Integrin α2-Deficient Mice Develop Normally, Are Fertile, but Display Partially Defective Platelet Interaction with Collagen

The integrin α2-subunit was ablated in mice by targeted deletion of the ITGA2 gene. α2-Deficient animals develop normally, are fertile, and reproduce. Surprisingly, no obvious anatomical or histological differences were observed in mutant mice. Besides its significance in tissue morphogenesis, integrin α2β1 has been reported to play a major role in hemostasis by mediating platelet adhesion and activation on subendothelial collagen. To define its role in hemostasis, α2-deficient platelets were analyzed for their capacity to adhere to and aggregate in response to fibrillar or soluble collagen type I. We show that aggregation of α2-deficient platelets to fibrillar collagen is delayed but not reduced, whereas aggregation to enzymatically digested soluble collagen is abolished. Furthermore, α2-deficient platelets normally adhere to fibrillar collagen. However, in the presence of an antibody against GPVI (activating platelet collagen receptor), adhesion of α2-deficient but not wild type platelets is abrogated. These results demonstrate that integrin α2β1 significantly contributes to platelet adhesion to (fibrillar) collagen, which is further confirmed by the abolished adhesion of α2-deficient platelets to soluble collagen. Thus, α2β1 plays a supportive rather than an essential role in platelet-collagen interactions. These results are in agreement with the observation that α2β1-deficient animals suffer no bleeding anomalies.

Integrins are a large family of heterodimeric transmembrane receptors composed of noncovalently associated α- and β-subunits that function as receptors for extracellular matrix components and also bind to counter receptors on other cells (1–3). Integrin receptors modulate critical cellular processes, including adhesion and spreading, migration, survival, gene expression, and differentiation. These processes are physiologically relevant to growth and development, angiogenesis, and hemostasis but may also be significant in pathological conditions such as tumor metastasis and thrombosis (4–6).

The essential role of β1 integrins for development and differentiation was clearly demonstrated by the peri-implantation lethality of mouse embryos lacking β1 integrin (7). Four collagen-binding β1 integrin receptors have been identified, α1β1, α2β1, α10β1, and α11β1 (8), which interact with collagens via their individual I domains (9–12). α2β1 integrin (VLA-2, platelet GPIIbIIa) was thought to play a pivotal role in development, differentiation, and tissue morphogenesis. It is widely expressed, especially on cell types entering the final stages of differentiation (13, 14). α2β1 receptors bind with high affinity to collagen I (15) and also to collagens II–V (16, 17), and they mediate adhesion to laminins-1 and -5 (18, 19). Contact with collagen of α2β1 on fibroblasts and epithelial cells induces synthesis and activation of several matrix metalloproteinases (20–22) and is therefore thought to play an essential role in connective tissue remodeling and resurfacing of wounds.

The α2 protein was initially isolated from platelets, where it is involved in the adhesion to subendothelial collagen at sites of vascular injury and thereby contributes to the formation of a hemostatic plug (23). However, the interaction between platelets and collagen is complex and can either occur indirectly via immobilized von Willebrand factor binding to platelet receptors glycoprotein (GP)1 Ib-V-IX and/or activated αIIbβ3 integrin (24) or by direct interaction of collagen with specific receptors, including the Ig-like receptor GPVI (25, 26) and α2β1 integrin. GPVI is essential for this process as it mediates the activation of β1 and β3 integrins, which is a prerequisite for firm adhesion and thrombus growth (27). In contrast, the role of α2β1 in both platelet adhesion and activation on collagen has been controversially debated. Although previous studies (28, 29) emphasized an essential role of this integrin in platelet-collagen interactions and hemostasis, we have recently shown that mice lacking β1 integrins on their platelets display no major hemostatic defect. In vitro, however, β1-deficient platelets failed to interact with enzymatically digested collagen and displayed partial defects in their activation by and adhesion to native fibrillar collagen (27). In these studies, however, it could not be clarified definitively whether these defects were based on the absence of α2β1 alone, because β1-deficient platelets also lack α2β1 and α2β3.

To assess the function of the α2β1 receptor in vivo, particularly in hemostasis, we generated α2 integrin-deficient mice. In contrast to previous reports that suggested that homozygous deletion of the ITGA2 gene results in embryonic lethality (5, 30, 31), these mice develop normally and reproduce. Strikingly,
platelet counts and bleeding times are normal in α2 integrin-deficient mice. Although the interaction of α5β1-deficient platelets with soluble collagen is abrogated, they display only subtle defects in response to native fibrillar collagen.

**EXPERIMENTAL PROCEDURES**

**Chemicals and Antibodies—**Fibrillar type I collagen from equine tendon (Horn, Nycomed, Munich, Germany), high molecular weight heparin, and soluble, non-fibrillar type I collagen from rat tail were from Sigma.

The following antibodies were used: FITC anti-β3 integrin (H931/8), FITC anti-α2 integrin (Hα12/9), rat anti-α2 integrin (5H11-27), FITC anti-α3 integrin (GoH3) (all from BD PharMingen). Polyclonal rabbit anti-α5 integrin antibodies were kindly provided by U. Mayer (Manchester, UK). Rat anti-mouse β3 integrin (EDL4), GpIb-IX (pop1), GPV (DOM1), CD9 (ULF1), and GPVI (J4A1) have been described (32–34). Fab fragments of JAQ1 were prepared as described (35). FITC-conjugated rabbit anti-rat IgG (Dako) and horseradish peroxidase-conjugated swine anti-rabbit IgG were used as secondary reagents.

**Production of ITGA2 (−/−) Mice—**A 439-bp cDNA fragment of mouse α2 integrin corresponding to exons 1–3 was used to screen a λ FIX II genomic library (Stratagene) of the 129SVJ mouse strain. A targeting vector was generated in pBluescript KS II (Stratagene) containing 3 kb of the promoter region, the first exon including the translation start, and 4 kb of the first intron. A HindIII and loxP site was placed 1 kb upstream of the first exon in a single StuI site, and a phosphoglycerate kinase-driven neomycin resistance (neo+) cassette, flanked by loxP sites, was inserted 0.4 kb downstream of the exon into a single SacII site.

Embryonic stem (ES) cells of the E14 subclone IB-10 were grown under standard conditions. ES cells were electroporated (Bio-Rad Gene Pulser II) with the linearized targeting vector. G418-resistant clones, cut with HindIII, were analyzed by RFLP analysis using the SacdIII-digested λfix vector as a probe (Fig. 1), resulting in a single 7.5-kb band. Single copy integration was confirmed by probing with a neo+ probe (Int), resulting in a single 7.5-kb band. Deletion of the neo+ cassette was achieved by electroporating correctly targeted ES cells with the plasmid pIC Cre expressing Cre recombinase. Loss of the neo+ cassette reduced the 7.5-kb mutated fragment to 6 kb. Three individual clones then generated germ line chimeras as described (36).

Mice homozygous for the loxP-flanked exon were bred to mice expressing Cre recombinase in the zygote,2 giving rise to offspring with a heterozygous deletion of the first exon of the ITGA2 gene, ITGA2 (+/−) mice were intercrossed to generate mice with a null mutation of the α2 integrin.

**Analysis of Integrin Expression—**Platelets and tissue samples from ITGA2 (−/−) and wild type mice were homogenized in standard lysis buffer, and proteins were separated by SDS-PAGE under reducing conditions and transferred to nitrocellulose membranes. Following blocking, membranes were incubated with polyclonal rabbit antibodies against mouse α2 integrin. Bound antibodies were detected by horse radish peroxidase-conjugated anti-rabbit IgG and ECL (Amersham Biosciences).

**Results and Discussion**

**Generation of Integrin α2-Deficient Mice—**A targeting vector was constructed in which the first exon of the ITGA2 gene along with the translation start were flanked by loxP sites to generate a mouse line that enables conditional inactivation of the ITGA2 gene (Fig. 1A). The vector was used to produce ES cell clones with a single homologous recombination event, as confirmed by Southern blot analysis (Fig. 1B). Deletion of the neo+ cassette was achieved by transiently transfecting these ES cell clones with a vector expressing Cre recombinase. Southern blot analysis of resulting G418-sensitive clones revealed 7 clones lacking the neo+ cassette and harboring the loxP-flanked first exon (Fig. 1B, Flox).

Three individual clones were used to generate germ line chimeras. Germ line transmission in progeny of the chimeras was confirmed by Southern blot analysis of HindIII-digested tail DNA, and mice heterozygous for the mutation in the ITGA2 gene were intercrossed to produce mice homozygous for the loxP-flanked first exon (Fig. 1C, flo/+.). These animals (ITGA2flox) appeared normal, and Western blot analysis of several mouse organs confirmed that insertion of the loxP sites did not interfere with integrin α2 expression (Fig. 1D).

To produce mice with a heterozygous ablation of the ITGA2 gene, ITGA2flox animals were bred to mice expressing Cre recombinase in the zygote,2 leading to deletion of loxP-flanked regions. Deletion of the first exon was confirmed by Southern blot analysis, and heterozygous animals were intercrossed to produce α2 (−/−) mice (Fig. 1E).

**Integrin α2-Deficient Mice Develop Normally, Are Fertile, and Display No Obvious Anatomical Defects—**Homozygous α2-deficient mice are viable and show no striking phenotypical differences when compared with their heterozygous and wild type littermates. Complete loss of the α2-subunit was confirmed by Western blot analysis of proteins extracted from several mouse organs and from platelets (Fig. 1F).

Litter sizes from breedings of heterozygotes are comparable with those of wild type animals, and genotyping of the viable offspring revealed normal Mendelian ratios, demonstrating that the loss of the integrin α2-subunit does not result in embryonic lethality. Therefore, the difference in size or weight at birth nor at 3 weeks of postnatal life between α2 (−/−) mice (females, 13.1 ± 0.7 g; males, 15.1 ± 1.6 g) and wild type animals (females, 12.8 ± 1.8 g; males, 15.3 ± 1.7 g). Integrin α2 (−/−) mice are fertile, and intercrossing these mice produced normal litter sizes. Notably, the progeny of α2 (−/−) mice also developed normally, indicating that the α2 (−/−)
females have no severe defects in placenta formation or lactation. No morphological or histological changes were obvious in these mice. That mice lacking $\alpha_2\beta_1$ receptors were viable and fertile was surprising, given in vitro data suggesting that tissue morphogenesis could be impaired due to lack of proper adhesion, spreading, and migration. Further work will demonstrate whether subtle temporal alterations are present in these mice.

Functional compensation by other collagen or laminin receptors, e.g., $\alpha_1\beta_1$ or the discoidin domain receptors, may be an explanation for this subtle phenotype. Interestingly, ablation of both collagen receptors, $\alpha_1\beta_1$ (38) and $\alpha_2\beta_1$, present mice with only subtle phenotypes, differing thereby from other integrin-deficient mice, most of which display severe defects (39, 40).

Normal Platelet Counts and Bleeding Time in $\alpha_2$-Deficient Mice—While integrin $\alpha_2\beta_1$ has long been recognized as a platelet collagen receptor, its exact role in hemostasis has been controversial (41). To address this question, we analyzed platelets from $\alpha_2$-deficient mice. First, peripheral platelet counts were determined to assess platelet production in mutant mice. As shown in Fig. 2A, platelet counts were similar in control, $\alpha_2$ (+/+), and $\alpha_2$ (−/−) mice. Flow cytometric analysis confirmed the absence of integrin $\alpha_2$-subunits on homoygous mutant platelets, whereas the expression levels in heterozygous platelets were reduced by −50% when compared with wild type (Table I). Interestingly, the levels of integrin $\beta_1$ were reduced by −30% in $\alpha_2$ (−/−) and −15% in $\alpha_2$ (+/−) platelets as compared with controls, whereas the expression of $\alpha_5$ and $\alpha_6$ was significantly increased in mutant platelets (Table I). In contrast to $\beta_1$ integrins, the expression levels of other membrane glycoproteins, such as integrin $\beta_3$, GPVI, or the GPIX-V-IX complex, were not altered in mutant platelets (Table I). Western analyses of platelet lysates confirmed the absence of $\alpha_2$ (Fig. 1F) and normal expression of GPVI (not shown) in platelets from $\alpha_2$ (−/−) mice. These findings demonstrate that integrin $\alpha_2$ is not essential for megakaryocyte development and platelet production. However, its absence significantly alters the expression levels of other subunits of the $\beta_1$ integrin family, suggesting that different $\alpha$-subunits compete for association with $\beta_1$ in platelets.

Previous reports (28, 29) showed markedly increased bleeding in patients with reduced expression of $\alpha_2\beta_1$ integrin on platelets, suggesting a pivotal role of the integrin in hemostasis. In contrast to this hypothesis, we have shown recently (27) that bone marrow-chimeric mice with $\beta_1$ integrin-deficient platelets display no increased bleeding tendency. To test directly these contrasting findings in a defined system, bleeding times were determined in $\alpha_2$ (−/−) mice. Strikingly, bleeding times were found comparable for $\alpha_2$ (−/−) and control mice (Fig. 2B), demonstrating that the lack of $\alpha_2\beta_1$ integrin on platelets, and also on other cells of the cardiovascular system, has no major effect on normal hemostasis in mice. This finding confirms and extends the observations made in $\beta_1$ integrin mutant mice but stands in sharp contrast to the reported severe bleeding in patients with reduced $\alpha_2\beta_1$ levels on their platelets. The most likely explanation for this discrepancy is that these very few patients had additional defects in their platelets, although species-specific differences cannot be excluded.
Delayed Aggregation of α2β1-Deficient Platelets in Response to Fibrillar Collagen—Several reports (28, 29, 42) suggested a central role of α2β1 integrin during collagen-induced platelet aggregation. In contrast to these findings, the analysis of integrin β1 (−/−) platelets demonstrated a supportive rather than an essential role of β1 integrins in platelet-collagen interactions (27). To define unequivocally the role of α2β1 integrin in this process, we induced aggregation of control, α2 (+/−), and α2 (−/−) platelets using fibrillar type I collagen. Dose-response and maximum aggregation of mutant platelets did not differ from normal platelets (Fig. 3C). However, onset of aggregation was significantly delayed in α2 (−/−) platelets, and this was particularly evident at low collagen concentrations. Interestingly, no significant delay was observed in α2 (+/−) platelets (Fig. 3B).

It is established that platelet activation by collagen strictly depends on functional GPVI (27, 35, 43). To test this directly, we induced aggregation of control (Fig. 3A), α2β1-heterozygous (+/−), α2β1-deficient (−/−), white bars, squares) mice was stimulated with different concentrations of fibrillar collagen. Light transmission was recorded on a standard aggregometer. The delay (B) in platelet aggregation is expressed as time (seconds) between addition of collagen and maximal shape change. Results are expressed as mean ± S.D. (n = 6). D, heparinized PRP from the above mice was stimulated with the indicated concentrations of CRP. Results are expressed as mean ± S.D. (n = 6).

These results clearly demonstrate that integrin α2 is not essential for platelet activation by collagen, although the process is slightly delayed in the absence of the integrin. A similar delay in collagen-induced aggregation has been observed on human platelets in the presence of α2β1-blocking antibodies (45), in integrin β1-deficient mouse platelets (27), or in mouse platelets lacking GPV (46). These defects most likely reflect a reduced stability of the initial platelet-collagen interaction due to the lack of collagen-binding sites on the cells.

Defective Activation of α2β1-Deficient Platelets by Soluble Collagen—In vivo, secreted procollagen is proteolytically converted into collagen and assembled into insoluble, cross-striated fibrils (47). In vitro, collagen fibrils can be partly digested by pepsin, which cleaves the molecule in the non-triple helical region, thereby releasing “soluble” collagen (48). In numerous studies, such preparations of soluble collagen have been used to characterize the interaction of individual platelet receptors with collagen (48–50). We have shown recently that aggregation in response to soluble collagen is abrogated in integrin β1 (−/−) platelets, but α2 (−/−) platelets, whereas robust aggregation of control platelets was already seen at 5 μg/ml (Fig. 4A). The critical role of α2β1 integrin in this process was further confirmed by an 5-fold right shift of the dose-response curve of the α2 (−/−) platelets when compared with the control (Fig. 4B). Because platelet activation by

|               | Control | α2 (+/−) | α2 (−/−) |
|---------------|---------|----------|----------|
| GPIa (α2)     | 69.7 ± 8.3 | 34.6 ± 7.9 | 5.1 ± 1.3 |
| GPIc (α2)     | 22.3 ± 6.1 | 28.7 ± 9.5 | 29.5 ± 9.8 |
| GPIb (α2)     | 63.5 ± 4.7 | 82.3 ± 12.4 | 89.5 ± 6.2 |
| GPIa (β1)     | 200.3 ± 13.4 | 169.1 ± 9.7 | 142.2 ± 8.9 |
| GPIb (β1)     | 251.4 ± 16.9 | 247.8 ± 21.3 | 255.4 ± 18.6 |
| GPV           | 63.1 ± 8.4 | 60.9 ± 5.9 | 61.7 ± 8.5 |
| GPIb           | 411.7 ± 26.3 | 422.2 ± 34.1 | 412.8 ± 29.3 |
| GPV           | 190.7 ± 13.9 | 198.8 ± 11.6 | 194.1 ± 6.5 |
| CD9           | 743.3 ± 45.5 | 748.9 ± 43.1 | 737.8 ± 38.4 |
action of platelets with collagen and demonstrate that $\alpha_\beta_2$ is essential for platelet adhesion to soluble collagen, whereas adhesion to fibrillar collagen is only dependent on the integrin when the major collagen-binding site on GPVI is blocked. Although in vivo collagens can be degraded in certain pathological situations, the majority of collagens is deposited in fibrillar form in normal vessel walls. Therefore, GPVI interaction with collagen at sites of vascular injury should not be dependent on integrin $\alpha_\beta_2$. Once the platelets are activated through GPVI, other adhesive receptors, most importantly integrin $\alpha I\beta_3$, can mediate firm attachment and thrombus growth (24, 27).

In conclusion, we show that mice lacking $\alpha_\beta_2$ integrin receptors develop normally, are fertile, and exhibit surprisingly subtle alterations. More sophisticated analyses will be required to illustrate whether other subtle defects are present and which other receptor(s) may compensate for loss of $\alpha_\beta_2$ function. Possibly $\alpha_\beta_2$ integrins are not essential for development but may be needed for tissue repair, host defense, or other challenges that the adult organism has to meet. The analysis of $\alpha_\beta_2$-deficient platelets revealed a subtle rather than a major defect which is in line with recent studies on $\beta_2$-deficient platelets. The mice described here will allow detailed studies on the involvement of integrin $\alpha_\beta_2$ in thrombotic diseases where it has been proposed to play a major role (23).

Acknowledgments—We thank Ulrike Mayer (Manchester, UK) for the generous gift of monoclonal antibodies; Kerstin Elias and Marion Reibetanz (Cologne) for excellent technical assistance; and Reinhard Fassler and Cord Brakebusch (Lund, Sweden) and Roswitha Nischt, Christoph Leuker, and Monzur Murshed (Cologne) for critical discussion.

REFERENCES

1. Hemler, M. E. (1990) Ann. Rev. Immunol. 8, 365–400
2. Hynes, R. O. (1992) Cell 70, 11–25
3. Humphries, M. J. (2000) Biochem. Soc. Trans. 28, 311–319
4. van der Flier, A., and Sannenberingen, A. (2001) Cell Tissue Res. 305, 285–298
5. De Arcangelis, A., and Georges-Labouesse, E. (2000) Trends Genet. 16, 389–395
6. Varner, J. A., and Cherek, D. A. (1996) Curr. Opin. Cell Biol. 8, 724–730
7. Fassler, R., and Meyer, M. (1995) Genes Dev. 9,1986–1990
8. Heino, J. (2000) Matrix Biol. 19, 319–323
9. Calderwood, D. A., Tuckwell, D. S., Eble, J., Kuhn, K., and Humphries, M. J. (1997) J. Biol. Chem. 272, 12131–12137
10. Knight, C. G., Morton, L. F., Peachey, A. R., Tuckwell, D. S., Farndale, R. W., and Barnes, M. J. (2000) J. Biol. Chem. 275, 35–40
11. Emsley, J., Knight, C. G., Farndale, R. W., Barnes, M. J., and Liddington, R. C. (2000) Cell 101, 47–56
12. Tullia, M., Pentikainen, O. T., Viatasalo, T., Kapyla, J., Impola, U., Nykvist, P., Nissinen, L., Johnson, M. S., and Heino, J. (2001) J. Biol. Chem. 276, 48296–48312
13. Zutter, M. M., and Santoro, S. A. (1999) Am. J. Pathol. 157, 113–120
14. Wu, J. E., and Santoro, S. A. (1994) Dev. Dyn. 199, 292–314
15. Kern, A., Eble, J., Golbik, R., and Kuhn, K. (1993) Eur. J. Biochem. 215, 151–159
16. Kamata, T., and Takada, Y. (1994) J. Biol. Chem. 269, 26006–26010
17. Nykvist, P., Tu, H., Ivaska, J., Kapyla, J., Pihlajaniemi, T., and Heino, J. (2000) J. Biol. Chem. 275, 8255–8261
18. Pfaff, M., Gehring, W., Brown, J. C., and Timp, R. (1994) Eur. J. Biochem. 225, 975–984
19. Decline, P., and Boussele, P. (2001) J. Cell Sci. 114, 811–823
20. Langholz, O., Rockel, D., Mauch, C., Kozlowska, E., Bank, I., Krieg, T., and Eckes, B. (1995) J. Cell Biol. 131, 1903–1915
21. Ravanti, L., Heino, J., Lopez-Onin, C., and Kahari, V. M. (1999) J. Biol. Chem. 274, 2446–2455
22. Zigino, P., Dresscher, C., and Mauch, C. (2001) Eur. J. Cell Biol. 80, 68–77
23. Santoro, S. A. (1999) Blood 93, 3575–3577
24. Savage, B., Alonso-Jacobs, F., and Ruggeri, Z. M. (1998) Cell 94, 657–666
25. Mori, M., Jung, S. M., Okuma, M., and Shimazaki, K. (1989) J. Clin. Invest. 84, 1440–1445
26. Clemetson, J. J., Polgar, J., Magenat, E., Wells, T. N., and Clemetson, K. J. (1999) J. Biol. Chem. 274, 29019–29024
27. Niewandt, B., Brakebusch, C., Bergmeier, W., Schulte, V., Bouvard, D., Mokhtari-Nejad, R., Lindhout, T., Heemskerk, J. W., Zirngibl, H., and Fassler, R. (2001) EMBO J. 20, 2120–2130
28. Nieuwenhuis, H. K., Akkerman, J. W., Houdijk, W. P., and Sixma, J. J. (1985) Nature 318, 470–472
29. Kehrel, B., Balleisen, L., Kockert, R., Mestere, R., Stenzinger, W., Clemetson, K. J., and van de Loo, J. (1988) Blood 71, 1074–1078
30. Sheppard, D. (2000) Matrix Biol. 19, 203–209
31. de Fougereoles, A. R., Sprague, A. G., Nickerson-Nutter, C. L., Chi-Rasso, G., Renertt, P. D., Gardner, H., Gottsals, P. J., Lobb, R. R., and Koteliansky, V.
Platelets from Integrin α2β1-Deficient Mice

V. E. (2000) *J. Clin. Invest.* **105**, 721–729
32. Bergmeier, W., Rackebrandt, K., Schroder, W., Zirngibl, H., and Nieswandt, B. (2000) *Blood* **95**, 886–893
33. Nieswandt, B., Bergmeier, W., Rackebrandt, K., Gesnser, J. E., and Zirngibl, H. (2000) *Blood* **96**, 2520–2527
34. Nieswandt, B., Bergmeier, W., Schulte, V., Rackebrandt, K., Gesnser, J. E., and Zirngibl, H. (2000) *J. Biol. Chem.* **275**, 23998–24002
35. Nieswandt, B., Schulte, V., Bergmeier, W., Mokhtari-Nejad, R., Rackebrandt, K., Cazenave, J. P., Ohlmann, P., Gachet, C., and Zirngibl, H. (2001) *J. Exp. Med.* **193**, 459–469
36. Smyth, N., Vatansever, H. S., Murray, P., Meyer, M., Frie, C., Paulsson, M., and Edgar, D. (1999) *J. Cell Biol.* **144**, 151–160
37. Carmeliet, P., Stassen, J. M., Schoonjans, L., Ream, B., van den Oord, J. J., De Mol, M., Mulligan, R. C., and Collen, D. (1993) *J. Clin. Invest.* **92**, 2756–2760
38. Gardner, H., Kreidberg, J., Kotelyansky, V., and Jaenisch, R. (1996) *Dev. Biol.* **175**, 301–313
39. Fassler, R., Georges-Labouesse, E., and Hirsch, E. (1996) *Curr. Opin. Cell Biol.* **8**, 641–646
40. Hynes, R. O. (1996) *Dev. Biol.* **180**, 402–412
41. Watson, S., Berlanga, O., Best, D., and Frampton, J. (2000) *Platelets* **11**, 252–258
42. Saelman, E. U., Nieuwenhuis, H. K., Hese, K. M., de Groot, P. G., Heijnen, H. F., Sage, E. H., Williams, S., McKeown, L., Grahnick, H. R., and Sixma, J. J. (1994) *Blood* **83**, 1244–1250
43. Poole, A., Gibbins, J. M., Turner, M., van Vugt, M. J., van de Winkel, J. G., Saito, T., Tybulewicz, V. L., and Watson, S. P. (1997) *EMBO J.* **16**, 2333–2341
44. Asselin, J., Gibbins, J. M., Achison, M., Lee, Y. H., Morton, L. F., Farndale, R. W., Barnes, M. J., and Watson, S. P. (1997) *Blood* **89**, 1235–1242
45. Coller, B. S., Beer, J. H., Scudder, L. E., and Steinberg, M. H. (1989) *Blood* **74**, 182–192
46. Moog, S., Mangin, P., Lenain, N., Strassel, C., Ravanat, C., Schuhler, S., Freund, M., Santer, M., Kahn, M., Nieswandt, B., Gachet, C., Cazenave, J. P., and Lanza, F. (2001) *Blood* **98**, 1038–1046
47. Kadler, K. E., Holmes, D. F., Trotter, J. A., and Chapman, J. A. (1996) *Biochem. J.* **316**, 1–11
48. Savage, B., Ginsberg, M. H., and Ruggeri, Z. M. (1999) *Blood* **94**, 2704–2715
49. Morton, L. F., Peachey, A. R., Zijenah, L. S., Goodall, A. H., Humphries, M. J., and Barnes, M. J. (1994) *Biochem. J.* **299**, 791–797
50. Siljander, P., and Lasila, R. (1999) *Arterioscler. Thromb. Vasc. Biol.* **19**, 3033–3043
51. Schulte, V., Snell, D., Bergmeier, W., Zirngibl, H., Watson, S. P., and Nieswandt, B. (2001) *J. Biol. Chem.* **276**, 364–368