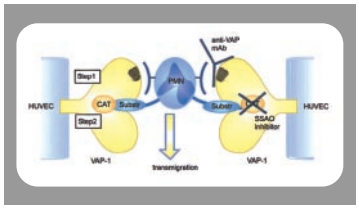
tissue inflammation is regulated by a complex network of signals involving adhesion molecules and chemotactic cytokines. The first stage of the extravasation cascade involves leukocyte tethering to the endothelial surface, followed by rolling, events largely mediated via selectins. If additional activation signals are received, the leukocytes proceed to the second stage and firmly adhere. This phase is mediated by a variety of cell adhesion molecules. Leukocytes then transmigrate through the endothelial layer, and it is this third stage of the extravasation cascade that is perhaps the least well understood. In this issue of *Blood*, Koskinen and colleagues (page 3388) provide new insight into how leukocyte transmigration may be regulated. Vascular adhesion protein-1 (VAP-1) is unique amongst endothelial cell–expressed adhesion molecules because it possesses semicarbazide-sensitive amine oxidase (SSAO) activity. Until now it has not been clear whether the enzymatic activity of VAP-1 contributes to its actions as an adhesion molecule. Koskinen and colleagues provide the first evidence that VAP-1 SSAO activity is required for leukocyte rolling and transmigration through the endothelium.

The plethora of signals regulating the extravasation cascade of leukocytes can complicate assessment of the contributions made by individual molecules. The studies of Koskinen et al were facilitated by the fact that human umbilical vein endothelial cells (HUVECs) are normally VAP-1–negative, enabling expression of wild-type VAP-1 or catalytically inactive (Y471F) VAP-1 without the complication of endogenous VAP-1. The effects of SSAO inhibitors and anti–VAP-1 antibodies on rolling, firm adhesion, and transmigration of PMNs on the transduced HUVECs were quantified using a laminar shear flow capillary model designed to recapitulate the conditions of shear stress in postcapillary venules. BTT-2027, a newly described SSAO inhibitor, reduced leukocyte rolling on HUVECs expressing wild-type VAP-1, as did the prototypical SSAO inhibitors semicarbazide and hydroxylamine. Although the features of BTT-2027 have yet to be fully reported, this is the first evidence linking the enzymatic activity of VAP-1 to a role in mediating PMN migration. More striking and perhaps




significant is the demonstration that VAP-1 SSAO activity is also required for leukocyte transmigration. Remarkably, BTT-2027 reduced transmigration through wild-type VAP-1–transduced HUVECs by almost 50%, quite surprising considering the wide range of cell adhesion molecule interactions occurring between PMNs and endothelial cells. The authors propose a sequential model for adhesion and SSAO activity in facilitating transmigration, but how the adhesive and enzymatic properties of VAP-1 collaborate to facilitate transmigration remains enigmatic. Clearly, further investigations will be required to determine whether one or more of the products of VAP-1 enzymatic activity are required to facilitate transmigration and, if so, their mechanism of action. Identification of molecules that interact with and activate VAP-1 are also important future goals. Critically for this study, preadministration of BTT-2027 reduced PMN extravasation in an in vivo model of acute inflammation. This "proof of principle" identifies VAP-1 SSAO activity as a potential new target for anti-inflammatory therapy, a particularly attractive prospect since VAP-1 is one of the few molecules currently known to possess SSAO activity in humans.

**—Melanie J. Welham**
University of Bath

HEMOSTASIS, THROMBOSIS, AND VASCULAR BIOLOGY

## Of men; why not mice?

Platelet glycoprotein (GP) VI and integrin $\alpha_2\beta_1$ cooperate to mediate effective adhesion of platelets to collagens. When platelets engage collagen, it is thought that GPVI predominantly mediates outside-in signaling leading to activation of $\alpha_2\beta_1$ that then mediates a more stable adhesion. While debate remains as to the relative contribution of each receptor, it is clear that in mice the genetic elimination of either receptor alone does not completely inhibit collagen-induced platelet responses.[1,2]

The independent function of $\alpha_2\beta_1$ and its potential role in collagen-induced signal transduction may be underestimated. Recently, Inoue et al[3] looked more carefully at the role of $\alpha_2\beta_1$ in adhesion and spreading of platelets on a collagen-coated surface. A key observation was that the intracellular signaling cascade used by $\alpha_2\beta_1$ shares many of the features of the GPVI signaling cascade, including participation of Src kinases, Syk, SLP-76, and PLCγ2. The only component that is apparently not involved is LAT. Moreover, engagement of $\alpha_2\beta_1$ initiates signals through an independent pathway involving FAK, PMCA, and $Ca^{2+}$ mobilization that is not detectable when collagen binds to platelets in suspension.

For several years, it has been known that platelet $\alpha_2\beta_1$ levels in humans can vary by roughly 4-fold due to the inheritance of 1 or more of 3 major human *ITGA2* haplotypes.[4] Haplotype 1 ($T_{807}$; $G_{1648}$) is associated with the highest density of $\alpha_2\beta_1$; haplotype 2 ($C_{807}$; $G_{1648}$) confers the lowest density; and the low-frequency haplotype 3 ($C_{807}$; $A_{1648}$) is associated with intermediate receptor density. The molecular basis for differences in expression associated with each of these haplotypes remains to be precisely determined. In addition, 2 single nucleotide polymorphisms (SNPs) within the promoter region of human *ITGA2* can decrease binding of the transcription factor Sp1, resulting in decreased transcription of *ITGA2* and further reductions in densities of platelet $\alpha_2\beta_1$.[5]

Until now, these genetic differences in $\alpha_2\beta_1$ expression had been thought to be unique to humans. However, Li and colleagues (page 3396) in this issue of *Blood* describe yet another experiment of nature

that adds a new perspective to the study of platelet integrin $\alpha_2\beta_1$ expression and its role in platelet function in vivo. They have made the very intriguing observation that genetic variation in $\alpha_2$ expression exists between mouse strains and accounts for differences in platelet responses to collagen. Compared with 4 other common strains, platelets from FVB/N mice show a 50% reduction in expression of $\alpha_2\beta_1$ but normal levels of other key receptors, including GPVI, GPIb, and the integrin $\alpha_{IIb}\beta_3$. Platelets from FVB/N mice exhibit reduced aggregation and a prolonged lag phase in their response to collagen. Linkage analysis demonstrates that the reduction in $\alpha_2\beta_1$ levels is linked to the D13mit260 marker that is upstream from the $\alpha_2$ gene. The discovery of differences in $\alpha_2\beta_1$ expression in mouse strains provides a new vehicle to investigate the molecular basis of this phenomenon and its importance to platelet function in vivo.

**—Thomas J. Kunicki**
The Scripps Research Institute

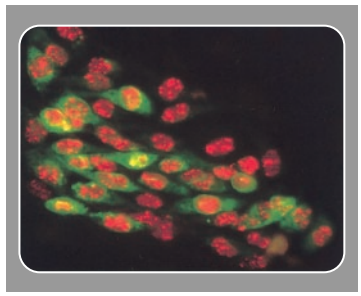
1. Kato K, Kanaji T, Russell S, et al. The contribution of glycoprotein VI to stable platelet adhesion and thrombus formation illustrated by targeted gene deletion. Blood. 2003;102:1701-1707.
2. Holtkotter O, Nieswandt B, Smyth N, et al. Integrin α2-deficient mice develop normally, are fertile, but display partially defective platelet interaction with collagen. J Biol Chem. 2002;277:10789-10794.
3. Inoue O, Suzuki-Inoue K, Dean WL, Frampton J, Watson SP. Integrin alpha2beta1 mediates outside-in regulation of platelet spreading on collagen through activation of Src kinases and PLCgamma2. J Cell Biol. 2003;160:769-780.
4. Kritzik M, Savage B, Nugent DJ, et al. Nucleotide polymorphisms in the alpha 2 gene define multiple alleles which are associated with differences in platelet alpha 2 beta 1. Blood. 1998;92:2382-2388.
5. Jacquelin B, Rozenshteyn D, Kanaji S, et al. Characterization of inherited differences in transcription of the human integrin alpha 2 gene. J Biol Chem. 2001;276:23518-23524.

NEOPLASIA

## KSHV induces heme oxygenase: another trick by a wily virus

Research on Kaposi sarcoma (KS) was given a shot in the arm with the discovery in 1994 of Kaposi sarcoma–associated herpesvirus (KSHV), also known as human herpesvirus-8 (HHV-8).[1] This virus is an etiological agent for KS and also for primary effusion lymphoma (PEL), multicentric Castleman disease (MCD), and possibly pulmonary hypertension. Like other herpesviruses, KSHV encodes for several unique genes that have their origin in the host genome. Some, including viral interleukin 6 (vIL-6), viral macrophage inflammatory protein-1 (vMIP-1), vMIP-2, and vMIP-3, have direct proangiogenic activity and contribute to KS pathogenesis.[2] vIL-6 is also responsible for much of the symptomatology of MCD and acts as an autocrine growth factor for PEL cells. Other KSHV genes act indirectly by influencing host genes or pathways. In particular, a constitutively active viral G protein–coupled receptor (ORF74) can act indirectly to promote angiogenesis by inducing vascular endothelial growth factor (VEGF) production. This occurs at least in part through enhancement of the activity of hypoxia inducible factor (HIF).[3]

In this issue, McAllister and colleagues (page 3465) define a novel mechanism by which KSHV can induce growth of endothelial cells: by increasing the activity of heme oxygenase-1 (HO-1). The authors found the enhanced expression of this protein by comparing the protein expression profiles of KSHV-infected and uninfected endothelial cells. HO-1 is responsible for the physiologic breakdown of heme into carbon monoxide, iron, and biliverdin (which is then converted to bilirubin). In normal cells, HO-1 is induced by heme. It can also be up-regulated by hypoxia and HIF. In tissues affected by injury and bleeding, HO-1 helps improve vascular flow through the production of carbon monoxide; provides protection against free heme, a prooxidant, by producing biliverdin, an antioxidant; mediates antiapoptotic effects; and has anti-inflammatory activities. HO-1 also up-regulates a number of angiogenic growth factors including VEGF, [4] and, as shown in this paper, can enhance the proliferation of KSHV-infected dermal microvascular endothelial cells (DMVECs). Its induction by KSHV thus provides a new mechanism by which the virus can promote angiogenesis and the growth of infected endothelial cells. There is substantial blood extravasation in KS tissues (blood gives KS its characteristic color), and HO-1 induction also protects KSHV-infected cells against heme toxicity. Its importance in KS pathogenesis was underscored by the finding in the current paper



that HO-1 mRNA and protein are highly expressed in KS lesions. These findings might, with further study, lead to new therapeutic avenues for treating KS with specific HO inhibitors that are already being used experimentally for the treatment of hyperbilirubinia.

The ability to promote angiogenesis through multiple pathways is one of the most distinctive features of KSHV. Very few KSHV-infected patients develop KS (outside a setting of HIV coinfection or other immunosuppression), and KS can probably best be viewed as a biologic accident resulting in part from the production of proangiogenic factors by KSHV-infected cells. But what forces have led KSHV to adopt the strategy of inducing angiogenesis? Endothelial cells are important targets for KSHV infection, and production of angiogenic factors may promote KSHV infection of and replication in these cells. The recent finding that KSHV lytic replication is up-regulated by hypoxia and HIF[5] suggests that the virus may be particularly well poised to replicate in wounds (where it may find a portal of entry). The vascular compromise, heme deposition, and hypoxia at the edge of wounds lead to HO-1 expression, HIF activation, and angiogenesis with endothelial cell growth. This would appear to provide a