless, certain antiangiogenic actions of endostatin have no molecular explanation and remain as open questions. In this issue of *Blood*, Shi and colleagues address 3 of these questions. Why does endostatin specifically target angiogenic blood vessels, but not quiescent blood vessels? Why does endostatin inhibit tumor angiogenesis with virtually no toxicity in animal studies and clinical trials? Why has the antiangiogenic activity of endostatin appeared to be heparin-dependent in previous studies? These questions are answered by a novel and important finding: that nucleolin is expressed on the surface of proliferating angiogenic human microvascular endothelial cells, but not on the surface of quiescent endothelium. In angiogenic endothelial cells, the cell-surface nucleolin binds endostatin and transports it to the nucleus, where endostatin inhibits phosphorylation of nucleolin. Phosphorylation of nucleolin induced by VEGF or bFGF has been reported to be essential for cell proliferation. Furthermore, endostatin does not inhibit proliferation of many types of tumor cells per se, possibly because while they express nucleolin on their surfaces, they do not internalize it in the presence of endostatin. The heparin binding sites on nucleolin were found to be critical for endostatin. Increasing concentrations of exogenous heparin dissociated the binding of endostatin to nucleolin.

The article by Shi and colleagues is also thought-provoking because the endostatin-nucleolin connection is now fertile soil for future studies. For example, it will be interesting to learn how specific the binding of endostatin is to nucleolin compared with other proteins that also bind to endostatin, as reported by the authors. Furthermore, it will be helpful if the relationship of nucleolin to other cell-surface endostatin-binding proteins can be uncovered, particularly $\alpha_5\beta_1$. Will this endostatin-nucleolin connection lead to the uncovering of a mechanism

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