Journal of Thrombosis and Haemostasis, **5**: 599–603

ORIGINAL ARTICLE

Generation of soluble P- and E-selectins *in vivo* is dependent on expression of P-selectin glycoprotein ligand-1

P. F. BODARY,*[1] J. W. HOMEISTER,†[1] F. B. VARGAS,* K. J. WICKENHEISER,* S. S. CUDNEY,* K. L. BAHROU,* M. ÖHMAN,* A. B. RABBANI* and D. T. EITZMAN*

*Division of Cardiovascular Medicine, Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, MI; and †Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC, USA

To cite this article: Bodary PF, Homeister JW, Vargas FB, Wickenheiser KJ, Cudney SS, Bahrou KL, Öhman M, Rabbani AB, Eitzman DT. Generation of soluble P- and E-selectins *in vivo* is dependent on expression of P-selectin glycoprotein ligand-1. *J Thromb Haemost* 2007; **5**: 599–603.

Summary. *Background:* Factors contributing to the generation of soluble P- and E-selectins remain unclear. *Results:* This work demonstrates that mice lacking P-selectin glycoprotein ligand-1 (*Psgl-1*$^{-/-}$) are deficient in soluble P-selectin (sP-sel), which is due to a defective binding interaction between PSGL-1 and P-sel, because mice lacking α(1,3)-fucosyltransferase-VII are also deficient in sP-sel. *Psgl-1*$^{-/-}$ mice are also deficient in soluble E-selectin (sE-sel) indicating that leukocyte interactions with endothelial cells lead to the generation of sE-sel. The generation of sE-sel requires an interaction between PSGL-1 and P-sel, as deficiency of sE-sel is observed in both *Psgl-1*$^{-/-}$ and *P-sel*$^{-/-}$ mice. Bone marrow transplantation from *Psgl-1*$^{-/-}$ to *Psgl-1*$^{+/+}$ mice leads to deficiency of sP-sel and sE-sel in recipient mice, establishing the importance of bone marrow-derived PSGL-1 toward the generation of sP-sel and sE-sel. Bone marrow transplantation from *P-sel*$^{-/-}$ to *P-sel1*$^{+/+}$ mice does not lead to a significant reduction in sP-sel, confirming the importance of the endothelium toward the liberation of sP-sel. *Conclusion:* sP-sel and sE-sel reflect an interaction between leukocyte PSGL-1 and endothelial P-sel.

Keywords: adhesion, endothelium, leukocyte, platelets, thrombosis.

Introduction

The selectins (P, E, and L) are a class of adhesion molecules that play important roles in many physiologic processes, including leukocyte rolling and adhesion on endothelial cells [1]. P-selectin (P-sel) is present in platelet α-granules and endothelial cell Weibel–Palade bodies and is rapidly expressed on the cell surface following stimulation [2,3]. E-selectin (E-sel) is expressed on endothelial cells following cytokine stimulation [4] and L-selectin (L-sel) is expressed on leukocytes [5]. Deficiency of one or more of these selectins has been shown to alter vascular disease processes in several preclinical models [6]. Although the membrane-bound selectins mediate cell–cell interactions in the vasculature, each selectin also has a soluble form (sE-sel, sP-sel, and sL-sel) that can be measured in the plasma [5,7,8]. The soluble selectins have been used as markers of vascular disease processes [9] and may play direct roles in inflammatory disease processes [5,7,8].

A physiologically important endogenous ligand for the selectins is P-sel glycoprotein ligand-1 (PSGL-1), which is expressed primarily on leukocytes [10] and requires α(1,3)-fucosylation for binding activity [11,12]. Several studies have demonstrated that adhesive interactions between selectins and PSGL-1 facilitate leukocyte rolling on endothelial cells [13,14] and mediate the formation of platelet–leukocyte aggregates [15]. Deficiency of PSGL-1 in mice is associated with impaired leukocyte rolling and reduced generation of procoagulant microparticles [16,17]. Because elevated levels of soluble selectins are associated with disease processes that require selectin/selectin-ligand interactions, we tested the hypothesis that generation of soluble selectins is regulated by interaction with a physiologic ligand.

Methods

Mice

Psgl-1$^{-/-}$ and *P-sel*$^{-/-}$ mice were purchased from Jackson Laboratory, Bar Harbor, ME, USA. α(1,3)-Fucosyltransferase-VII-deficient mice (*FucT-VII*$^{-/-}$) were previously generated [11] and compared with wild-type mice from the same colony. All mice had previously been backcrossed several generations to the C57BL6/J background strain (*P-sel*$^{-/-}$ mice >10 generations; *Psgl-1*$^{-/-}$ mice $= 6$ generations; *FucT-VII*$^{-/-}$ mice $= 10$ generations).

Correspondence: Daniel T. Eitzman, 1150 W. Medical Center Dr., 7301 MSRB III, Ann Arbor, MI 48109-0644, USA.
Tel.: +1 734 763 7838; fax: +1 734 936 2641; e-mail: deitzman@umich.edu

[1]Contributed equally to this work.

Received 7 November 2006, accepted 30 December 2006

Measurement of circulating selectins (P, E, and L) and vascular cell adhesion molecule-1

Enzyme-linked immunosorbent assays (R&D Systems, Inc., Minneapolis, MN, USA) were used to determine the concentrations of soluble murine P-, E-, and L-selectin, and vascular cell adhesion molecule-1 (VCAM-1) in mouse serum. Measurement of platelet P-sel was performed as previously described [18] using Triton X-100 on washed platelets followed by measurement of P-sel in the platelet supernatant.

Bone marrow transplantation

Bone marrow transplantation experiments from *P-sel*$^{-/-}$, *Psgl-1*$^{-/-}$ and wild-type donors to irradiated wild-type recipients were performed as previously described [19].

Results

Effect of PSGL-1 expression and binding capacity on generation of soluble selectins

To determine if selectin interactions with their ligands affects the generation of soluble selectins, sP-sel from mice deficient in the corresponding ligand, PSGL-1, was measured. Serum samples from *Psgl-1*$^{+/+}$ mice contained 4.6-fold greater sP-sel than serum from *Psgl-1*$^{-/-}$ mice (Fig. 1A). In contrast, total platelet P-sel levels were not reduced in *Psgl-1*$^{-/-}$ mice compared with wild-type mice (1.97 ± 0.06 vs. 1.56 ± 0.09 ng mL^{-1}). Concentrations of sP-sel were not different between PSGL-1 heterozygotes (*Psgl-1*$^{+/-}$) and *Psgl-1*$^{+/+}$ mice (123 ± 3.3 vs. 116 ± 2.3 ng mL^{-1}; $n = 6$ per genotype; $P =$ ns).

Because α(1,3)-fucosylation of PSGL-1 by the myeloid α(1,3)-fucosyltransferase-VII directs expression of the PSGL-1 binding glycoform toward P-sel [11,20], sP-sel was also measured in *FucT-VII*$^{-/-}$ mice. Consistent with a role for ligand binding to P-sel in the generation of sP-sel, *FucT-VII*$^{-/-}$ mice were also deficient in sP-sel (Fig. 1B).

Concentrations of the circulating endothelial-specific selectin, sE-sel, were also measured in *Psgl-1*$^{+/+}$ and *Psgl-1*$^{-/-}$ mice. Serum from *Psgl-1*$^{+/+}$ mice contained 3.2-fold greater sE-sel than serum from *Psgl-1*$^{-/-}$ mice (Fig. 2A). Similarly, *FucT-VII*$^{-/-}$ mice showed reduced sE-sel (Fig. 2B). Circulating levels of VCAM-1, an endothelial adhesion molecule with expression that is under regulatory influences similar to those of E-sel [21], were not different between *Psgl-1*$^{-/-}$ and *Psgl-1*$^{+/+}$ mice (947 ± 49 vs. 913 ± 49 ng mL^{-1}; $n = 5$ per group; $P =$ ns). sE-sel levels in *P-sel* $^{-/-}$ mice were reduced to levels similar to those observed in *Psgl-1*$^{-/-}$ mice (Fig. 2C).

To determine if PSGL-1 affected generation of sL-sel, we measured sL-sel in *Psgl-1*$^{+/+}$ and *Psgl-1*$^{-/-}$ mice. sL-sel was actually increased in *Psgl-1*$^{-/-}$ mice compared with *Psgl-1*$^{+/+}$ mice (1.85 ± 0.068 vs. 1.63 ± 0.030 μg mL^{-1}; $n = 6$ per group; $P = 0.01$).

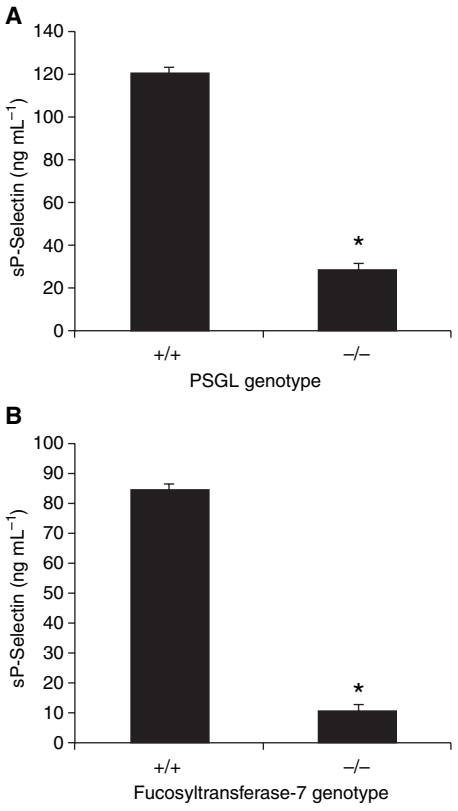

Fig. 1. Effect of P-selectin glycoprotein ligand-1 (PSGL-1) or α(1,3)-fucosyltransferase-VII (FucT-VII) deficiency on soluble P-selectin (sP-sel) levels. (A) sP-sel levels in *Psgl-1*$^{+/+}$ mice ($n = 6$) compared with *Psgl-1*$^{-/-}$ mice ($n = 6$); *$P < 0.00001$. (B) sP-sel levels in *FucT-VII*$^{+/+}$ mice ($n = 6$) compared with *FucT-VII*$^{-/-}$ mice ($n = 6$); *$P < 0.00001$.

Effect of PSGL-1- and P-sel-deficient bone marrow transplantation on levels of soluble selectins

To determine the relevant PSGL-1 tissue compartment for generation of sP-sel, we performed bone marrow transplantation from *Psgl-1*$^{-/-}$ into *Psgl-1*$^{+/+}$ mice. sP-sel and sE-sel were markedly reduced compared with transplanted wild-type controls (Fig. 3A,B). In contrast, *P-sel*$^{-/-}$ bone marrow did not significantly affect sP-sel levels in *P-sel*$^{+/+}$ recipients ($n =$ 4; sP-sel $= 157 \pm 15$ ng mL^{-1}).

Discussion

Selectins have previously been shown to play important roles in the development of atherosclerosis and thrombosis [22–24]. Circulating soluble forms of selectins have direct effects on vascular events such as thrombosis [7] and have been used as markers of disease processes [9], although the factors involved in the generation of sP-sel and sE-sel are not well-characterized.

The primary ligand for P-sel, PSGL-1, is expressed predominantly on leukocytes. To test the physiologic role of PSGL-1 in regulating sP-sel concentrations *in vivo*, we measured sP-sel in *Psgl-1*$^{-/-}$ mice. Mice deficient in PSGL-1 have markedly

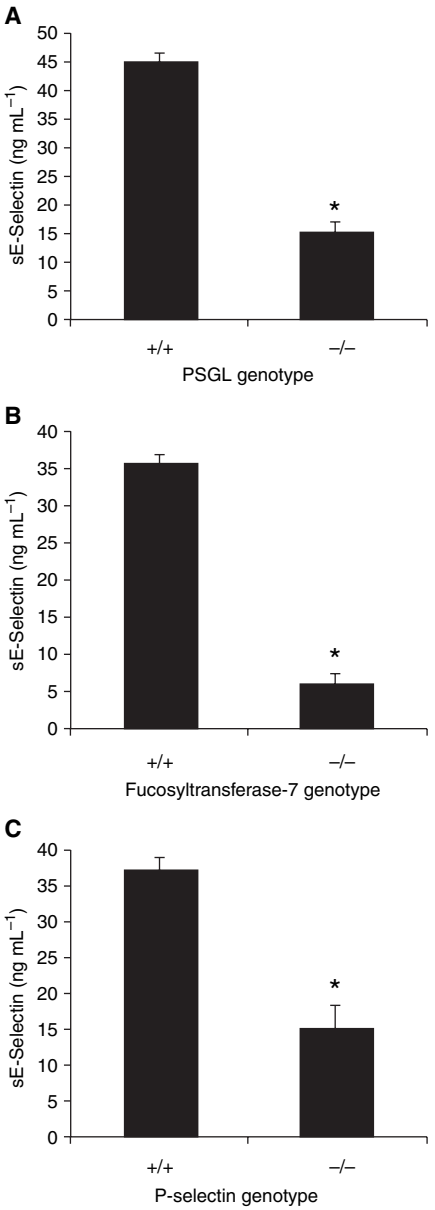

Fig. 2. Effect of P-selectin glycoprotein ligand-1 (PSGL-1) or α(1,3)-fucosyltransferase-VII (FucT-VII) deficiency on soluble E-selectin (sE-sel) levels. (A) sE-sel levels in *Psgl-1*$^{+/+}$ mice ($n = 4$) compared with *Psgl-1*$^{-/-}$ mice ($n = 4$); *$P < 0.00001$. (B) sE-sel levels in *FucT-VII*$^{+/+}$ mice ($n = 4$) compared with *FucT-VII*$^{-/-}$ mice ($n = 4$); $P < 0.00001$. (C) sE-sel levels in *P-sel*$^{+/+}$ ($n = 5$) compared with *P-sel*$^{-/-}$ ($n = 4$); *$P < 0.001$.

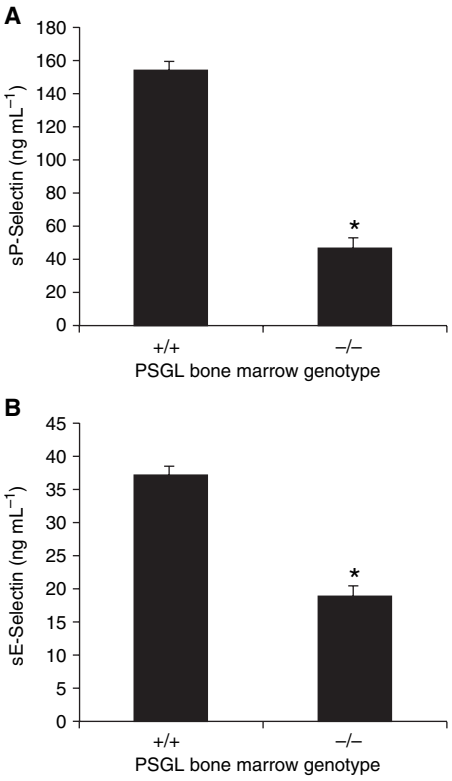

Fig. 3. Effect of bone marrow transplantation on soluble P-selectin (sP-sel) and soluble E-selectin (sE-sel) levels. (A) sP-sel levels in P-selectin glycoprotein ligand-1 (*Psgl-1*$^{+/+}$) mice transplanted with *Psgl-1*$^{+/+}$ or *Psgl-1*$^{-/-}$ bone marrow ($n = 5$ per genotype); *$P < 0.00001$. (B) sE-sel levels in *Psgl-1*$^{+/+}$ mice transplanted with *Psgl-1*$^{+/+}$ or *Psgl-1*$^{-/-}$ bone marrow ($n = 10$); *$P < 0.001$.

reduced levels of sP-sel, but slightly increased total platelet P-sel levels, indicating that the ligand is playing a role in the generation of sP-sel. The selectin-binding glycoform of PSGL-1 is regulated by α(1,3)-fucosylation, and leukocytes from mice deficient in FucT-VII have been shown to lack the capacity to bind P-sel [11]. Our finding that *FucT-VII*$^{-/-}$ mice are deficient in sP-sel demonstrates that a functional binding interaction between PSGL-1 and P-sel is required for the generation of sP-sel *in vivo*. The modest differences between baseline levels of sP-sel in the wild-type controls of the *Psgl-1*$^{-/-}$ and *FucT-VII*$^{-/-}$ mice may reflect minor differences in serum collection techniques.

It has been previously demonstrated, using bone marrow transplantation techniques, that the predominant source of sP-sel in atherosclerotic-prone mice is the endothelium, with only a minor contribution from platelets [25]. We have now confirmed in non-atherosclerotic mice, using bone marrow transplantation, that the vast majority of sP-sel in mice is derived from a non-bone marrow source (i.e. endothelium) as no significant effect was observed when *P-sel*$^{-/-}$ bone marrow was transplanted into *P-sel*$^{+/+}$ mice.

To further explore potentially relevant tissue sources of circulating selectins specifically regulated by PSGL-1, concentrations of the circulating endothelial-specific selectin, sE-sel, were also measured in *Psgl-1*$^{+/+}$ and *Psgl-1*$^{-/-}$ mice. Although PSGL-1 may be a relatively minor E-sel ligand [26], serum from *Psgl-1*$^{-/-}$ mice was deficient in sE-sel. Similarly, serum from *FucT-VII*$^{-/-}$ mice was also deficient in sE-sel compared with *FucT-VII*$^{+/+}$ mice. Thus, sE-sel levels are dependent on PSGL-1 expression and α(1,3)-fucosylation, again indicating an important role for binding interactions between PSGL-1 and an endothelial selectin. In contrast, circulating levels of VCAM-1 were not different between *Psgl-1*$^{-/-}$ and *Psgl-1*$^{+/+}$ mice, demonstrating specificity for the selectin–ligand interaction.

The endothelial-specific expression of E-sel indicates that the endothelial cell is the source of PSGL-1-dependent sE-sel generation. To determine whether generation of sE-sel is due to a direct interaction of PSGL-1 with E-sel or secondary to a facilitative role of PSGL-1 with P-sel, sE-sel levels were measured in *P-sel*$^{-/-}$ mice. sE-sel levels in *P-sel*$^{-/-}$ mice were reduced to levels similar to those observed in *Psgl-1*$^{-/-}$ mice, indicating that generation of sE-sel is due to ligands other than PSGL-1 and that interaction of these other ligands with E-sel may require initial interactions between PSGL-1 and P-sel. For example, a previous study demonstrated reduced leukocyte rolling on E-sel following treatment with an antibody to block P-sel [27]. Alternatively, based on the present work, we cannot rule out that an interaction between PSGL-1 and P-sel could lead to endothelial secretion of sE-sel. sP-sel and sE-sel may therefore be generated during leukocyte interactions, such as rolling, with the endothelium, and selectin shedding may actually be required for efficient rolling. L-sel shedding has been previously shown to occur rapidly during the process of leukocyte rolling in an *in vitro* hydrodynamic flow model, and inhibition of the shedding process with a metalloprotease inhibitor reduced neutrophil rolling, leading to increased neutrophil accumulation [28]. However, it is not clear from our data at which stage of leukocyte interaction with endothelial cells soluble selectin shedding occurs. For example, P-sel may be cleaved after leukocyte arrest or during transmigration.

In addition to leukocytes, PSGL-1 has also been shown to be present on endothelial cells and to bind P-sel [29]. To test the role of bone marrow-derived PSGL-1 on the generation of sP-sel and sE-sel, we performed bone marrow transplantation from *Psgl-1*$^{-/-}$ to *Psgl-1*$^{+/+}$ mice. This transplant produced the deficiency state of sP-sel and sE-sel, supporting the role of leukocyte PSGL-1 in the generation of soluble selectins.

Although we have shown the importance of α(1,3)-fucosylated PSGL-1 in the generation of sP-sel and sE-sel *in vivo*, *Psgl1*$^{-/-}$ mice are not completely deficient in these circulating selectins. This suggests either that there is ligand-independent shedding or that ligands other than PSGL-1 also contribute to sP-sel and sE-sel generation. It is also possible that during inflammatory states in which soluble selectins are elevated, the mechanisms responsible for their generation are different from the mechanisms responsible for the generation of basal levels of circulating selectins. These important issues will need to be addressed in various models of inflammatory diseases.

In conclusion, this study demonstrates a requirement for bone marrow-derived PSGL-1 and α(1,3)-fucosyltransferase activity in the generation of sP-sel and sE-sel levels *in vivo*. These findings indicate that circulating selectins are specific markers of leukocyte interactions with endothelial cells, and will serve as an extremely valuable tool in tracking ligand–selectin interactions *in vivo*. The presence of persistent circulating adhesion molecules in the circulation indicates that generation of circulating selectins is part of an ongoing physiologic process reflecting cell–cell interactions, and that selectin shedding may play an important regulatory role in leukocyte adhesive interactions with endothelial cells.

Author contributions

P. F. Bodary: design and performance of experiments, manuscript preparation; J. W. Homeister: design and performance of experiments, manuscript preparation; F. B. Vargas: performance of experiments; K. J. Wickenheiser: design and performance of experiments; S. S. Cudney: performance of experiments; K. L. Bahrou: performance of experiments; M. Öhman: performance of experiments; A. B. Rabbani: performance of experiments; D. T. Eitzman: design of experiments, manuscript preparation.

Acknowledgements

The authors thank D. Ginsburg for thoughtful discussion regarding this manuscript.

Disclosure of Conflict of Interests

This work was supported by funds from the National Heart, Lung and Blood Institute (HL057346), American Diabetes Association (7-05-JF-04), and American Heart Association (0275023N).

References

1 Bevilacqua MP, Nelson RM. Selectins. *J Clin Invest* 1993; **91**: 379–87.
2 Stenberg PE, McEver RP, Shuman MA, Jacques YV, Bainton DF. A platelet alpha-granule membrane protein (GMP-140) is expressed on the plasma membrane after activation. *J Cell Biol* 1985; **101**: 880–6.
3 McEver RP, Beckstead JH, Moore KL, Marshall-Carlson L, Bainton DF. GMP-140, a platelet alpha-granule membrane protein, is also synthesized by vascular endothelial cells and is localized in Weibel-Palade bodies. *J Clin Invest* 1989; **84**: 92–9.
4 Bevilacqua MP, Stengelin S, Gimbrone Jr MA, Seed B. Endothelial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. *Science* 1989; **243**: 1160–5.
5 Schleiffenbaum B, Spertini O, Tedder TF. Soluble L-selectin is present in human plasma at high levels and retains functional activity. *J Cell Biol* 1992; **119**: 229–38.
6 Etzioni A, Doerschuk CM, Harlan JM. Of man and mouse: leukocyte and endothelial adhesion molecule deficiencies. *Blood* 1999; **94**: 3281–8.
7 Andre P, Hartwell D, Hrachovinova I, Saffaripour S, Wagner DD. Pro-coagulant state resulting from high levels of soluble P-selectin in blood. *Proc Natl Acad Sci U S A* 2000; **97**: 13835–40.
8 Ruchaud-Sparagano MH, Walker TR, Rossi AG, Haslett C, Dransfield I. Soluble E-selectin acts in synergy with platelet-activating factor to activate neutrophil beta 2-integrins. Role of tyrosine kinases and Ca2+ mobilization. *J Biol Chem* 2000; **275**: 15758–64.
9 Atalar E, Aytemir K, Haznedaroglu I, Ozer N, Ovunc K, Aksoyek S, Kes S, Kirazli S, Ozmen F. Increased plasma levels of soluble selectins in patients with unstable angina. *Int J Cardiol* 2001; **78**: 69–73.
10 McEver RP, Cummings RD. Perspectives series: cell adhesion in vascular biology. Role of PSGL-1 binding to selectins in leukocyte recruitment. *J Clin Invest* 1997; **100**: 485–91.
11 Homeister JW, Thall AD, Petryniak B, Maly P, Rogers CE, Smith PL, Kelly RJ, Gersten KM, Askari SW, Cheng G, Smithson G, Marks RM, Misra AK, Hindsgaul O, von Andrian UH, Lowe JB. The alpha(1,3)fucosyltransferases FucT-IV and FucT-VII exert collaborative control over selectin-dependent leukocyte recruitment and lymphocyte homing. *Immunity* 2001; **15**: 115–26.

12 Huang MC, Zollner O, Moll T, Maly P, Thall AD, Lowe JB, Vestweber D. P-selectin glycoprotein ligand-1 and E-selectin ligand-1 are differentially modified by fucosyltransferases Fuc-TIV and Fuc-TVII in mouse neutrophils. *J Biol Chem* 2000; **275**: 31353–60.
13 Norman KE, Moore KL, McEver RP, Ley K. Leukocyte rolling in vivo is mediated by P-selectin glycoprotein ligand-1. *Blood* 1995; **86**: 4417–21.
14 Moore KL, Patel KD, Bruehl RE, Li F, Johnson DA, Lichenstein HS, Cummings RD, Bainton DF, McEver RP. P-selectin glycoprotein ligand-1 mediates rolling of human neutrophils on P-selectin. *J Cell Biol* 1995; **128**: 661–71.
15 Huo Y, Schober A, Forlow SB, Smith DF, Hyman MC, Jung S, Littman DR, Weber C, Ley K. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. *Nat Med* 2003; **9**: 61–7.
16 Yang J, Hirata T, Croce K, Merrill-Skoloff G, Tchernychev B, Williams E, Flaumenhaft R, Furie BC, Furie B. Targeted gene disruption demonstrates that P-selectin glycoprotein ligand 1 (PSGL-1) is required for P-selectin-mediated but not E-selectin-mediated neutrophil rolling and migration. *J Exp Med* 1999; **190**: 1769–82.
17 Hrachovinova I, Cambien B, Hafezi-Moghadam A, Kappelmayer J, Camphausen RT, Widom A, Xia L, Kazazian Jr HH, Schaub RG, McEver RP, Wagner DD. Interaction of P-selectin and PSGL-1 generates microparticles that correct hemostasis in a mouse model of hemophilia A. *Nat Med* 2003; **9**: 1020–5.
18 Kamath S, Blann AD, Caine GJ, Gurney D, Chin BS, Lip GY. Platelet P-selectin levels in relation to plasma soluble P-selectin and beta-thromboglobulin levels in atrial fibrillation. *Stroke* 2002; **33**: 1237–42.
19 Eitzman DT, Bodary PF, Shen Y, Khairallah CG, Wild SR, Abe A, Shaffer-Hartman J, Shayman JA. Fabry disease in mice is associated with age-dependent susceptibility to vascular thrombosis. *J Am Soc Nephrol* 2003; **14**: 298–302.
20 Maly P, Thall A, Petryniak B, Rogers CE, Smith PL, Marks RM, Kelly RJ, Gersten KM, Cheng G, Saunders TL, Camper SA, Camphausen RT, Sullivan FX, Isogai Y, Hindsgaul O, von Andrian UH, Lowe JB. The alpha(1,3)fucosyltransferase Fuc-TVII controls leukocyte trafficking through an essential role in L-, E-, and P-selectin ligand biosynthesis. *Cell* 1996; **86**: 643–53.
21 Cernuda-Morollon E, Ridley AJ. Rho GTPases and leukocyte adhesion receptor expression and function in endothelial cells. *Circ Res* 2006; **98**: 757–67.
22 Dong ZM, Brown AA, Wagner DD. Prominent role of P-selectin in the development of advanced atherosclerosis in ApoE-deficient mice. *Circulation* 2000; **101**: 2290–5.
23 Dong ZM, Chapman SM, Brown AA, Frenette PS, Hynes RO, Wagner DD. The combined role of P- and E-selectins in atherosclerosis. *J Clin Invest* 1998; **102**: 145–52.
24 Yokoyama S, Ikeda H, Haramaki N, Yasukawa H, Murohara T, Imaizumi T. Platelet P-selectin plays an important role in arterial thrombogenesis by forming large stable platelet-leukocyte aggregates. *J Am Coll Cardiol* 2005; **45**: 1280–6.
25 Burger PC, Wagner DD. Platelet P-selectin facilitates atherosclerotic lesion development. *Blood* 2003; **101**: 2661–6.
26 Zanardo RC, Bonder CS, Hwang JM, Andonegui G, Liu L, Vestweber D, Zbytnuik L, Kubes P. A down-regulatable E-selectin ligand is functionally important for PSGL-1-independent leukocyte-endothelial cell interactions. *Blood* 2004; **104**: 3766–73.
27 Xia L, Sperandio M, Yago T, McDaniel JM, Cummings RD, Pearson-White S, Ley K, McEver RP. P-selectin glycoprotein ligand-1-deficient mice have impaired leukocyte tethering to E-selectin under flow. *J Clin Invest* 2002; **109**: 939–50.
28 Walcheck B, Kahn J, Fisher JM, Wang BB, Fisk RS, Payan DG, Feehan C, Betageri R, Darlak K, Spatola AF, Kishimoto TK. Neutrophil rolling altered by inhibition of L-selectin shedding in vitro. *Nature* 1996; **380**: 720–3.
29 Rivera-Nieves J, Burcin TL, Olson TS, Morris MA, McDuffie M, Cominelli F, Ley K. Critical role of endothelial P-selectin glycoprotein ligand 1 in chronic murine ileitis. *J Exp Med* 2006; **203**: 907–17.