The $\alpha 1\beta 1$ Integrin Has a Mechanistic Role in Control of Interstitial Fluid Pressure and Edema Formation in Inflammation

Ø.S. Svendsen, M.M. Barczyk, S.N. Popova, Å Lidén, D. Gullberg, H. Wiig

Objective—Collagen-binding integrins may be involved in controlling interstitial fluid pressure (Pif), transcapillary fluid flux, and tissue fluid volume. Our aim was to explore whether the newly discovered collagen binding $\alpha 1\beta 1$ integrin has a mechanistic role in inflammatory edema formation.

Methods and Results—In collagen matrices seeded with a mixture of mast cells and fibroblasts, fibroblasts lacking the $\alpha 1$ integrin subunit ($\alpha 1^{−/−}$) contracted collagen gels less efficiently than control fibroblasts, suggesting that the $\alpha 1\beta 1$ integrin is able to mediate tensile force in connective tissues. In $\alpha 1^{−/−}$ mice, control Pif in skin did not differ from the pressure found in wild-type mice. Whereas a reduction in Pif was found in control mice after inducing inflammation, thereby contributing to fluid extravasation and edema formation, such a reduction was not seen in $\alpha 1^{−/−}$ mice. That this effect is mediated through the extracellular compartment is suggested by a similar plasma protein extravasation ratio in $\alpha 1^{−/−}$ and wild-type mice.

Conclusions—Our data suggest that $\alpha 1\beta 1$ integrins on dermal fibroblasts mediate collagen lattice remodeling and have a mechanistic role in controlling Pif in inflammation and thereby fluid extravasation and edema formation in vivo. (Arterioscler Thromb Vasc Biol. 2009;29:1864-1870.)

Key Words: collagen ■ endothelium ■ extracellular matrix ■ genetically altered mice ■ microcirculation

Fluid flux across the capillaries is governed by hydrostatic and colloid osmotic pressure gradients between plasma and interstitium. Edema (ie, extravascular fluid accumulation in the tissue) is one of the key features of inflammation and is the result of increased transcapillary fluid transport in this condition. During inflammation there is an increased capillary leakage, but also initially a rapid decrease in the hydrostatic pressure outside the capillary, the interstitial fluid pressure (Pif), which is quantitatively more important than increased capillary permeability as a driving force in the initial phase of edema generation.1,2

Traditionally, cells are not included in the interstitium, defined as the extracellular compartment between the blood vessels and lymphatics of a tissue.3,4 In recent studies, however, we have shown an active role of the interstitium in edema generation during inflammation (reviewed in5), and in the present context we include cells (ie, fibroblasts) when addressing the mechanisms for edema generation. Furthermore, our studies have suggested a role for integrins in controlling interstitial fluid volume (Vif) by their ability to modulate force from the cytoskeleton inside the cells to the structural proteins (ie, collagens) of the extracellular matrix outside the cell. The normal fluid homeostasis is dependent on functioning collagen-binding integrins, and the result of perturbed integrin function can be edema.2,5,6

There are four collagen-binding integrins: $\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 10\beta 1$ and $\alpha 11\beta 1$, and in previous studies we have shown a role for $\alpha 2\beta 1$ in control of Vif.6,7 The $\alpha 11\beta 1$ integrin has, similar to the $\alpha 2\beta 1$ integrin, a higher affinity for fibrillar than network-forming collagens, and is expressed in vivo on fibroblasts where the collagens are arranged in a highly ordered manner.8

The integrins are also involved in regulation of vascular permeability9 and transmigration of leukocytes.10 Several subtypes of integrins, including the collagen-binding $\alpha 1\beta 1$ and $\alpha 2\beta 1$ integrins, are found on endothelial cells where they are expressed primarily on their abluminal surface.9 The protein leakage across the capillaries is dependent on the integrity of the vascular endothelium, again affected by different physiological and pathological conditions like inflammation and tumor growth. The possible role of $\alpha 11\beta 1$ integrin in the regulation of capillary permeability is unknown.

We have recently generated a mouse deficient for the $\alpha 1$ chain ($\alpha 1^{−/−}$) which displays a phenotype involving incisors and resulting in dwarfism.11 Also, the $\alpha 11\beta 1$ integrin has...
been shown to be commonly overexpressed in nonsmall-cell lung carcinoma cells. We hypothesized that the α11β1 integrin, because of the affinity for fibrillar collagens, also has a role in controlling Pif and thus Vif and edema formation in inflammation. To address this issue we used the α11−/− mouse model and were able to demonstrate that the α11β1 integrin, most likely through its collagen lattice remodeling ability, has a significant role in controlling Pif and thus fluid extravasation in vivo.

Methods

Animals

Age- and sex-matched C57BL/6J wild-type and α11 integrin knockout mice (α11−/−) bred on a C57BL/6J background were used in the experiments. Experiments were performed with the approval of and in accordance with the recommendations of the Norwegian State Commission for Laboratory Animals.

For a more detailed description of animal handling, immunohistochemistry, cells, transfection, PCR, collagen matrices, measurement of tissue fluid volumes, pressures and plasma extravasation, please see the supplemental materials (available online at http://atvb.ahajournals.org).

Results

Expression of α11 Integrins in Skin and Fibroblasts

We analyzed mRNA and protein expression of different collagen-binding integrins in the mouse paw and mouse dermal fibroblasts (Figures 1 and 2). In the mouse paw skin, mRNA for the α subunits of all 4 collagen-binding integrins were detected (Figure 1A). The strongest signal was observed for the integrin subunits α1 and α2 in both wild-type and α11−/− tissues. mRNA analysis of dermal fibroblasts from passage 2 revealed the presence of α1 and α10 integrin subunits in both wild-type and α11−/− cells. The levels of the α2 integrin subunit were very low and difficult to detect in both cell types. Strong signal for α11 was detected in wild-type cells (Figure 1B). In a separate experiment, quantitative PCR did not show any compensatory increase in mRNA of other collagen binding integrins, or of the αvβ3 integrin in dermal fibroblasts under conditions used (Figure 1C). Immunohistochemical staining revealed expression of α1 mainly in capillaries and α2 in capillaries and basal keratinocytes (Figure 2). The α11 chain was weakly expressed in the dermis (Figure 2), whereas the signal for α10 was too weak to allow its detection by immunohistochemistry (data not shown). Please note that intracellular staining with affinity purified antibodies to α11 in muscle fibers is non-specific and was not paralleled by α11 staining at the sarcolemma.

Collagen Matrices

Fibroblasts in a 3-dimensional (3D) collagen matrix are able to contract and thereby reduce the total matrix area in vitro. We used a modified assay, where a mixture of mastocytoma cells (hereafter referred to as mast cells) and fibroblasts were seeded in matrices of collagen I. The skin fibroblasts used were isolated from α11−/− and wild-type mice. The experiments were repeated 3 times with reproducible results, and Figure 3A shows the typical findings from one experiment. The matrices where α11−/− fibroblasts were used contracted significantly less efficiently than the matrices containing wild-type cells (2 hours: α11−/− fibroblasts: 0.8±0.02, n=8, wild-type fibroblasts: 0.5±0.01 n=8, P<0.001).

To study whether there was an effect of C 48/80 (a mast cell degranulator and an inflammatory agent14,15 used in animal experiments described below) on the contraction of the collagen matrices, we added this agent to some of the wells. C 48/80 improved contraction of lattices for both kinds of fibroblasts, but the difference between them was still

![Figure 1](image1.png)

**Figure 1.** Reverse transcriptase PCR of mouse integrin chains α1, α2, α10, and α11 mRNA isolated from paw skin (A) and dermal skin fibroblasts (B) from α11−/− (-/-) and wild-type (+/+) mice. C, Relative expression of integrin chains in dermal fibroblasts (error bars represent calculations of 2^−ΔΔCT from ΔΔCT standard deviations).

![Figure 2](image2.png)

**Figure 2.** Strong expression of integrins α1 and α2 in capillary endothelium in the dermis and muscle layer (A, B, D, and E); signal for integrin α2 in the basal keratinocytes (arrow on B and E) and signal for integrin α11 in the dermis (bracket on C) in wild-type (+/+) mouse paw. Integrin α11 signal is lacking in dermis of integrin α11−/− (-/-) mice (F). epi indicates epidermis; d, dermis; m, muscle layer. Scale bar=100 μm.
significant (2 hours: $\alpha_{11}^{-/-}$ fibroblasts: $0.6\pm0.02$, n=4, wild-type fibroblasts: $0.4\pm0.01$ n=4, $P<0.001$). Taken together, the gel contraction experiments suggest that the $\alpha_{11}\beta_1$ integrin on dermal fibroblasts in a coculture with mast cells is involved in tension generation in a collagen matrix. Also, C 48/80 stimulated contraction for both cocultures of mast cells and wild-type fibroblasts, as well as cocultures of mast cells and $\alpha_{11}^{-/-}$ fibroblasts.

Nothing is known about the detailed mechanism whereby $\alpha_{11}\beta_1$ mediates collagen matrix contraction and whether there is a specific contribution from the intracellular part of the $\alpha_{11}$ subunit to the contractile and signaling events involved in the integrin–mediated contraction process. A universal downstream mediator of $\beta_1$ integrin–mediated events is the soluble tyrosine kinase focal adhesion-kinase (FAK). To investigate whether FAK in part could rescue the collagen gel contraction in the absence of $\alpha_{11}\beta_1$, we transfected cells with constitutively activated FAK.17 $\alpha_{11}^{-/-}$ skin fibroblasts expressing activated FAK contracted collagen matrices more efficiently (Figure 3B). Interestingly, also the $\alpha_{11}^{-/-}$ cells expressing CD2-FAK displayed an improved capacity to contract the collagen lattices.

**Pif and Tissue Fluid Volumes**

Having shown a role in vitro, we next studied a potential effect of the $\alpha_{11}\beta_1$ integrin in intact animals. In these experiments, a control Pif was measured before the mice were euthanized. The circulation was arrested to minimize fluid filtration and a following increase in interstitial fluid volume as a result of the inflammatory process. If pressure measurements are performed in vivo with intact circulation, the contributing role of Pif to the inflammatory edema is severely underestimated.2 Previous experiments have shown that the Pif measured during the hour after cardiac arrest is similar to the corresponding pressure in vivo.18

The in vitro collagen matrix experiments suggested that the integrin-collagen interaction retains 3D matrices contraction in steady state conditions, and a release of this contraction in vivo could result in a reduced Pif and swelling. We first tested whether blocking of the $\beta_1$-integrin chain was reflected in the recorded Pif, because a blockage of this integrin chain will abolish the mechanoreceptor effect of all $\beta_1$-integrins. A needle (33 or 34 G) was tunneled under the paw skin in control mice, and $\beta_1$ integrin blocking antibodies or the corresponding control antibodies were injected. The pressure measurements were done at the edge of the injected volume.19 As evident from Figure 4A, control IgM antibodies had no effect on Pif when injected intradermally in wild-type mice (n=6). In contrast, anti-integrin $\beta_1$ had an effect on Pif, which was visible already after the first 15 minutes after injection (n=7, $P=0.008$; Figure 4). Interestingly, this fall in Pif was not seen when injecting $\beta_1$ integrin blocking antibodies in $\alpha_{11}^{-/-}$ mice, suggesting that compensation mecha-
nisms not involving the classical collagen binding integrins are active in this situation.

We next went on to test the role of the \(\alpha 1\beta 1\) integrin in the context of inflammation in the intact animal. After inducing a generalized inflammation by injection of C 48/80, we followed the Pif profile after circulatory arrest. Before injection of 200 \(\mu\)g C 48/80 i.v., control pressures were measured in wild-type and \(\alpha 1\beta 1\)-null mice, and were found to be similar between the strains (controls: \(-0.5 \pm 0.2\) mm Hg, \(n=7\) and \(\alpha 1\beta 1\)-null: \(-0.3 \pm 0.1\) mm Hg, \(n=5\) \(P=0.39\)). Two minutes after inducing the generalized inflammation, circulatory arrest was induced (0.2 mL KCl i.v.). As evident from Figure 4B, Pif decreased significantly in wild-type animals, from \(-0.5 \pm 0.2\) mm Hg to \(-2.1 \pm 0.3\) mm Hg \(P=0.002\). The decrease was stable through the whole observation period. In contrast, Pif in \(\alpha 1\beta 1\)-null animals did not change after inflammation. There were significant differences in Pif measured in wild-type and \(\alpha 1\beta 1\)-null mice in all periods after induced inflammation.

We also tested whether \(\alpha 1\beta 1\) integrin–collagen interaction affects the control of Vif in the steady state condition. Thus we determined Vif, using the extracellular tracer \(^{51}\)Cr-EDTA, and total tissue water (Tw; including the intracellular fluid phase). There was a tendency toward increased fluid volumes in paw compared to back skin in \(\alpha 1\beta 1\)-null mice \(n=6\) compared to wild-type mice \(n=6\); Figure 5A and 5B), but this difference did not reach statistical significance. We next went on to measure Tw after 30 minutes of local inflammation induced by injecting C 48/80 in thigh skin \(n=5\) for both strains), using NaCl injected in the other limb as control. Although C 48/80 induced a significant increase in Tw, there was no significant difference between the 2 mouse strains (Figure 5C) in this experiment.
Dermal fibroblasts were shown to contain α11 mRNA, in agreement with previous published data. Our immunohistochemical experiments show at protein level, that the α11β1 integrin actually is expressed in dermis, although at low levels. This fact, combined with the observation that this integrin bind fibrillar collagens with high affinity, led us to hypothesize that the α11β1 integrin also could play a significant role in regulating tension of the extracellular matrix and thereby Pif and tissue fluid balance in the intact animal.

We used C 48/80 to induce general and local inflammation. The substance works as a mast cell degranulating agent, and i.v. administration in mice gives a massive anaphylactoid reaction accompanied with fluid extravasation and edema. The physiological responses of C 48/80 are the results of massive release of mediators from the mast cells (ie, histamine and leukotrienes). Surprisingly, C 48/80 actually increased the contraction of the collagen matrices populated by fibroblasts and mast cells, which in this context would represent a tendency to increase Pif. If a similar process occurs in vivo, it will partly counteract the rapid decrease in Pif seen in acute inflammation. C 48/80 is an activator of phosphoinositide 3-kinase (PI3K) pathways. It has been shown earlier that this signaling pathway is important for both counteracting decrease in dermal Pif and also for stimulating contraction of collagen lattices. An important role for the αvβ3 integrin in this setting has been suggested. Also, reorganization and depolymerization of the actin cytoskeleton is stimulated by C 48/80, leading to degranulation in mast cells. It is possible that any of these mechanisms are involved in the modulation of the collagen lattice contraction observed in our experiments. In vivo, additional cell types such as neutrophils, macrophages, and endothelial cells probably contribute to generating signals affecting the collagen-remodeling properties of the fibroblasts.

To understand the mechanism whereby α11β1 mediates collagen contraction in some more detail, we tested the possible involvement of FAK in this event. In agreement with previous results implicating FAK in TGF-β-stimulated fibroblast-mediated collagen contraction, we found that also in dermal fibroblasts, FAK takes part in the contraction process. Whether this contribution is via the recently discovered inside-out signaling role of FAK in integrin activation, or via its more conventional outside-in signaling function, remains to be determined.

The fact that the collagen reorganizing capacity of α11 cells can be rescued is compatible with other collagen receptors, such as α2β1, mediating the linkage to collagen in the absence of α11β1. In support of a role for α2β1 in the collagen lattice contraction, previous data have documented a dynamic upregulation of α2 inside a 3D collagen lattice. The finding that the α11 cells, expressing both α2β1 and α11β1, are stimulated by the introduction of constitutively activated FAK demonstrates that also in these cells FAK activation is a limiting factor for maximal contractile activity of the cells. It will be interesting to determine whether integrin turnover rates are different.
inside the 3D collagen gel compared to 2D substrates and whether this is a rate-limiting factor in FAK activation.

Although Pif under normal conditions in skin is slightly negative, the extracellular matrix is kept under constant tension. 3D gels consisting of collagen and fibroblasts are supposed to mimic this process, and the tensile strength will determine the ability of the gels to expand. When the contraction becomes weaker, more fluid will enter, and in the intact animal the consequence may be edema. In accordance with previous studies, we show that blocking antibodies against β1 integrins decrease Pif, in contrast to control antibodies. Also, we show that anti-β1 integrin antibodies do not have any significant effect on integrin function in α1β1 mice, suggesting a β1 integrin-independent compensating mechanism in control of Pif in α1β1 mice. Unfortunately, no available blocking antibodies against the α1 integrin chain exist, and therefore we were not able to test the response on blocking the α1β1 integrin directly. A plausible other candidate integrin to mediate contraction of the collagen matrix in the absence of α1 in vivo is αvβ3, which can mediate contraction of collagen lattices via an indirect mechanism.

In exploring the possible role of the α1β1 integrin expressed in dermis, we compared Pif in paw skin from wild-type and α1β1−/− mice. In the normal steady state situation, there was no difference between the strains whereas during inflammation, a reduction in Pif was not observed in the α1β1−/− mice. Our interpretation is that the α1β1 integrin–collagen tension, which is deficient in mice lacking the α1β1 integrin, is affected by the inflammatory reaction in the wild-type mouse. We suggest that the α1β1 integrin is involved in maintaining normal tension, and when this connection is lost in acute inflammation, the consequence is reduced Pif and edema formation. This interpretation implies that the α1β1 integrin plays a role in the fluid homeostasis and is involved in the process of inflammation when Pif decrease and thereby “aspirate” plasma from the capillaries out into the tissue. The fall in Pif after administration of anti-β1 IgM was of the same magnitude as the decrease after C 48/80 induced inflammation in wild-type mice, which did not occur in α1β1−/− mice. These facts suggest that the α1β1 integrin is at least as important as the α2β1 integrin in the fluid regulation in mouse skin.

One might assume that if the α1β1 integrin is involved in maintaining tension of the extracellular matrix, it should also affect fluid volumes. Measured volumes were, however, not significantly different in the wild-type and α1β1−/− mice in control situation and after induction of inflammation in vivo. The explanation is probably redundancy in capacity to accommodate an increased fluid volume in the interstitium and by the complex vascular response to inflammation. During control or steady state conditions, the interstitial fluid volume is “autoregulated” and determined by capillary filtration and lymph flow. A lower tension induced by abrogated binding of α1β1 integrins will tend to reduce Pif and increase Vif. This situation will be compensated by dilution of interstitial fluid proteins and an increase in lymph flow. These effects have the capacity to compensate for increased filtration pressures many times the observed increase of net filtration pressure of ≈1.5 mm Hg induced by reduced tension observed in our study. These compensatory mechanisms are also active during an acute inflammation akin to that induced here by C48/80. Moreover, during an acute inflammation, an array of vascular reactions are activated (reviewed in), increasing the filtration several fold. Thus, in addition to the reduction in Pif studied here, the hydraulic conductance and capillary and interstitial fluid colloid osmotic pressure will increase. The increase in the latter will reduce the transcapillary oncotic gradient. Furthermore the osmotic reflection coefficient, σ, will decrease. All these reactions will mask the Pif effect, which in our experiments was unmasked by arresting the circulation.

The mechanisms for protein extravasation from plasma to the interstitium are still debated, putting different emphasis on paracellular or transcellular transport. The interaction between basement membrane, integrins, and endothelial cells has been shown to be important for the endothelial barrier function. Having shown a role for the α1β1 integrin in regulation of fluid transport, we performed microdialysis experiments to investigate a possible role for α1β1 integrins in regulating protein extravasation under inflammatory conditions. C 48/80 was administered locally through an active dialysis membrane, and we could demonstrate a significant increase in albumin extravasation around this membrane. However, the response pattern and increase in capillary albumin leakage was identical in wild-type and α1β1−/− mice. In accordance with the immunochemical data, our experiments do not therefore suggest any role for the α1β1 integrin in regulation of protein extravasation across the capillary endothelium.

In summary, we have shown that the α1β1 integrins expressed on fibroblasts in dermis are capable of increasing the contraction of collagen gels in vitro, suggesting an effect of these integrins in regulating the tension of the extracellular matrix and accordingly fluid extravasation. The α1β1 integrin do not seem have any role in regulation of capillary permeability. Whereas an induced inflammatory reaction resulted in a reduction in Pif that will contribute to increase transcapillary fluid mobilization, such an effect was not observed in α1β1−/− mice. Based on the present data we suggest that the α1β1 integrin has a mechanistic role in fluid extravasation and edema formation in the initial phase of inflammation and may act as a target for edema therapy.

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Disclosures
None.

References
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The α1β1 integrin has a mechanistic role in control of interstitial fluid pressure and edema formation in inflammation

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ONLINE SUPPLEMENTAL METHODS

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Methods

Animals

Age and sex matched C57BL/6J wild type and α11 integrin knockout mice (α11−/−) bred on a C57BL/6J background were used in the experiments. Mice were fed *ad libitum* before experiments and anesthetized with 0.2-0.3 mL ketamin (12.2 mg/mL) combined with medetomidin (24.3 µg/mL) injected *subcutaneously* (s.c.) on the lower back of the animals. Supplemental anesthesia was added when needed. Care was taken to avoid injection in the areas where pressure and volume measurements were done. The mice were catheterized in the jugular vein and circulatory arrest was induced by an *intravenous* (i.v.) injection of saturated KCl. Experiments were performed with the approval of and in accordance with the recommendations of the Norwegian State Commission for Laboratory Animals.

Immunohistochemistry.

For immunohistochemistry, specimens were snap-frozen in liquid nitrogen and stored in –70°C until used. Tissues were sectioned on a Leica cryostat and 5-7 µm thin sections collected on SuperFrost glass slides. The primary antibodies were hamster anti-α1 integrin (Ha31/8; BD Pharmingen), rat anti-α1 integrin (Emfret Analytics), rabbit anti-α11 integrin. The secondary antibodies were Cy3 conjugated goat anti-armenian hamster IgG, goat anti-rat IgG and goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories Inc.). The staining procedure was performed as described earlier. Images were visualized under a Zeiss Axioscope microscope equipped with optics for observing fluorescence and were captured using a digital AxioCam MRm camera.
**Cells**

Primary dermal fibroblasts were isolated and cultured as previously described. The fibroblasts were used between passage 1 and 3. Transplantable mouse mastocytoma cells (hereafter referred to as mast cells) described originally by Furth et al. were used in co-culture together with dermal fibroblasts.

**Transfection**

Primary mouse dermal fibroblasts were isolated from newborn wildtype (wt) or α11−/− mice. 10 µg CD2-FAK in pCMD8 plasmid was added to each 75cm² flask of subconfluent wt or α11−/− cells at low passage number using FuGENE ® HD (Roche) as transfection reagent according to the recommendations of the manufacturer. Following overnight recovery, cells were trypsinized and used in the collagen gel contraction assay.

**PCR**

Total RNA isolation from mouse tissue and primary mouse dermal fibroblasts as well as cDNA synthesis was performed as described elsewhere. The sequences of the primers amplifying collagen-binding integrins have been described earlier.

Quantitative PCR (QPCR) analysis of integrin mRNA expression was performed using primers described in the Supplemental Table and iQ SYBR Green Supermix (BioRad) following manufacturer-recommended protocol. The comparative Ct method was used to calculate the relative mRNA expression. Target genes quantifications were normalized to GAPDH. QPCR was performed in a LightCycler® 480 Instrument II (Roche). Each of the genes was run in quadruplets on the 96-well plate (Roche) under the same conditions (45 cycles of 20s, 58°C). mRNA expression of integrin subunits was analyzed by LightCycler®
480 Software version 1.5 (Roche). The comparative Ct method\textsuperscript{6} was used to calculate relative mRNA expression by determination of \(\Delta\Delta\text{Ct}\) values. Expression of the different integrin chains in control cells were chosen as reference.

**Collagen Matrices**

The collagen gel contraction procedure was performed as described by Gullberg et al\textsuperscript{7} with some modifications: Wild type or \(\alpha 11^{-/-}\) fibroblasts were suspended together with mast cells in the collagen solution. DMEM containing 5\(\mu\)g/ml of C 48/80 or DMEM alone was used to detach gels from the plate. Free-floating gels were incubated at 37\(^\circ\)C, and gel diameter was measured under microscope at the indicated time points.

**Measurements of tissue fluid volumes**

The interstitial fluid volume (Vif) and total tissue water (Tw) were measured as described in\textsuperscript{8}, with the exception of using 90 minutes equilibrium time of the extracellular tracer instead of 120 minutes. Furthermore, we induced local inflammation by injecting 5.0 \(\mu\)L C 48/80 (Sigma) (10 mg/mL) diluted in NaCl (9.0 mg/mL) and NaCl (9.0 mg/mL) (control) in thigh skin, using Hamilton Microliter Syringe with a 33G needle. Samples were taken after 30 minutes, using an 8 mm dermal biopsy punch (Miltex), and Tw were measured.

**Measurements of interstitial fluid pressures (Pif)**

Pif was measured in hind paw skin by a micropuncture technique as described in detail elsewhere\textsuperscript{9}. Pressures were recorded intermittently during the experimental period, and the mean pressures obtained in 15 min intervals were averaged. We injected 1.0 \(\mu\)L purified hamster IgM isotype standard (1 mg/mL) (BD Biosciences) as control and purified hamster
anti-rat CD29 (integrin β1 chain) IgM (1 mg/mL) (BD Biosciences) s.c. C 48/80 (2 mg/mL) diluted in NaCl (9 mg/mL) was given i.v.

*Microdialysis and plasma albumin extravasation ratio (AER)*

Capillary permeability was measured by a microdialysis technique as described by Iversen et al. 10. Dialysates were collected for 10 minutes periods, and after a baseline period, one of the fibers was perfused with C 48/80 (4 mg/mL) diluted in NaCl (9 mg/mL). Values in the experimental periods were expressed relative to the basal albumin extravasation rate.

*Statistical methods*

Values are given as means ± S.E.M. unless otherwise stated. Mann-Whitney and Wilcoxon Signed Ranks tests were used to compare volumes. One-way analysis of variance (ANOVA) and subsequent Bonferroni tests were used where appropriate. A P-value < 0.05 was considered significant.

*References for Supplemental Methods*


## Supplemental Table. Primers sequences used in QPCR analysis

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<th>mRNA</th>
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