

RAPID REPORT

Rapid changes in shear stress induce dissociation of a $G\alpha_{q/11}$ -platelet endothelial cell adhesion molecule-1 complex

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It has been recently shown that endothelial platelet endothelial cell adhesion molecule-1 (PECAM-1) expression is pro-atherogenic. PECAM-1 is involved in sensing rapid changes in fluid shear stress but the mechanisms for activating signalling complexes at the endothelial cell junction have yet to be elucidated. Additional studies suggest the activation of membrane-bound G proteins $G\alpha_{q/11}$ also mediate flow-induced responses. Here, we investigated whether PECAM-1 and $G\alpha_{q/11}$ could act in unison to rapidly respond to fluid shear stress. With immunohistochemistry, we observed a co-localization of $G\alpha_{q/11}$ and PECAM-1 at the cell–cell junction in the atheroprotected section of mouse aortae. In contrast, $G\alpha_{q/11}$ was absent from junctions in atheroprone areas as well as in all arterial sections of PECAM-1 knockout mice. In primary human endothelial cells, temporal gradients in shear stress led to a rapid dissociation of the $G\alpha_{q/11}$ –PECAM-1 complex within 30 s and a partial relocalization of the $G\alpha_{q/11}$ staining to perinuclear areas within 150 min, whereas transitioning fluid flow devoid of temporal gradients did not disrupt the complex. Inhibition of G protein activation eliminated temporal gradient flow-induced $G\alpha_{q/11}$ –PECAM-1 dissociation. These results allow us to conclude that $G\alpha_{q/11}$ –PECAM-1 forms a mechanosensitive complex and its localization suggests the $G\alpha_{q/11}$ –PECAM-1 complex is a critical mediator of vascular diseases.

(Received 18 March 2009; accepted after revision 27 March 2009; first published online 30 March 2009)

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Abbreviations FSS, fluid shear stress; HUVEC, human umbilical vein endothelial cell; KO, knockout; NO, nitric oxide; PECAM-1, platelet endothelial cell adhesion molecule-1; VEGF-R2, vascular endothelial growth factor-receptor 2; TBST, Tris-buffered saline with Tween 20; WT, wild-type.

Detailed analyses of blood flow in atherosclerosis-susceptible vascular regions suggest that the balance of temporal gradients and mean shear stress determines the atherogenicity of haemodynamic signal (Zarins *et al.* 1983). This pattern of plaque formation, independent of other associated risk factors, has led a number of investigators to suggest that high steady fluid flow within the vasculature is atheroprotective, whereas unsteady flow with a low mean is pro-atherogenic (White *et al.* 2007).

Given the pulsatile nature of the cardiac cycle, flow patterns are comprised of two distinct and superimposed components: steady and dynamic (temporal). Temporal gradients in shear stress are defined as a rapid increase or decrease of shear stress over a small period of time (<0.3 s) at the same location. For steady shear stress, although levels

of shear vary throughout the cardiac cycle, time-averaged fluctuations lack rapid changes in absolute levels of shear stress.

The molecular basis of shear-induced mechanochemical signal transduction and the endothelium's ability to discriminate between flow profiles remain largely unclear. Given that fluid shear stress (FSS) does not involve a traditional receptor–ligand interaction, identification of the molecule(s) responsible for sensing fluid flow and mechanical force discrimination has been difficult (Dai *et al.* 2004). In addition to the glycocalyx (Tarbell & Ebong, 2008), it has been proposed that the lipid bilayer itself may act as a flow sensing receptor (Gudi *et al.* 1998). Fluid shear stress is a frictional force that acts on the apical surface of the endothelial monolayer. In an endothelial monolayer,

the region of greatest tension has been shown to be at the cell–cell junction (Fung & Liu, 1993) where temporal gradients induce membrane deformation and subsequent signalling (White *et al.* 2001; Dusserre *et al.* 2004).

A number of membrane-associated proteins are specifically localized to the cell–cell junction. Platelet endothelial cell adhesion molecule-1 (PECAM-1) is the cell–cell adhesion molecule most abundantly expressed in endothelial cells. In endothelial cells forming a confluent monolayer, it is localized along the cell–cell border (Albelda *et al.* 1990). When endothelial cells are exposed to physiological levels of fluid shear stress, PECAM-1 has been shown to be tyrosine phosphorylated (Osawa *et al.* 2002). Given the subcellular localization of PECAM-1 to regions of high mechanical tension, shear-induced PECAM-1 signalling may result from force-induced deformational changes in the molecule (Osawa *et al.* 2002), suggesting a role in sensing atheroprone haemodynamic flow. This is supported by the recent demonstration that PECAM-1 is a critical mediator of atherosclerosis (Goel *et al.* 2008; Harry *et al.* 2008; Stevens *et al.* 2008). There is also increasing evidence that PECAM-1 serves as a scaffold for other mechanochemical signalling. Nitric oxidase synthase III (eNOS) is localized at the endothelial cell junction and complexes with PECAM-1 (Dusserre *et al.* 2004). The eNOS–PECAM-1 complex is rapidly disrupted by atherogenic temporal gradients in FSS but not by steady atheroprotective FSS. A mechanosensitive complex involving PECAM-1, the vascular endothelial growth factor-receptor 2 (VEGF-R2) and the vascular endothelial (VE) cadherin has also been shown (Tzima *et al.* 2005). Here we describe a mechanosensitive complex involving the G protein $G\alpha_{q/11}$ with PECAM-1.

The rapid activation of heterotrimeric G proteins is known as one of the earliest flow-mediated responses in endothelial cells (Gudi *et al.* 1996, 2003), and may also play a role in the ability of the endothelium to discriminate between flow profiles. The sudden temporal onset of flow induces a burst of nitric oxide (NO) production (Frangos *et al.* 1996). This process is calcium-, G-protein- and PECAM-1-dependent (Dusserre *et al.* 2004; Bagi *et al.* 2005). In contrast, the prolonged steady shear stress that follows induces a sustained release of NO, and is calcium-, G-protein- and PECAM-1-independent (Kuchan *et al.* 1994; Dusserre *et al.* 2004; Bagi *et al.* 2005). This suggests that shear-induced stimulation of endothelial cells is derived from the superposition of two independent mechanical stimuli (steady shear and temporal changes in shear), which are in turn transduced by two different mechanochemical pathways. Furthermore, membrane-linked force transduction appears to be mediated, at least in part, by the $G\alpha_{q/11}$ heterotrimeric G protein (Gudi *et al.* 2003).

In the present study, we hypothesized a combined role of $G\alpha_{q/11}$ and PECAM-1 in mechanochemical transduction in endothelial cells. We observed that (1) both

proteins are co-localized at the cell–cell junctions and (2) signalling events of $G\alpha_{q/11}$ activation are associated with PECAM-1. This study is the first to suggest a role of the $G\alpha_{q/11}$ –PECAM-1 complex in the mechano-transduction response to fluid shear stress. We believe that this study provides additional understanding of how the endothelium senses haemodynamic forces in both normal physiology and vascular disease.

Methods

Cell culture and shear stress

Primary human umbilical vein endothelial cell (HUVEC) isolation was performed as previously described (Frangos *et al.* 1988). Human umbilical cords were obtained from Sharp Memorial Hospital (San Diego, CA, USA) under the auspices of Sharp Healthcare Institutional Review Board protocol no. 011081. Cells were seeded onto glass microscope slides and grown to confluence within 4 days in M199 media (Irvine Scientific). Prior to all experimental procedures, the HUVECs were serum-starved overnight in ATP-free M199 with 2% BSA to establish quiescence in the monolayer. Similar culture media was used in a conventional parallel-plate flow chamber where confluent HUVEC monolayers were subjected to varying periods of fluid shear stress via a computer-controlled syringe pump: 1 s square-wave (impulse) at 14 dynes cm^{-2} , a continuous series of impulses (pulsatile) at a frequency of 1 Hz, or smoothly ramped transient FSS consisting of a 15 s increase in slowly transitioning FSS from 0–14 dynes cm^{-2} , maintained for 1 s, and a 14–0 dynes cm^{-2} decrease over 14 s (White *et al.* 2001). For longer periods of FSS, HUVEC slides were mounted on similar flow chambers connected to a flow loop providing a sustained 19 dynes cm^{-2} shear stress, maintained at 37°C and ventilated with 95% humidified air with 5% CO_2 (Frangos *et al.* 1988). An oscillatory flow was generated by the addition of an oscillator to create a mean FSS of ± 19 dynes cm^{-2} at a frequency of 1 Hz. A minimal forward flow was provided every hour to deliver nutrients and remove waste products from cells. Slides were quickly transferred to ice-cold PBS (6.7 mM KH_2PO_4 , 150 mM NaCl, pH 7.4) with 2 mM sodium orthovanadate for 1 min and harvested for lysate preparation (see below). Time-matched static or sham (slides mounted on chamber without flow) were performed for all experimental procedures. For G protein inhibition studies, HUVECs were incubated with 900 μM GDP βS (Calbiochem) 4 h prior to shear stress stimulation.

En face and immunohistochemistry microscopy studies

All mouse procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of LJBI

(Assurance number A3432) which is authorized by the Office of Laboratory Animal Welfare of the US National Institutes of Health, as required by the Health Resource Extension Act of 1985. PECAM-1 knockout mice (B6; 129S-PECAM^{tm1Lex}) and wild-type mice (B6;129SF2/J) were purchased from Jackson Labs. Mice were anaesthetized with 90 mg kg⁻¹ ketamine and 10 mg kg⁻¹ xylazine, i.p., and then perfused with ice-cold Stefanini buffer (2% paraformaldehyde and 15% picric acid in 0.1 M phosphate buffer, pH 7.6) for 10 min. Aortas were carefully dissected, branches (renal artery bifurcation) and blood flow were recorded, and cross-sectional segments were cut and opened longitudinally, then permeabilized with 0.1% Triton X-100 for 10 min. For double labelling, aortae were successively incubated with: (1) 5% donkey serum to block for non-specific staining; (2) goat anti-PECAM-1 antibody (Santa Cruz Biotechnology, 1/50) and rabbit anti- $G\alpha_{q/11}$ antibody (SC, 1/25); (3) donkey anti-goat IgG (Alexa Fluor 488, Molecular Probes) or donkey anti-rabbit (Alexa Fluor 568, Molecular Probes) and nuclei were stained with DAPI (Molecular Probes). Vessels were mounted in Gelvatol (Polyvinyl Alcohol) mounting media, internal side up. All samples were observed under a DeltaVision deconvolution microscope system (Applied Precision Inc.) at the UCSD Cancer Center Digital Imaging Shared Resource.

For *in vitro* studies, HUVECs were fixed in ice-cold methanol/acetone for 10 min and processed as described above with a monoclonal anti-PECAM-1 antibody (R&D systems, 1/1000) and a rabbit anti- $G\alpha_{q/11}$ antibody (Santa Cruz Biotechnology, 1/100). Slides were then examined under a confocal fluorescent microscope (Zeiss Pascal LSM5, Carl Zeiss, Germany) equipped with a Plan-Apochromatic 63/1.4 objective.

Preparation of cellular lysates

HUVECs were harvested in octyl glucoside (OG) lysis buffer (60 mmol l⁻¹ OG, 50 mM Tris-HCl pH 7.5, 50 μ mol l⁻¹ EGTA, 125 mmol l⁻¹ NaCl, 2 mmol l⁻¹ dithiothreitol, 2 mmol l⁻¹ sodium orthovanadate and protease inhibitors) and incubated on ice for 30 min. Lysates were centrifuged (14 000 g, 20 min, 4°C) and the detergent-soluble supernatant fractions retained.

Coimmunoprecipitation

Aliquots of cell lysates were pre-cleared for 45 min at 4°C with 20 μ l of rabbit anti-goat beads (Sigma). Supernatants were incubated overnight with goat anti-PECAM-1 (Santa Cruz Biotechnology, clone M-20) at a final concentration of 8 μ g ml⁻¹. Rabbit anti-goat beads were then added and mixed for 1.5 h at 4°C. Bound immune complexes were washed 4 times with lysis buffer. To decrease non-specific

binding, bound proteins were released from the beads with an acid elution buffer (0.1 M glycine, pH 2.5, 60 mmol l⁻¹ OG) and immediately neutralized with 1 : 10 volume of 1 M Tris buffer, pH 8.0.

Immunoblot

Denatured immunoprecipitated proteins or crude lysates were separated on NuPAGE 4–12% Bis-Tris gels and transferred to PVDF membrane (Millipore). Membranes were blocked at 1 h with 5% non-fat milk in Tris-buffered saline with 0.1% (v/v) Tween 20 (TBST) and then incubated with primary antibodies (from Santa Cruz Biotechnology) against $G\alpha_{q/11}$ (1 : 10 000), PECAM-1 (1 : 5000) for 1 h in 5% milk-TBST. Bound primary antibodies were detected by horseradish peroxidase-conjugated secondary antibodies: anti-rabbit IgG True Blot (eBioscience, 1 : 5000) and anti-goat IgG True Blot (eBioscience, 1 : 5000), respectively. Band intensity was quantified on unsaturated X-ray film by a digital image analyser (Bio-Rad Laboratories). All comparisons were made relative to static or sham controls.

Image analysis and statistics

$G\alpha_{q/11}$ perinuclear staining was quantified in *en face* preparations using Image J software (NIH, USA) and each cell showing a full distinct ring of $G\alpha_{q/11}$ staining around the nucleus was counted as a positive cell and a ratio was done over the total number of cells observed. A similar quantification was performed in HUVECs cultured *in vitro* and for each experiment 15 fields were counted with an average of 10 cells per field. The quantification of the $G\alpha_{q/11}$ and PECAM-1 co-staining was performed by measuring the intensity of the co-staining (ranges of yellow).

All experimental values are given as mean and standard deviation of the mean. All reported values of *n* refer to the number of separate and independent experiments from multiple primary HUVEC cultures. Non-parametric statistical comparisons between groups were performed with significance level of *P* < 0.05.

Results

$G\alpha_{q/11}$ localization in endothelial cells from mice aorta

To determine whether $G\alpha_{q/11}$ and PECAM-1 co-localize within endothelial cells *in vivo*, a deconvolution microscopy, double-label immunofluorescence study was performed in freshly isolated aortae from wild-type (WT) and PECAM-1 knockout (KO) mice. In wild-type mice, PECAM-1 was localized along the cell periphery in branch-free areas of the descending aorta (straight section, Fig. 1Aa), described as atheroprotective areas with

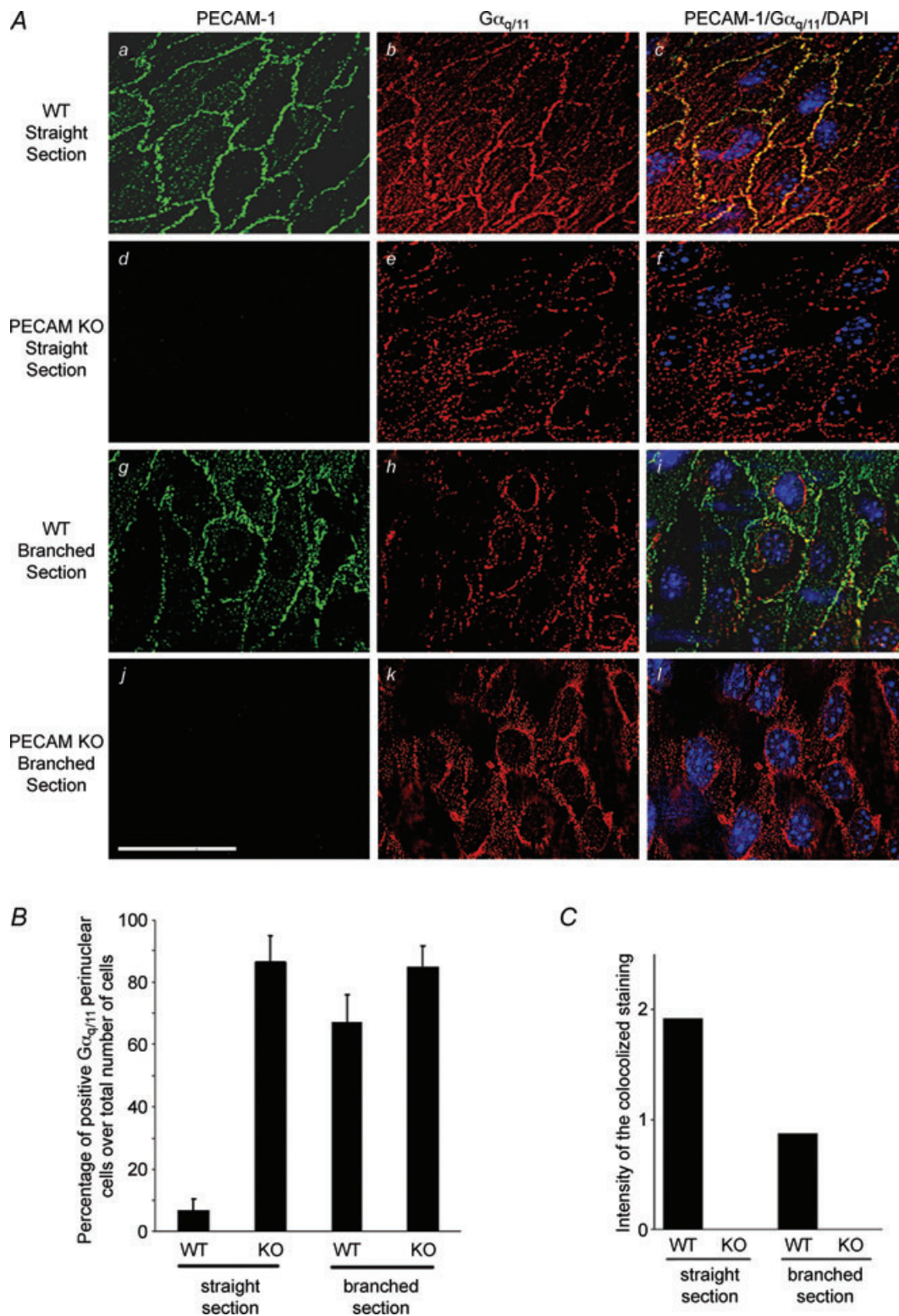


Figure 1. *En face* deconvolution microscopy of PECAM-1 and $G\alpha_{q/11}$ staining in PECAM-1 knockout (KO) mouse aorta preparations

A, staining was performed on the descending aorta (straight section) of wild-type (WT) mouse (a–c), PECAM-1 KO mouse (d–f) and branch points of the aorta (renal artery bifurcation) of WT (g–i) and KO mouse (j–l). Double staining was performed to localize PECAM-1 (green) and $G\alpha_{q/11}$ (red). Superposition of PECAM-1 and $G\alpha_{q/11}$

unidirectional flow and small temporal gradients of fluid shear stress (Nakashima *et al.* 1998). Similarly, staining with anti- $G\alpha_{q/11}$ also reveals strong $G\alpha_{q/11}$ staining along the cell periphery and a predominant co-localization of the two proteins was observed at the cell–cell junction (Fig. 1*Ab* and *c*). No labelling of PECAM-1 was detected in KO mice and $G\alpha_{q/11}$ was absent from the cell–cell junction and instead localized perinuclearly (Fig. 1*Ae*). Similarly, in WT mouse aortae, junctional $G\alpha_{q/11}$ staining was absent at the renal artery bifurcation, which is an atheroprone area exposed to large temporal gradients (Fig. 1*Ah*). Quantitative studies showed that the percentage of cells with $G\alpha_{q/11}$ perinuclear staining was significantly higher in KO mice ($86.4 \pm 8.5\%$ (S.E.M.), $n = 3$; Fig. 1*B*) and renal artery bifurcation (WT: $67.2 \pm 8.8\%$, $n = 3$; KO: $84.7 \pm 6.9\%$) than WT descending aorta ($6.5 \pm 3.9\%$, $n = 3$). Even though some $G\alpha_{q/11}$ staining was still localized to the cell–cell junction, co-localization of $G\alpha_{q/11}$ and PECAM-1 in the WT was reduced by half at the renal artery bifurcation as compared to the descending aortae (Fig. 1*C*).

Oscillatory flow induces $G\alpha_{q/11}$ perinuclear localization *in vitro*

To determine whether shear stress has a role in mediating $G\alpha_{q/11}$ translocation *in vitro*, confluent serum-starved HUVECs were subjected to temporal gradients in FSS for 150 min, a sufficient period of time to allow $G\alpha_{q/11}$ translocation (Drmotá *et al.* 1998). The immuno-co-localization of $G\alpha_{q/11}$ and PECAM-1 at the cell–cell junction was seen in all conditions and no significant decrease in $G\alpha_{q/11}$ junctional staining was detected after 2 h of stimulation. However, under oscillatory flow or slowly ramped FSS of similar value, distinct rings of $G\alpha_{q/11}$ staining were detected perinuclearly (arrow, Fig. 2*A*). Quantification of the $G\alpha_{q/11}$ staining shows a significant elevation of cells positive for $G\alpha_{q/11}$ perinuclear staining under oscillatory condition *versus* ramp or static condition (Fig. 2*B*, $P < 0.01$, in all cases).

A single impulse but not ramped-transient flow promotes time-dependent dissociation of $G\alpha_{q/11}$ -PECAM-1 complex

Co-immunoprecipitation of PECAM-1 and $G\alpha_{q/11}$ in HUVEC *in vitro* demonstrates that they form a complex

(sham, Fig. 3*A*). In order to dissect the kinetics of shear-induced dissociation of the $G\alpha_{q/11}$ -PECAM-1 complex, cells were subjected to a single impulse flow. A single impulse of flow represents a single cycle of pulsatile flow and has previously been shown to stimulate endothelial responses such as BrdU uptake and eNOS-PECAM-1 dissociation as longer term pulsatile flow (White *et al.* 2001; Dusserre *et al.* 2004). A rapid disruption of the $G\alpha_{q/11}$ -PECAM-1 complex was observed 15 s after exposure to a 1 s impulse flow and was maximal 30 s after impulse ($42 \pm 7\%$ of the control, Fig. 3*B*). The complex rapidly re-associated after 30 s and the amount of $G\alpha_{q/11}$ immunoprecipitated with PECAM-1 subsequently returned to the control (sham) level. Interestingly, $G\alpha_{q/11}$ -PECAM-1 dissociation was sustained when cells were exposed for 10 min pulsatile flow while a smoothly transitioning ramped flow devoid of temporal gradients did not induce dissociation (Fig. 4*A*). Similarly, ramped transient flow did not induce the dissociation of the $G\alpha_{q/11}$ -PECAM-1 complex at 30 s as compared to a single impulse ($\dagger P < 0.05$, Fig. 4*B*, filled bars). As similar levels of FSS were achieved in ramped flow, it is clear that FSS itself did not induce complex dissociation. Rather, the dynamic component (temporal gradient in shear stress) appears responsible for the dissociation, making the *in vitro* findings consistent with the *in vivo* observations.

Shear-induced $G\alpha_{q/11}$ -PECAM-1 complex dissociation is dependent on G protein activation

To determine whether G protein activation is required to mediate the dissociation of the $G\alpha_{q/11}$ -PECAM-1 complex, cells were treated with GDP β S, an inhibitor of G protein activation. GDP β S abolished impulse flow-induced $G\alpha_{q/11}$ -PECAM-1 dissociation (Fig. 4*B*, open bars). Complex dissociation, therefore, is dependent on G protein activation. GDP β S treatment had no significant effect on the association/dissociation of the complex in cells subjected to ramped flow or sham controls ($87 \pm 15\%$ of the non-treated sham control; n.s., $n = 3$).

Discussion

Vascular mechanotransduction has long been recognized as mediating the localization of lesions in atherosclerosis.

staining demonstrates co-localization in yellow–orange (right column). Cell nuclei were labelled using DAPI (blue). Scale bar is 20 μ m. *B*, in WT descending aorta only a few cells expressed $G\alpha_{q/11}$ staining in perinuclear areas. The number of cells with $G\alpha_{q/11}$ perinuclear staining was significantly increased in PECAM-1-deficient mice and in branched regions of WT mice ($n = 3$ animals for each condition). *C*, the quantification of the $G\alpha_{q/11}$ and PECAM-1 co-staining intensity (ranges of yellow) was reduced to half in branched section compared to straight section of the aorta. KO mice did not express PECAM-1.

There is a consensus that haemodynamic forces represent two distinct biomechanical stimuli: unidirectional fluid shear which is atheroprotective, while rapidly changing and/or reverse flow which is atherogenic (White *et al.* 2001). The ability of the endothelium to discriminate between these flow components strongly suggests the

existence of distinct molecular mechanisms of mechanochemical signal transduction (Frangos *et al.* 1996). The molecular components distinguishing these transduction pathways, however, remain largely unclear. Both PECAM-1 and G proteins have independently been proposed as mechanosensors (Masuda *et al.* 1997).

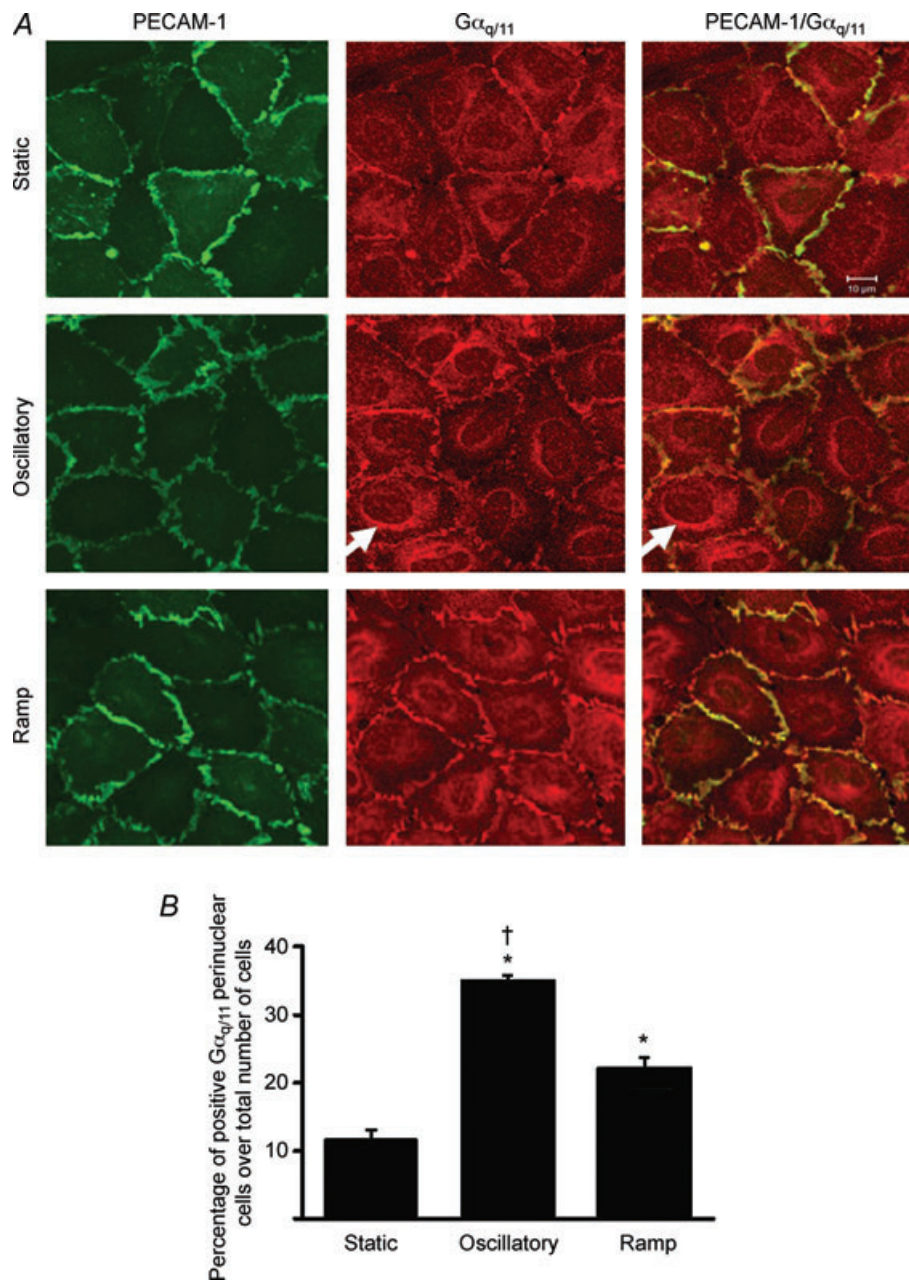


Figure 2. Perinuclear expression of G $\alpha_{q/11}$ under shear stress *in vitro*

A, coimmunostaining for G $\alpha_{q/11}$ (red) and PECAM-1 (green) of serum-starved HUVECs after exposure for 150 min of no flow (Static, upper lane), oscillatory flow (± 19 dynes cm^{-2} , 1 Hz, middle lane) or ramped-transient flow (2 min slow ramping to 19 dynes cm^{-2} , maintained for 146 min, then 2 min slow ramping down to 0 dynes cm^{-2} , lower lane). Arrow indicates positive cells with a distinct ring of G $\alpha_{q/11}$ perinuclear staining. *B*, the number of cells with G $\alpha_{q/11}$ perinuclear staining increases under flow conditions. Values are mean \pm s.d. ($*P < 0.01$ from static control, $\dagger P < 0.01$ from ramp; $n = 4, 3$ and 3 experiments for static, oscillatory and ramp flow conditions, respectively). Scale bar in *A* is 10 μm .

This is the first study to demonstrate that PECAM-1 and $G\alpha_{q/11}$ form a complex which is also mechano-sensitive. In addition, the association between PECAM-1 and $G\alpha_{q/11}$ was shown to discriminate between specific components of fluid flow both *in vivo* and *in vitro*. In the atheroprotected region of the descending aorta, $G\alpha_{q/11}$ co-localized with PECAM-1 at the cell-cell junctions, yet was localized perinuclearly in atheroprone regions. *In vitro*, while flow devoid of temporal gradients of shear stress could not induce $G\alpha_{q/11}$ -PECAM-1 dissociation, dynamic flow was a potent and rapid mediator of PECAM-1 and $G\alpha_{q/11}$ dissociation.

The $G\alpha_{q/11}$ -PECAM-1 complex may be part of a larger macromolecular structure assembled at the endothelial cell-cell junction. Regions of PECAM-1 form a scaffold for the G protein-coupled receptor bradykinin 2 receptor (Yeh *et al.* 2008), eNOS (Dusserre *et al.* 2004), VEGFR2 and VE-cadherin (Tzima *et al.* 2005). Inhibition of dissociation

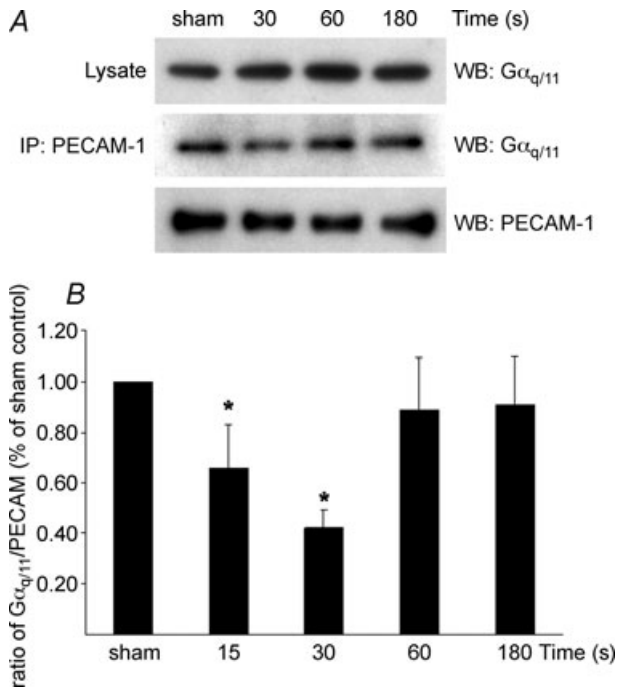


Figure 3. Time-dependent dissociation of $G\alpha_{q/11}$ -PECAM-1 complex in response to impulse flow

Confluent serum-starved HUVECs were harvested 15, 30, 60 or 180 s after exposure to a single 1 s impulse (temporal gradient) using a syringe pump delivering a 14 dynes cm^{-2} FSS rate. *A*, representative Western blot (WB) of one experiment of sham and after 30, 60 and 180 s exposure to the impulse showing lysate $G\alpha_{q/11}$, immunoprecipitated (IP) PECAM-1 blotted with $G\alpha_{q/11}$ and PECAM-1. *B*, densitometric analysis of three independent experiments displays the kinetics of association-dissociation of the $G\alpha_{q/11}$ -PECAM-1 complex following impulse flow. Levels of $G\alpha_{q/11}$ co-immunoprecipitated with PECAM-1 were normalized to lysate $G\alpha_{q/11}$ levels and immunoprecipitated PECAM-1 levels. Results are percentage of sham controls. Values are mean \pm s.d. (* P < 0.05 from sham control).

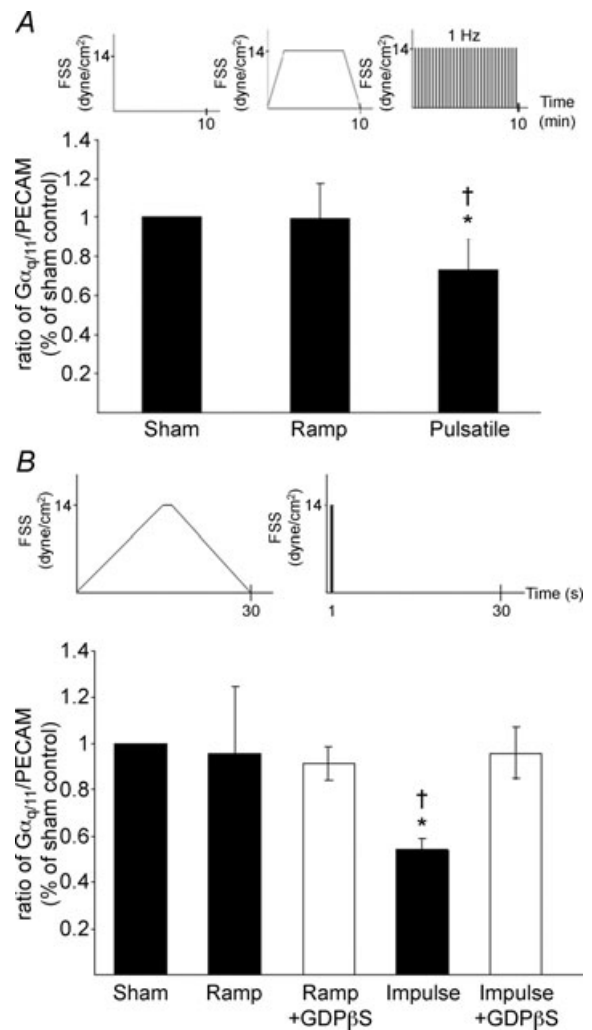


Figure 4. Impulse but not ramped-transient flow promotes dissociation of the $G\alpha_{q/11}$ -PECAM-1 complex after 30 s; this dissociation is dependent on G protein activation

A, pulsatile FSS induces dissociation of the $G\alpha_{q/11}$ -PECAM-1 complex, whereas slowly transitioning ramped FSS does not. Confluent serum-starved HUVECs were subjected to ramped-transient flow (60 s slow ramping to 14 dynes cm^{-2} , maintained for 8 min, 60 s slow ramping down to 0 dynes cm^{-2}) or pulsatile flow (14 dynes cm^{-2} , 400 pulses of 0.5 s with 1 s intervals for 10 min). Immunoprecipitated $G\alpha_{q/11}$ levels were normalized to lysate $G\alpha_{q/11}$ levels and immunoprecipitated PECAM-1 levels. Results are percentage of sham controls. Values are mean \pm s.d. (* P < 0.01 from sham control, † P < 0.01 from ramp; n = 3 experiments). *B*, confluent serum-starved HUVECs were pretreated with or without GDP β S for 4 h, subjected to impulse flow (temporal gradient, 1 s at 14 dynes cm^{-2}) or ramped-transient flow (devoid of temporal gradients). Immunoprecipitated $G\alpha_{q/11}$ levels were normalized to lysate $G\alpha_{q/11}$ levels and immunoprecipitated PECAM-1 levels. Results are percentage of sham controls. Densitometric analysis of three independent experiments shows a significant dissociation of the $G\alpha_{q/11}$ -PECAM-1 complex following impulse flow in non-treated samples. GDP β S treatment had no effect on the association/dissociation of the complex in sham controls. Inhibition of G proteins with GDP β S completely eliminated impulse flow-induced $G\alpha_{q/11}$ -PECAM-1 complex dissociation. Values are mean \pm s.d. (* P < 0.05 from sham control, † P < 0.05 from ramp).

with GDP β S shows that flow-induced G $\alpha_{q/11}$ -PECAM-1 complex dissociation requires G protein activation and this mechanism may represent the initiation signal leading to activation of the macromolecular complex at the membrane. Indeed, heterotrimeric G protein activation is one of the earliest known responses to shear (Berthiaume & Frangos, 1992; Gudi *et al.* 1996), with activation occurring within 1 s of flow onset. The activation of G proteins may be direct (Gudi *et al.* 1998) or indirectly by flow-induced activation of the bradykinin B₂ receptor (Chachisvilis *et al.* 2006).

Although no significant change in G $\alpha_{q/11}$ junctional staining was observed *in vitro* we have shown here that dissociation of the G $\alpha_{q/11}$ -PECAM-1 complex is accompanied by increased localization of G $\alpha_{q/11}$ in the perinuclear region upon 2 h of stimulation with dynamic shear stress. This may be an indication of an initial G $\alpha_{q/11}$ translocation, consistent with the reversible trafficking of G protein subunits from the plasma membrane to intracellular sites upon activation (Marrari *et al.* 2007). In addition, G protein redistribution from the plasma membrane to the Golgi is correlated to the rate of GTP/GDP exchange (Azpiazu *et al.* 2006). G $\alpha_{q/11}$ induces the transcription factor NF κ B activation through a Ras-Raf-Erk pathway and subsequently stimulates production of pro-inflammatory cytokines (Gudi *et al.* 2003; Williams *et al.* 2007). Furthermore, G $\alpha_{q/11}$ inhibition significantly reduces systemic blood pressure and neointima formation after carotid artery injury in mice (Kawasaki *et al.* 2005). Thus, G protein translocation may be an early step in endothelial pro-inflammatory activation. Conversely, the inability to form junctional G $\alpha_{q/11}$ in animals deficient in the junctional scaffold PECAM-1 leads to disruption of G $\alpha_{q/11}$ trafficking and pro-inflammatory signalling. This is consistent with the recent findings that PECAM-1 is a critical mediator of atherogenic signalling (Goel *et al.* 2008; Harry *et al.* 2008; Stevens *et al.* 2008).

In summary, this is the first study to demonstrate that PECAM-1 and G $\alpha_{q/11}$ form a mechanosensitive complex. The localization of the G $\alpha_{q/11}$ -PECAM-1 complex to the endothelial cell-cell junction positions this mechanosensor complex to a region of greatest shear-induced mechanical tension (Fung & Liu, 1993; White & Frangos, 2007). Temporal gradients in shear stress lead to a rapid dissociation of the G $\alpha_{q/11}$ -PECAM-1 complex, whereas fluid flow devoid of temporal gradients does not disrupt the complex. Furthermore, G protein activation is required for G $\alpha_{q/11}$ -PECAM-1 dissociation. Together with the role of PECAM-1 as a critical mediator of atherogenic signalling, the finding of this flow-dependent complex dissociation may represent a missing link between the primary force-sensing elements of the cell and the downstream mechanochemical transduction pathway.

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Author contributions

L.A.O., K.S.B., L.L., J.-C.Y., B.M., D.N.D, H.Y.S and C.R.W performed the experiments, L.A.O, B.M. and J.A.F prepared the manuscript, and J.A.F designed the experiments and secured the funding.

Acknowledgements

We thank Ronald Y. Kwon for critical reading of the manuscript. This study was supported by NIH-NHLBI Grant HL-40696.