British Journal of Haematology, 2003, **123**, 896–902

Endothelial microparticles released in thrombotic thrombocytopenic purpura express von Willebrand factor and markers of endothelial activation

Joaquin J. Jimenez,[1] Wenche Jy,[1] Lucia M. Mauro,[1] Lawrence L. Horstman,[1] Carl Soderland[2] and Yeon S. Ahn[1] [1]*Wallace H Coulter Platelet Laboratory, Division of Hematology/Oncology, University of Miami School of Medicine, Miami, FL, and* [2]*Applied Cell Biology Research Institute, Kirkland, WA, USA*

Received 23 June 2003; accepted for publication 12 September 2003

Summary. It has been suggested that endothelial apoptosis is a primary lesion in the pathogenesis of thrombotic thrombocytopenic purpura (TTP). We tested this hypothesis by examining the phenotypic signatures of endothelial microparticles (EMP) in TTP patients. In addition, the effect of TTP plasma on microvascular endothelial cells (MVEC) in culture was further delineated. EMP released by endothelial cells (EC) express markers of the parent EC; EMP released in activation carry predominantly CD54 and CD62E, while those in apoptosis CD31 and CD105. We investigated EMP release *in vitro* and in TTP patients. Following incubation of MVEC with TTP plasma, EMP and EC were analysed by flow cytometry for the expression of CD31, CD51, CD54, CD62E, CD105, CD106 and von Willebrand factor (VWF) antigen. EMP were also analysed in 12 TTP patients. In both EC and EMP, CD62E and CD54 expression were increased 3- to 10-fold and 8- to 10-fold respectively. However, CD31 and CD105 were reduced 40–60% in EC but increased twofold in EMP. VWF expression was found in 55 ± 15% of $CD62E^+$ EMP. Markers of apoptosis were negative. In TTP patients, $CD62E^+$ and $CD31^+/CD42b^-$ EMP were markedly elevated, and preceded and correlated well with a rise in platelet counts and a fall in lactate dehydrogenase. $CD62E^+$ EMP (60 ± 20%) co-expressed VWF and CD62E. The ratio of $CD31^+/42b^-$ to $CD62E^+$ EMP exhibited a pattern consistent with activation. In conclusion, our studies indicate endothelial activation in TTP. EMP that co-express VWF and CD62E could play a role in the pathogenesis of TTP.

Keywords: von Willebrand factor, endothelial microparticles, thrombotic thrombocytopenic purpura, endothelium, endothelial cell activation.

In thrombotic thrombocytopenic purpura (TTP) intravascular platelet adhesion, aggregation and formation of platelet rich thrombi in microvasculature lead to severe thrombocytopenia, microangiopathic haemolytic anaemia and multiple organ dysfunction (Ridolfi & Bell, 1981; Ruggenenti & Remuzzi, 1996; Moake, 2002). Moake (1986, 2002) reported unusually large von Willebrand factor multimers (ULVWFM) in patients with relapsing TTP and suggested that ULVWFM released from disturbed endothelia play a pivotal role in TTP, promoting platelet adhesion and aggregation in the microvasculature. This observation supported the theory that endothelial injury is the primary lesion in the pathogenesis of TTP (Ridolfi & Bell, 1981). However, the detailed nature of the endothelial lesion in TTP remains to be elucidated.

We previously demonstrated (Jimenez *et al*, 2001) that plasma from patients with acute TTP, when incubated with brain or renal microvascular endothelial cells (MVEC), induced expression of endothelial adhesins such as vascular cell adhesion molecule-1 (VCAM-1, CD106) and intracellular adhesion molecule-1 (ICAM-1, CD54), and promoted release of endothelial microparticles (EMP), small membrane-derived vesicles ($\lesssim$1·0 μm) shed from disturbed endothelium. EMP carry antigens, such as platelet-endothelial cell adhesion molecule-1 (PECAM-1, CD31) and vitronectin receptor (CD51), of the parent EC, which can be detected by flow cytometry, employing antibodies specific to these antigens (Combes *et al*, 1999; Jimenez *et al*, 2001, 2003; Minagar *et al*, 2001; Bernal-Mizrachi *et al*, 2003; Gonzalez-Quintero *et al*, 2003; Preston *et al*, 2003). Recently, we observed *in vitro* that the immunological phenotype of EMP reflected the status of the parent EC

Correspondence: Joaquin J. Jimenez MD, University of Miami School of Medicine, 1600 NW 10th Ave, Mail Code R-38 Miami, FL 33136, USA. E-mail: jjimenez@med.miami.edu

© 2003 Blackwell Publishing Ltd

(Jimenez *et al*, 2003). For example, during activation, EMP bearing inducible markers such as CD62E and CD54 were increased, in parallel with increased expression of these antigens on the parent EC. In contrast, in apoptosis, EMP expressed predominantly constitutive markers such as CD31 and CD105, with a concomitant depletion of such antigens on the parent EC. Thus, assay of a selected panel of antigenic markers on EMP could discriminate the nature of endothelial lesion, such as state of activation *versus* apoptosis.

MATERIALS AND METHODS

Antibodies. The following fluorescent-tagged anti-human monoclonal antibodies were obtained from the indicated commercial suppliers: anti-human CD31 [phycoerythrin (PE); Pharmingen, San Diego, CA, USA, cat. no. 555446], anti-human CD54 [fluorescein isothiocyante (FITC); Sigma, St Louis, MO, USA, cat. no. F-0549], anti-human CD105 (FITC; Ancell, Bayport, MN, USA, cat. no. 326-040), anti-human CD62E (PE; Pharmingen, cat. no. 551145), human von Willebrand factor:antigen (VWF:Ag) (FITC; Biodesign International, Saco, ME, USA, cat. no. K90054F) and anti-CD42 (FITC; Pharmingen, cat. no. 555472).

Patient and control populations. This study was based on data from 12 patients admitted to the University of Miami Medical Centre with a diagnosis of TTP. Approval by the Institutional Review Board of the University of Miami School of Medicine was obtained and all patients gave informed consent. Criteria for the diagnosis of TTP have been previously described (Ahn *et al*, 1996). Briefly, all patients with TTP presented with the following findings: severe thrombocytopenia (platelet count $<20 \times 10^9$/l), microangiopathic haemolytic anaemia, elevated lactate dehydrogenase (LDH) level and neurological dysfunction. As patient controls, 10 patients with chronic idiopathic thrombocytopenic purpura (ITP) were recruited. All had suffered chronic thrombocytopenia for over a year without any known underlying causes and had previously responded to glucocorticoids; bone marrow studies showed increased megakaryocyte counts, consistent with ITP. Normal control plasma was obtained from 10 healthy volunteers.

Sample preparation for clinical studies. Blood samples were collected in 5 ml sodium citrate tubes and were centrifuged 10 min at 160 ***g*** to prepare platelet-rich plasma (PRP). The PRP was further centrifuged 6 min at 1500 ***g*** to obtain platelet-poor plasma (PPP). PPP was prepared and assayed for EMP within 4 h of venepuncture. Aliquots of PPP (30 μl) in 12 × 75 mm polypropylene tubes were used to measure EMP as follows: (a) to measure $CD62E^+$ EMP, PPP was incubated with 5 μl of PE-labelled anti-CD62E; (b) to measure $CD31^+/CD42b^-$ EMP, PPP was incubated with 5 μl each of PE-labelled anti-CD31 and FITC-labelled anti-CD42b. The purpose of the anti-CD42b was to exclude platelet microparticles (PMP), as explained previously (Jimenez *et al*, 2001). Hereafter, these EMP are called simply $CD31^+$ EMP. After a 20 min incubation at room temperature with gentle shaking (orbital shaker, 120 rpm), 0·5 ml of phosphate-buffered saline (PBS) was added and EMP were analysed by flow cytometry (Coulter EPICS XL, Beckman Coulter, Miami, FL, USA), essentially as previously described (Jimenez *et al*, 2001).

Sample preparation for study of effect of TTP plasma on EC culture. Renal and brain MVEC (Cell Systems Corporation, Kirkland, WA, USA) were cultured and treated as previously described (Jimenez *et al*, 2001, 2003). Briefly, cells were resuspended in culture medium (Cell Systems) and replated in 12-well tissue culture multiwell clusters (Corning, Costar Corporation, Cambridge, MA, USA) precoated with attachment factor (Cell Systems) at a density of 1×10^5/well 48 h prior to experiments. Then, 200 μl of TTP or control plasmas were added to each well containing 1·0 ml media and maintained for 24 h. All plasmas were frozen at −70°C and filtered through a 0·1 μm filter (Whatman, Clifton, NJ, USA) immediately before use to eliminate ∼95% of detectable pre-existing EMP. As positive controls, renal and brain MVEC were cultured in the presence of 10 ng/ml of tumour necrosis factor-α (TNF-α; Sigma), or deprived of growth factors (GFD), for 24 h. These conditions have been shown to induce activation and apoptosis, respectively (Pober *et al*, 1986; Hogg *et al*, 1999), and were confirmed in our laboratory (Jimenez *et al*, 2001, 2003; Jy *et al*, 2002a). EMP in the supernatants were then measured by incubation of a 1:10 dilution with either anti-human CD31, CD54, CD62E, CD105, or anti-VWF:Ag. As no platelets were present in the culture, it was not necessary to use CD42b for *in vitro* experiments. Samples were then processed as described above for *in vivo* samples. Conversion of raw counts of EMP, given on the flow cytometer, to units of EMP per millilitre of original supernatant was calculated as previously described (Jimenez *et al*, 2003).

Detection of surface markers on EC. The above antigenic markers were also applied to the remnant EC. After supernatants were removed for testing, each plate was incubated with 10 μl of the above monoclonal antibodies for 30 min, then excess antibody was removed and the EC were washed three times with 1 ml of complete basal medium. The cells were then detached by addition of trypsin (Gibco Invitrogen, Carlsbad, CA, USA) (0·4 ml of 0·05% trypsin/0·53 mmol/l EDTA). After 3–5 min, the trypsin was neutralized with 0·3 ml of fetal bovine serum and the resulting 1:1 suspension of MVEC was examined by flow cytometry to measure fluorescent expression.

Statistical analysis. Student's *t*-test was used to evaluate significance between pairs of groups. In cases where the data failed the Kolmogorov–Smirnov normality test, the Mann–Whitney rank sum test was used. Statistical significance was defined as $P < 0{\cdot}05$. All data analyses were performed using the software Sigmaplot 4·0 and Statmost.

RESULTS

Effect of TTP plasma on EMP and remnant whole EC in cultures. GFD-induced apoptosis in MVEC, as indicated by TUNEL (terminal deoxynucleotidyl transferase mediated dUTP nick end labelling) positivity, and viability by trypan blue was 72 ± 12% and 8 ± 3% for renal MVEC, and 53 ± 12% and 15 ± 4% for brain MVEC respectively. Exposure of MVEC to

TNF-α did not result in apoptosis as indicated by TUNEL positivity of <2% and viability of >93% for all cultures, confirming our previous reports (Jimenez *et al*, 2001, 2003; Jy *et al*, 2002a).

Figure 1 summarizes the data on immunophenotypes of renal EC (Fig 1A,B) and the EMP counts (Fig 1C,D). Figure 1A shows that constitutive markers CD31 and CD105 on whole EC were substantially reduced in GFD when compared with untreated EC (Unt), while the EMP bearing these markers were greatly increased by GFD treatment (Fig 1C). However, as shown in Fig 1B, inducible markers CD54 and CD62E on the parent EC were increased by activation (TNF-α) but not by apoptosis (GFD), and likewise, the EMP released (Fig 1D). When these results were compared with those from plasma treatments, it was seen that TTP plasma from the acute stage (TTP-A) induced a phenotypic signature consistent with activation, not apoptosis, because of the expression of inducible markers on the EC surface and EMP (Fig 1B,D). Note that plasma from TTP patients in remission (TTP-R) had no such effect; nor did exposure to plasma from ITP patients or normal controls. Although $CD31^+$ and $CD105^+$ EMP were increased moderately by TTP plasma (Fig 1C), this effect was only marginally greater than that of TNF-α and the TTP plasma caused no decrease in CD105 surface expression (Fig 1A). We previously found a ratio of $CD62E^+$ to $CD31^+$ EMP of >4·0 in activation and <0·4 in apoptosis (Jimenez *et al*, 2003). In the present study, this ratio was 5·6 for MVEC stimulated with TTP plasma.

The above studies were conducted with renal MVEC. We also investigated brain MVEC by the same experiments and obtained data comparable with that given above (not shown).

EMP in TTP patients

Data on a representative patient are plotted in Fig 2A, together with the platelet counts (Fig 2B) and LDH (Fig 2C). All patients tested presented similar EMP patterns. It should be noted that both measures of EMP returned to normal after remission was achieved on day 18 (Fig 2).

As shown in Fig 3, Both $CD31^+/CD42b^-$ and $CD62E^+$ EMP counts were significantly elevated in TTP patients during the acute phase of TTP when compared with counts obtained in normal controls or in patients in remission. In three patients, EMP measurements were performed pre- and post-plasmapheresis. $CD62E^+$ and $CD31^+$ EMP counts fell

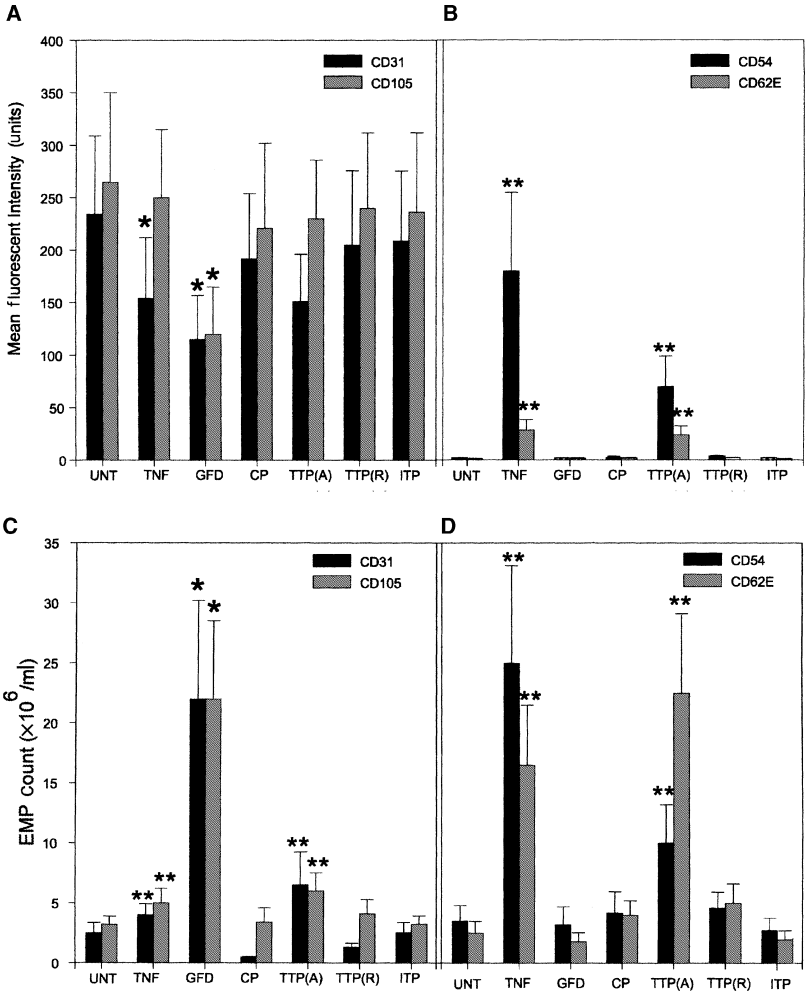

Fig 1. Expression of antigens on renal MVEC and EMP after stimulation with TTP plasma. This figure summarizes the immunophenotype results for two constitutive markers, CD31 and CD105 (A,C), and two inducible markers, CD54 and CD62E (B,D), in the remnant whole EC (A,B) and in EMP released to the supernatant (C,D). Results for whole EC are given as mean fluorescent intensity while those for EMP are counts ($\times 10^6$/ml). Constitutive markers are depicted on the left, while inducible are located on the right. UNT, untreated controls; GFD, growth factor deprivation; TNF, tumour necrosis factor-α; CP, control plasma ($n = 10$); TTP(A), TTP plasma, acute phase ($n = 10$); TTP(R), TTP plasma, remission ($n = 10$); ITP, ITP plasma, active phase ($n = 10$). Values shown are the means of three experiments (±SD) (*$P < 0{\cdot}0001$, **$P = 0{\cdot}01$).

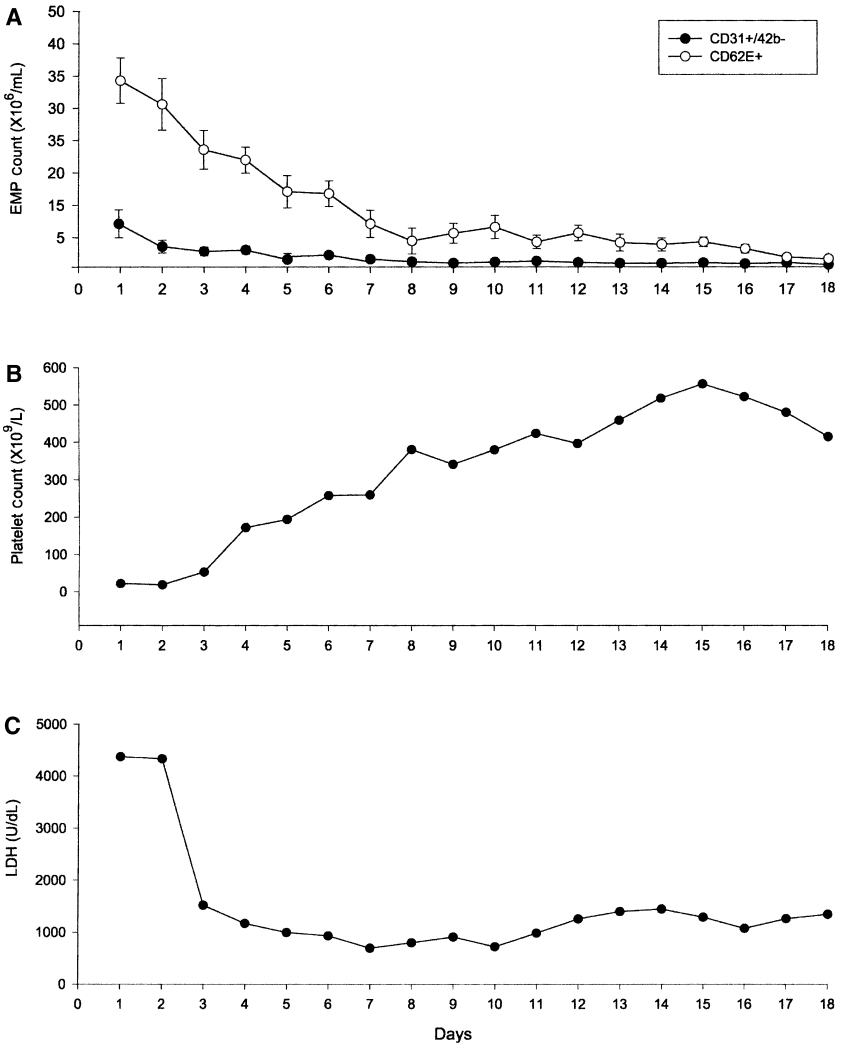

Fig 2. (A) Longitudinal measurement of EMP through the clinical course of a representative TTP patient. $CD31^+$ and $CD62E^+$ EMP were measured (±SD) on admission and when remission was achieved. Similar EMP patterns were observed in 10 patients with TTP. EMP counts correlated well with platelet counts (B) and LDH levels (C). Results are expressed as means of three measurements per time period.

sharply after the first treatment and thereafter declined steadily (Fig 4).

Association of VWF with EMP

As shown in Fig 5A, supernatants from cultured renal MVEC stimulated with plasma from five TTP patients yielded EMP that co-expressed VWF with CD62E at a level about 15-fold that of five normal controls. Figure 5B shows corresponding EMP plasma results in five TTP patients and five controls: VWF co-expressed with CD62E on EMP in the plasma of TTP patients was about fivefold of that found in normal control plasma.

DISCUSSION

Endothelial dysfunction plays a central role in the pathogenesis of many thrombotic and inflammatory disorders (Wu & Thiagarajan, 1996). However, existing laboratory tests to define or monitor endothelial disturbances in the clinical setting are limited. We have reported elevated $CD31^+/CD42b^-$ EMP in patients in active phases of TTP and multiple sclerosis (MS), and observed that the levels decreased to normal in remission (Jimenez *et al*, 2001; Minagar *et al*, 2001). We have also reported elevated EMP in pre-eclampsia, acute coronary syndromes and malignant hypertension (Bernal-Mizrachi *et al*, 2003; Gonzalez-Quintero *et al*, 2003; Preston *et al*, 2003). Others have reported elevated EMP in patients with lupus anti-coagulant, coronary artery disease and diabetes mellitus (Combes *et al*, 1999; Mallat *et al*, 2000; Boulanger *et al*, 2001; Sabatier *et al*, 2002a). Clinical applications of an EMP assay show promise for monitoring endothelial injury in thrombotic and inflammatory disorders.

We and others have shown that EMP are heterogeneous, as defined by antigenic phenotypes, and that their relative abundance and phenotype reflects the nature of the EC injury (Combes *et al*, 1999; Jimenez *et al*, 2001, 2003). In addition, EMP express tissue factor (TF) and provide a phospholipid surface, indicating procoagulant potential (Combes *et al*, 1999; Jimenez *et al*, 2001). Further investigation of

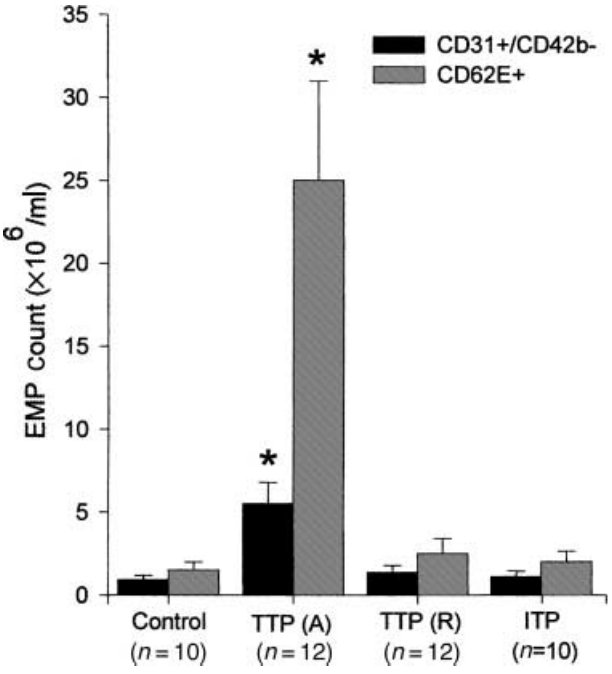

Fig 3. Quantification of $CD31^+/CD42b^-$ and $CD62E^+$ EMP in TTP patients during an acute episode. EMP were measured in 12 patients during the acute phase of TTP [TTP(A)], 12 patients during remission [TTP(R)], 10 controls and 10 patients during the acute phase of ITP. Both $CD31^+/CD42b^-$ and $CD62E^+$ were significantly elevated in TTP patients when compared with controls. Error bars show ±SD (*$P < 0{\cdot}0001$).

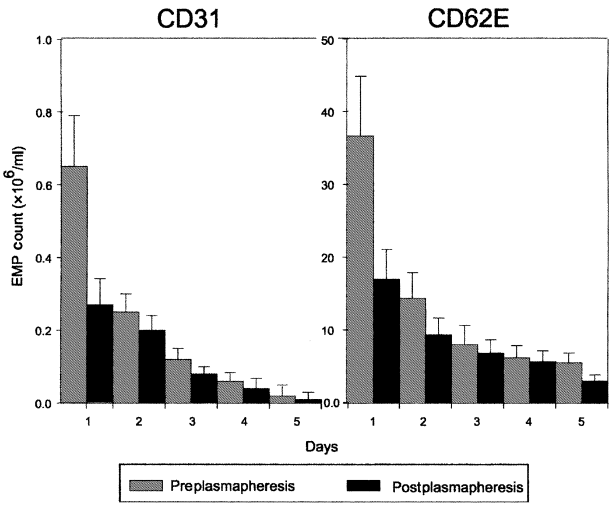

Fig 4. Quantification of $CD31^+/CD42b^-$ and $CD62E^+$ EMP in TTP patients pre- and post-plasmapheresis. EMP were measured pre- and post-plasmapheresis in three patients with TTP. Both $CD31^+/CD42b^-$ and $CD62E^+$ EMP decreased sharply after the first plasmapheresis, thereafter more gradually after each plasma exchange. Data were obtained upon admission and reflected the first 5 d of treatment (±SD).

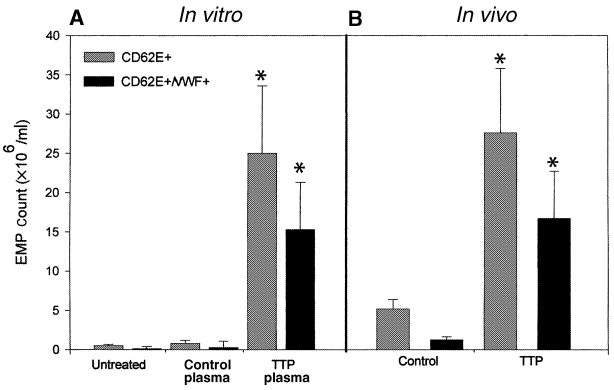

Fig 5. Co-expression of VWF on EMP (A) *in vitro* and (B) in TTP patients. (A) *in vitro* results are shown after 24 h of no treatment, or treatment with TTP ($n = 5$) or normal plasma ($n = 5$). Values shown are the means of three experiments. (B) EMP were measured in the plasma of five patients with TTP during the acute phase *versus* five normal controls. (±SD) (*$P < 0{\cdot}0001$).

the mechanisms of generation and functional characteristics of EMP in different disorders involving endothelial perturbation could lead to the improved understanding of the endothelial lesion in specific vascular, thrombotic and inflammatory conditions.

Endothelial injury is believed to be the primary underlying lesion in TTP, initiating intravascular platelet adhesion, aggregation and formation of platelet rich thrombi in microvasculature (Ridolfi & Bell, 1981). However, the precise nature of the endothelial lesion in TTP is poorly understood. It has been reported that TTP plasma induced apoptosis of EC derived from brain, renal, or dermal microvascular origin, but not EC derived from larger vascular beds (Laurence *et al*, 1996; Mitra *et al*, 1997). Thus, its has been argued that apoptosis may be the principal endotheliopathy in TTP (Laurence *et al*, 1996; Laurence & Mitra, 1997; Mitra *et al*, 1997, 1998).

In the present study, we investigated the phenotypic profiles of EMP that were released upon incubation of TTP plasma with EC *in vitro* as well as EMP in blood samples from patients with acute TTP. Our previous studies showed that CD62E and CD31 are the most useful markers of EMP found to date. In activation, $CD62E^+$ EMP are the most abundant, while in apoptosis, $CD31^+$ EMP predominate. Accordingly, the ratio of $CD62^+$ EMP to $CD31^+$ EMP provides a useful measure for discriminating a state of endothelial activation from apoptosis: this ratio is high in activation, low in apoptosis (Jimenez *et al*, 2003). This conclusion was based on findings obtained with TNF-α, a known activator, and deprivation of growth factor, conditions known to induce apoptosis (Pober *et al*, 1986; Hogg *et al*, 1999).

When plasma from TTP patients was incubated with MVEC, TTP plasma induced release of EMP expressing predominantly CD62E, hence the CD62E/CD31 ratio was consistent with endothelial activation, not apoptosis. Parallel clinical studies (*in vivo*) on EMP in TTP patients revealed that $CD62^+$ EMP were more abundant than $CD31^+$ EMP, indicating that EMP in TTP are released from

activated, not apoptotic, EC. Praprotnik *et al* (2001) recently reported that stimulation of EC with TTP plasma resulted in a state of endothelial activation.

The present report also includes data on EMP pre- and post-plasmapheresis. Interestingly, all four patients for whom this data were available showed a dramatic drop in EMP after the first treatment; thereafter, a more gradual reduction after each plasma exchange was observed. It is possible that the first plasmapheresis may remove large quantities of thrombogenic EMP accumulated in the circulation while subsequent procedures may clear only the newly released EMP. Alternatively, plasmapheresis may eliminate a factor present in TTP plasma that perturbs the endothelium, possibly an antibody or cytokine such as TNF-α. In this regard, Wada *et al* (1993) reported that TNF-α was elevated in the circulation of TTP patients during the onset of TTP. This would be consistent with our finding that TNF-α acts selectively on the microvasculature, eliciting a potent response in MVEC but having little effect on coronary artery EC at the same concentration (Jimenez *et al*, 2003).

Our study raises the question of the potential role of EMP in TTP. EMP are procoagulant, by virtue of both TF and procoagulant phospholipids (platelet factor three activity) (Combes *et al*, 1999; Jimenez *et al*, 2001). Our finding *in vitro* that TTP plasma induces release of EMP rich in VWF, and *in vivo* that EMP in TTP patients also express VWF, suggests a role of EMP in the pathogenesis of TTP. Impaired proteolysis of ULVWF multimers in TTP patients due to the decreased activity of the metalloprotease ADAMTS-13 (a disintegrin and metalloproteinase with thrombospondin motif-1) is currently a favoured hypothesis for the pathogenesis of TTP (Moake, 2002). Our finding that EMP are carriers of VWF suggest their possible involvement in aberrant VWF processing in TTP.

Studies are underway to clarify the issue of VWF abnormalities as related to EMP in TTP. It has been observed that microparticles, in general, act as activators of leucocytes. We, and others have documented that EMP bind to and activate monocytes, eliciting expression of TF (Jy *et al*, 2002b; Sabatier *et al*, 2002b). Thus, EMP may modulate the inflammatory response of monocytes by regulating the release of cytokines such as TNF-α or IL-1β, which could in turn result in a state of endothelial activation, as seen in TTP.

To summarize: (i) TTP plasma incubated with MVEC *in vitro* induced the release of EMP with phenotypes indicative of activation, and approximately 60% of them carried VWF; (ii) in parallel clinical studies, EMP levels in patients with TTP correlated well with the severity of TTP. EMP measured in TTP patients carried VWF and exhibited the phenotype of EC activation. The factor in TTP plasma responsible for release of EMP remains to be elucidated. The expression of VWF on EMP further implicates EMP in the pathophysiology of TTP.

ACKNOWLEDGMENTS

We would like to thank R.N. Micheline Aristide and M.T. Ariel Rodriguez from Transfusion Services at Jackson Memorial Hospital for their support. This work was supported by the Wallace H Coulter Foundation. We are also grateful for support from the Roz & Cal Kovens Research Fund, the Charles & Jane Bosco Research Fund and the Mary Beth Weiss Research Fund.

REFERENCES

Ahn, Y.S., Jy, W., Kolodny, L., Horstman, L.L., Mao, W.W., Valant, P.A. & Duncan, R.C. (1996) Activated platelet aggregates in thrombotic thrombocytopenic purpura: decrease with plasma infusions and normalization in remission. *British Journal of Haematology*, **95**, 408–415.

Bernal-Mizrachi, L., Jy, W., Jimenez, J.J., Pastor, J., Mauro, L.M., Horstman, L.L., de Marchena, E. & Ahn, Y.S. (2003) High levels of circulating endothelial microparticles in patients with acute coronary syndromes. *American Heart Journal*, **145**, 962–970.

Boulanger, C.M., Scoazec, A., Ebrahimian, T., Henry, P., Mathieu, E., Tedgui, A. & Mallat, Z. (2001) Circulating microparticles from patients with myocardial infarctions cause endothelial dysfunction. *Circulation*, **104**, 2649–2652.

Combes, V., Simon, A.C., Grau, G.E., Arnoux, D., Camoin, L., Sabatier, F., Mutin, M., Sanmarco, M., Sampol, J. & Dignat-George, F. (1999) In vitro generation of endothelial microparticles and possible prothrombotic activity in patients with lupus anticoagulant. *Journal of Clinical Investigation*, **104**, 93–102.

Gonzalez-Quintero, V.H., Jimenez, J.J., Jy, W., Mauro, L.M., Horstman, L.L., O'Sullivan, M.J. & Ahn, Y. (2003) Elevated endothelial microparticles in preeclampsia. *American Journal of Obstetrics and Gynecology*, **189**, 589–593.

Hogg, N., Browning, J., Howard, T., Winterford, C., Fitzpatrick, D. & Gobe, G. (1999) Apoptosis in vascular endothelial cells caused by serum deprivation, oxidative stress and transforming growth factor-beta. *Endothelium*, **7**, 35–39.

Jimenez, J.J., Jy, W., Mauro, L.M., Horstman, L.L. & Ahn, Y.S. (2001) Elevated endothelial microparticles in thrombotic thrombocytopenic purpura findings from brain and renal microvascular cell culture and patients with active disease. *British Journal of Haematology*, **112**, 81–90.

Jimenez, J.J., Jy, W., Mauro, L.M., Soderland, C., Horstman, L.L. & Ahn, Y.S. (2003) Endothelial cells release phenotypically and quantitatively distinct microparticles in activation vs. apoptosis. *Thrombosis Research*, **109**, 175–180.

Jy, W., Jimenez, J.J., Mauro, L.M., Ahn, Y.S., Newton, K.R., Mendez, A.J., Arnold, P.I. & Schultz, D.R. (2002a) Agonist-induced capping of adhesion proteins and microparticle shedding in cultures of human renal microvascular endothelial cells. *Endothelium*, **9**, 179–189.

Jy, W., Jimenez, J.J., Minagar, A., Mauro, L.M., Horstman, L.L., Bidot, C.J., Sheremata, W.A. & Ahn, Y.S. (2002b) Endothelial microparticles (EMP) enhance adhesion and transmigration of monocytes: EMP-monocyte conjugates as a marker of disease activity in multiple sclerosis (MS). *Blood*, **11**(part 2), 460 (abstract no. 1783).

Laurence, J. & Mitra, D. (1997) Apoptosis of microvascular endothelial cells in the pathophysiology of thrombotic thrombocytopenic purpura/sporadic hemolytic uremic syndrome. *Seminars in Hematology*, **34**, 98–105.

Laurence, J., Mitra, D., Steiner, M., Staiano-Coico, L. & Jaffe, E. (1996) Plasma from patients with idiopathic and human immunodeficiency virus-associated thrombotic thrombocytopenic purpura induces apoptosis in microvascular endothelial cells. *Blood*, **87**, 3245–3254.

Mallat, Z., Benamer, H., Hugel, B., Benessiano, J., Steg, P.G., Freyssinet, J.M. & Tedgui, A. (2000) Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. *Circulation*, **101**, 841–843.

Minagar, A., Jy, W., Jimenez, J.J., Sheremata, W.A., Mauro, L.M., Horstman, L.L. & Ahn, Y.S. (2001) Elevated plasma endothelial microparticles in multiple sclerosis. *Neurology*, **56**, 1319–1324.

Mitra, D., Jaffe, E.A., Weksler, B., Hajjar, K.A., Soderland, C. & Laurence, L. (1997) Thrombotic thrombocytopenic purpura and sporadic hemolytic-uremic syndrome plasmas induce apoptosis in restricted lineages of human microvascular endothelial cells. *Blood*, **89**, 1224–1234.

Mitra, D., Kim, J., MacLow, C., Karsan, A. & Laurence, J. (1998) Role of caspases 1 and 3 and Bcl-2-related molecules in endothelial cell apoptosis associated with thrombotic microangiopathies. *American Journal of Hematology*, **59**, 279–287.

Moake, J.L. (1986) von Willebrand factor in thrombotic thrombocytopenic purpura. *Blood*, **67**, 1523–1526.

Moake, J.L. (2002) Thrombotic microangiopathies. *New England Journal of Medicine*, **347**, 589–600.

Pober, J.S., Gimbrone, Jr, M.A., Lapierre, L.A., Mendrick, D.L., Fiers, W., Rothlein, R. & Springer, T.A. (1986) Overlapping patterns of activation of human endothelial cells by interleukin-1, tumor necrosis factor and immune interferon. *Journal of Immunology*, **137**, 1893–1896.

Praprotnik, S., Blank, M., Levy, Y., Tavor, S., Boffa, M.C., Weksler, B., Eldor, A. & Shoenfeld, Y. (2001) Anti-endothelial cell antibodies from patients with thrombotic thrombocytopenic purpura specifically activate small vessel endothelial cells. *International Immunology*, **13**, 203–210.

Preston, R.A., Jy, W., Jimenez, J.J., Mauro, L.M., Horstman, L.L., Valle, M., Aime, G. & Ahn, Y.S. (2003) Effect of severe hypertension on endothelial and platelet microparticles. *Hypertension*, **41**, 211–217.

Ridolfi, R.L. & Bell, W.R. (1981) Thrombotic thrombocytopenic purpura: report of 25 cases and review of the literature. *Medicine*, **60**, 413–428.

Ruggenenti, P. & Remuzzi, G. (1996) The pathophysiology and management of thrombotic thrombocytopenic purpura. *European Journal of Haematology*, **56**, 191–207.

Sabatier, F., Darmon, P., Hugel, B., Combes, V., Sanmarco, M., Velut, J.G., Arnoux, D., Charpiot, P., Freyssinet, J.M., Oliver, C., Sampol, J. & Dignat-George, F. (2002a) Type 1 and type 2 diabetic patients display different patterns of cellular microparticles. *Diabetes*, **51**, 2840–2845.

Sabatier, F., Roux, V., Anfosso, F., Camoin, L., Sampol, J. & Dignat-George, F. (2002b) Interaction of endothelial microparticles with monocytic cells in vitro induces tissue factor-dependent procoagulant activity. *Blood*, **99**, 3962–3970.

Wada, H., Kaneko, T., Ohiwa, M., Tanigawa, M., Tamaki, S., Minami, N., Takahashi, H., Deguchi, K., Nakano, T. & Shirakawa, S. (1992) Plasma cytokine levels in thrombotic thrombocytopenic purpura. *American Journal of Hematology*, **40**, 167–170.

Wu, K.K. & Thiagarajan, P. (1996) Role of endothelium in thrombosis and hemostasis. *Annual Review of Medicine*, **47**, 315–331.