Increased Levels of Circulating Endothelial-Derived Microparticles and Small-Size Platelet-Derived Microparticles in Psoriasis

Journal of Investigative Dermatology (2011) **131,** 1573–1576; doi:10.1038/jid.2011.57; published online 17 March 2011

TO THE EDITOR

Microparticles (MPs) are membrane vesicles of 0.1 to 1 μm diameter generated by budding or shedding from the plasma membrane and released by any cell type into the vascular compartment during activation or apoptosis (Théry *et al.*, 2009). Membrane lineage markers of their originating cell permit to distinguish several circulating MPs, such as platelet-derived MPs (PMPs) or endothelial cell-derived MPs (EMPs). Microparticles are involved in inflammatory and auto-immune diseases, as well as in cardiovascular disorders and the metabolic syndrome (Helal *et al.*, 2010; Leroyer *et al.*, 2010). MP implication in psoriasis pathophysiology is suggested by several features: (i) the pathogenic role of TNF-α (Nestle *et al.*, 2009), a powerful *in vitro* inducer of EMP generation (Combes *et al.*, 1999); (ii) the presence of activated endothelial cells within cutaneous lesions (Nestle *et al.*, 2009) and EMP generation that may reflect endothelium aggression; and (iii) the presence of platelet activation in psoriasis (Garbaraviciene *et al.*, 2010; Tamagawa-Mineoka *et al.*, 2010) that may generate PMPs (Italiano *et al.*, 2010). Moreover, the association between psoriasis and atherosclerotic risk factors (Boehncke *et al.*, 2010) may be related to excessive MP production. Based on this, we investigated circulating MPs in psoriatic patients.

The study design was approved by the local research ethics committee and written informed consent was provided before enrolment. The study adhered to the Declaration of Helsinki Principles. Fifty-two psoriatic patients analyzed before the introduction of systemic treatments and 30 healthy blood donors were investigated (Table 1). Patients with other dermatosis, with a history of cardiovascular disease, diabetes, chronic renal failure, or chronic inflammatory disease, were excluded. The severity of psoriasis was evaluated by the Psoriasis Area Severity Index (PASI) and the Dermatology Life Quality Index (DLQI) scores. We adapted the MP quantification method developed by Robert *et al.* (2009). Plasma was separated from whole blood by centrifugation at 1500 *g* for 15 minutes. Recovered plasma was centrifuged for 2 min at 13,000 *g* to remove residual cells and platelets. Microparticles were labeled using FITC-conjugated Annexin-V (AV) (BD Biosciences, Le Pont de Claix, France) and fluorescent mAbs were added to identify the MP cellular origin. The following mAbs were used: phycoerythrin (PE)-conjugated CD31 and PE-Texas Red-x (ECD)-conjugated CD41 (Beckman Coulter, Villepinte, France), PE-conjugated CD62E (BD Biosciences), and fluorescent-conjugated isotype control mAbs. After 30-minute incubation and addition of Flow-Set™ fluorosphere beads (Beckmann Coulter), samples were analyzed using a NAVIOS cytometer (Beckman Coulter). MP number was calculated on the basis of the known number of Flow-Set™ beads added to the sample. EMPs were identified as $CD31^+/CD41^-/AV^{+/-}$ or $CD62E^+/CD41^-/AV^{+/-}$ events, and PMPs as $CD31^+/CD41^+/AV^{+/-}$ events (Figure 1a–e, Supplementary Table S1 online). Two MP-size regions were determined and standardized daily with Megamix fluorescent beads (Biocytex, Marseille, France) containing two types of beads with a defined size (0.5 and 0.9 μm diameter, respectively). Two nonparametric statistical tests (Wilcoxon and Kruskal–Wallis tests) were used to compare the means of circulating MP counts.

Circulating EMP levels were increased in psoriatic patients (Table 1, Figure 1f and g). Circulating $CD31^+41^-$ EMPs were statistically higher compared with EMP levels in the control group ($80\,\mu l^{-1}$, ±121 vs. $12\,\mu l^{-1}$, ±10; $P=0.0002$). A trend toward significance was observed for $CD62E^+$ EMPs ($5\,\mu l^{-1}$, ±5 in patients vs. $3\,\mu l^{-1}$, ±2 in healthy subjects; $P=0.09$). When focusing on the smaller-size EMPs, we found a significantly higher number of both $CD31^+/CD41^-$ EMPs ($54\,\mu l^{-1}$, ±82 vs. $8\,\mu l^{-1}$, ±7; $P<0.0001$) and $CD62E^+$ EMPs ($4\,\mu l^{-1}$, ±4 vs. $2\,\mu l^{-1}$, ±2; $P=0.006$) in psoriatic patients. When considering larger-size EMPs (≥0.5 μm), we detected a significantly higher number of $CD31^+/CD41^-$ EMPs ($30\,\mu l^{-1}$, ±52) in patients than in the control group ($3\,\mu l^{-1}$, ±4; $P=0.0035$). No significant difference was observed for $CD62E^+$ EMPs. No correlation with psoriasis severity (assessed by PASI and DLQI scores) was observed. No difference was found in circulating $CD31^+/CD41^+$ PMP levels between psoriatic patients ($5{,}621\,\mu l^{-1}$, ±7,030) and healthy controls ($4{,}515\,\mu l^{-1}$, ±3,787; $P=0.35$). However, smaller-size PMP levels were significantly higher in psoriatic patients than in healthy patients ($3{,}528\,\mu l^{-1}$, ±5,122 vs. $1{,}508\,\mu l^{-1}$,

Abbreviations: AV, Annexin-V; DLQI, Dermatology Life Quality Index; EMP, endothelial-derived microparticle; MP, microparticle; PASI, Psoriasis Area Severity Index; PMP, platelet-derived microparticle; TNF-α, tumor necrosis factor-alpha

Table 1. Main clinical characteristics of subjects and plasma levels of EMPs, PMPs, and AV$^+$ MPs in patients with psoriasis and in healthy control subjects

	Control, *n*=30	Psoriasis, *n*=52	*P*
Age (years)[a]	50 (18–65)	51.8 (25–96)	NS
Sex (male/female)	15/15	29/23	NS
C-reactive protein (mg l^{-1})[a]	1.0 (1–1)	5.46 (1–11)	ND
PASI score[a]	ND	18 (14–25)	ND
DLQI[a]	ND	12.5 (0–26)	ND
EMPs[b]			
Total CD31$^+$/CD41$^-$ EMPs	12 ± 10 (2–44)[b]	80 ± 121 (3–543)	**0.0002**
0.5–0.9 μm CD31$^+$/CD41$^+$ EMPs	3 ± 4 (0–15)	26 ± 52 (0–222)	**0.0035**
<0.5 μm CD31$^+$/CD41$^-$ EMPs	8 ± 7 (0–29)	54 ± 82 (0–433)	**<0.0001**
Total CD62E$^+$/CD41$^-$ EMPs	3 ± 2 (0–12)	5 ± 5 (0–24)	0.09
0.5–0.9 μm CD62E$^+$/CD41$^-$ EMPs	1 ± 1 (0–6)	2 ± 3 (0–16)	0.21
<0.5 μm CD62E$^+$/CD41 EMPs	2 ± 2 (0–5)	4 ± 4 (0–13)	**0.006**
PMPs[b] *(CD31$^+$/CD41$^+$)*	4,515 ± 3,787 (829–16279)[b]	5,621 ± 7,030 (340–31,054)	0.35
0.5–0.9 μm PMPs	3,006 ± 2,921 (230–11332)	2,092 ± 2,331 (86–10,049)	0.12
<0.5 μm PMPs	1,508 ± 964 (321–4947)	3,528 ± 5,122 (254–22,291)	**0.007**
AV$^+$ MPs[b]	3,052 ± 2,850 (422–10568)[b]	3,177 ± 2,568 (129–11,537)	0.83
0.5–0.9 μm AV$^+$ MPs	2,020 ± 1,807 (153–6435)	1,428 ± 1,391 (43–7,280)	0.10
<0.5 μm AV$^+$ MPs	1,157 ± 860 (240–4144)	1,623 ± 1,631 (87–6,667)	0.09

Abbreviations: AV, Annexin-V; DLQI, Dermatology Life Quality Index; EMPs, endothelial-derived microparticles; MPs, microparticles; ND, not determined; NS, nonsignificant; PASI, Psoriasis Area Severity Index; PMPs, platelet-derived microparticles.
[a]Results are expressed as median (range).
[b]Results are expressed as the mean number ± standard deviation of microparticles per plasma μl (range). Microparticle size (that is, between 0.5 and 0.9 μm or <0.5 μm) is also considered.
Bold values are statistically significant.

± 964, $P=0.007$). We did not observe any correlation with psoriasis severity. Total circulating AV$^+$MP counts (whatever their origin) in psoriatic patients (3,052 μl^{-1}, ± 2,852) did not significantly differ from those quantified in the control group (3,177 μl^{-1}, ± 2,568, $P=0.83$). There was no difference depending on the size of AV$^+$MPs (Table 1).

Our study demonstrates that circulating EMPs and small-size PMPs are significantly increased in psoriatic patients as compared with healthy subjects. Tamagawa-Mineoka *et al.* (2010) previously showed that plasma PMP levels were significantly increased in 21 psoriasis patients. The discrepancy with our results may be related to the methods used for PMP quantification: ELISA versus flow cytometry. Cytometry permits to determine MP size (Orozco and Lewis, 2010). Small-size PMPs –identified here– may reflect platelet activation. Several evidences suggest that the majority of PMPs circulating in healthy subjects are derived from megakaryocytes, while PMPs derived from activated platelets have been separated into four size classes, including small-size PMPs (Italiano *et al.*, 2010), with different functional effects on platelets and endothelial cells (Dean *et al.*, 2009). Soluble P-selectin level, a platelet-activation hallmark, is a biomarker for inflammation in psoriasis (Garbaraviciene *et al.*, 2010). Increased EMP levels could be explained by endothelial cell activation by TNF-α, since TNF-α favors *in vitro* EMP generation (Combes *et al.*, 1999). We showed that EMPs induce plasmacytoid dendritic cell (pDC) maturation (Angelot *et al.*, 2009). As pDCs are essential to drive psoriasis development (Nestle *et al.*, 2005), EMPs could represent an activating factor for pDCs and thus contribute to inflammation. Systemic inflammation in turn causes insulin resistance in psoriasis, a state in which insulin is proatherogenic (Boehncke *et al.*, 2007). As both small-size PMPs (Dean *et al.*, 2009) and EMPs (Leroyer *et al.*, 2010) express procoagulant phosphatidylserine and tissue factor activities, increased small-size PMP and EMP levels may

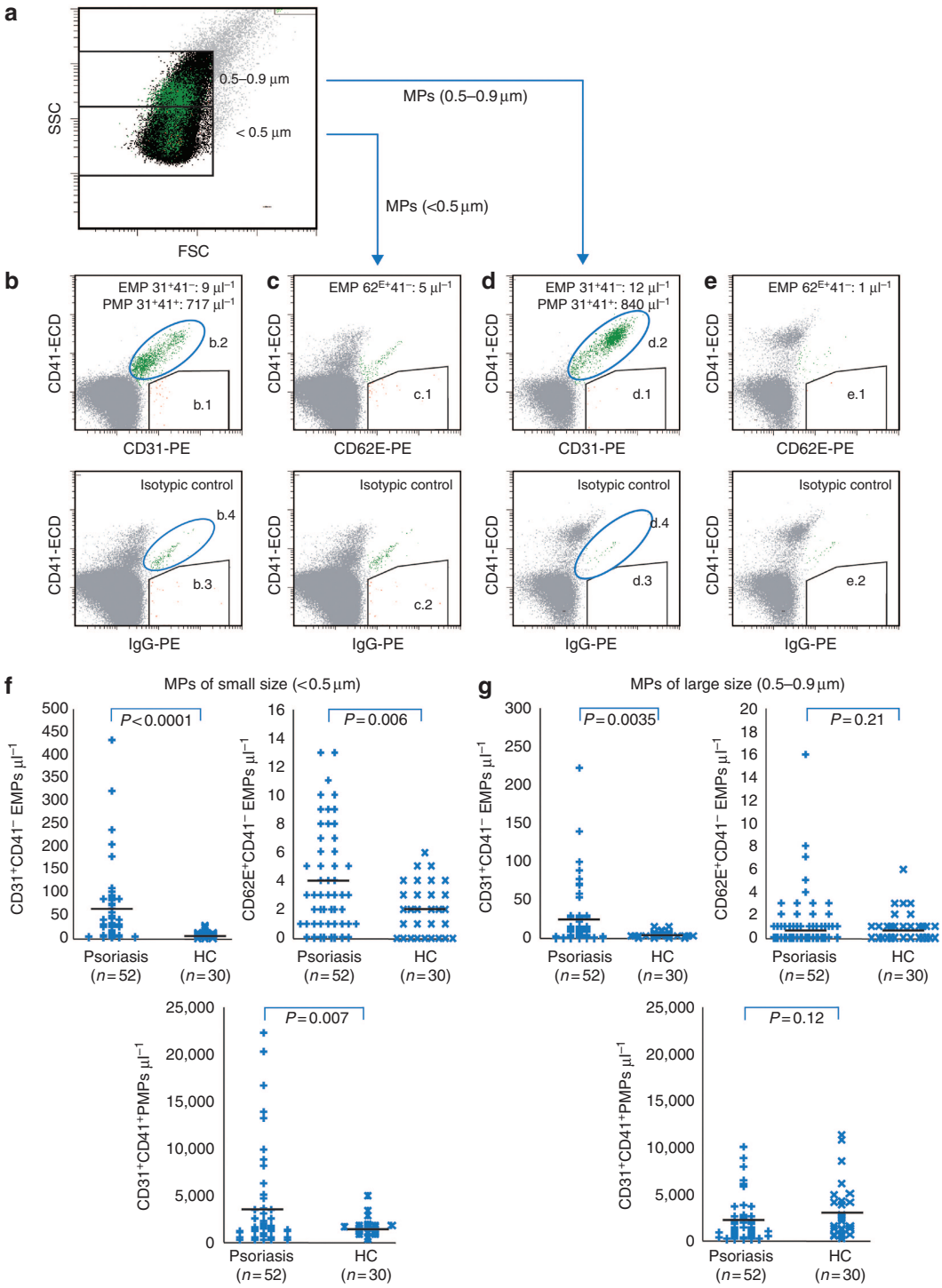

Figure 1. Analysis of circulating microparticles using a NAVIOS cytometer. (**a**) Standardized microparticle (MP) detection areas (0.5–0.9 and <0.5 µm) are represented in the side scatter/forward scatter dot plot. Platelet-derived microparticles (PMPs; 31^+ 41^+) are depicted in green, the endothelial-derived microparticles (EMPs; 31^+41^- or $62E^+41^-$) in red. All histograms represent the analysis of a sample from the same psoriatic patient. (**b, c**) Analysis of 31^+41^+, $41^+/62E^+$, or $IgG^+/41^+$ events in the MP size area <0.5 µm: 31^+41^- EMPs, PMPs, and $62E^+41^-$ EMPs are quantified in b.1, b.2, and c.1 areas, respectively. (**c.2, b.3–4**): IgG isotypic control. (**d, e**) Analysis in the 0.5–0.9-µm size area. (**f**) The small-size EMP and PMP levels in psoriatic patients compared with healthy subjects (HCs). (**g**) Idem for EMPs and PMPs quantified in the 0.5–0.9-µm size region.

contribute to accelerated atherosclerosis in psoriatic patients. However, the clinical relevance of our results remains still disputed: does EMP generation represent an epiphenomenon related to endothelium activation by TNF-α or do EMPs have a role in psoriasis pathophysiology –through pDC activation– leading to accelerated atherosclerosis?

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

The authors would like to thank Antoine Pachéco (Beckman Coulter), Elisabeth Homassel, and Stéphane Robert for technical support and manuscript editing. They are also grateful to Françoise Dignat-George for helpful discussion. This work was supported by the Fondation Transplantation (grants ET-051222, ET-060934, and ET-070636), the ''Société de Recherche Dermatologique (2008)'', the University of Franche-Comté (2008), and the University Hospital of Besançon (APICHU 2008).

***Fabien Pelletier*[1,2,3,4], *Francine Garnache-Ottou*[1,3,4], *Fanny Angelot*[1,3,4], *Sabeha Biichlé*[1,3,4], *Chrystelle Vidal*[5], *Philippe Humbert*[1,2,4], *Philippe Saas*[1,3,4,5], *Estelle Seillès*[1,3,4,7] and *François Aubin*[2,6,7]**

[1]*Université de Franche-Comté, UMR645, IFR133, Besançon Cedex, France;* [2]*Service de Dermatologie, CHU de Besançon, Besançon, France;* [3]*Établissement Français du Sang Bourgogne Franche-Comté, UMR645, Plateforme de Biomonitoring, Besançon, France;* [4]*INSERM, UMR645, Besançon Cedex, France;* [5]*Centre d'Investigation Clinique CBT506, CHU de Besançon, Besançon, France and* [6]*Université de Franche Comté, EA3181, IFR133, Besançon, France*
E-mail: fabien.pelletier@univ-fcomte.fr
[7]*These authors contributed equally to this work.*

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Antipruritic Effects of TRPV1 Antagonist in Murine Atopic Dermatitis and Itching Models

Journal of Investigative Dermatology (2011) **131,** 1576–1579; doi:10.1038/jid.2011.87; published online 7 April 2011

TO THE EDITOR

Transient receptor potential vanilloid type 1 (TRPV1) is a non-selective cation channel widely expressed in skin tissues, including keratinocytes and peripheral sensory nerve fibers (C and Aδ). Activated by noxious heat, capsaicin, or endogenous inflammatory mediators, TRPV1 can provoke neuropeptide releases and propagate neurogenic inflammation, which ultimately contributes to the development of diverse dermatoses and pruritus (Hutter *et al.*, 2005; Shim *et al.*, 2007; Imamachi *et al.*, 2009). Recently, we demonstrated that a novel and potent TRPV1 antagonist, PAC-14028 ((E)-N-((R)-1-(3,5-difluoro-4-methanesulfonylamino-phenyl)-ethyl)-3-(2-propyl-6-trifluoromethyl-pyridine-3-yl)-acrylamide) can alleviate atopic dermatitis (AD)-like symptoms through the acceleration of skin barrier recovery (Yun *et al.*, 2011). Of note, we discovered that PAC-14028 could also suppress scratching behavior significantly. Severe itch symptom is a hallmark of AD and at the same time, the representative unmet medical need in diverse skin diseases (Steinhoff *et al.*, 2006). Here, we investigated the antipruritic effects of PAC-14028 and explored the mechanism underlying them to examine the utility of a TRPV1 antagonist as a novel antipruritic therapy.

AD-like symptoms were induced in male NC/Nga mice (8-week old, twice a week for 3 weeks) with the repeated topical application of allergen, *Dermatophagoides farina* (Df) extract, the major species of house dust mites, on the shaved dorsum as previously described (Bae *et al.*, 2010). All animal experiment procedures were approved by the AmorePacific Institutional Animal Care and Use Committee. PAC-14028, which is a potent and selective TRPV1 antagonist as determined by resiniferatoxin-induced Ca^{2+} influx assay in rat TRPV1-expressed CHO cells (Figure 1a) and capsaicin-induced Ca^{2+} influx in dorsal root ganglia