EUROPEAN SOCIETY OF CARDIOLOGY®

Cardiovascular Research (2010) **87**, 211–217
doi:10.1093/cvr/cvq143

SPOTLIGHT REVIEW

Transcapillary exchange: role and importance of the interstitial fluid pressure and the extracellular matrix

Rolf K. Reed[1]* and Kristofer Rubin[2]

[1]Department of Biomedicine, University of Bergen, Jonas Lies vei 91, Bergen N-5009, Norway; and [2]Department of Medical Biochemistry and Immunology, Uppsala University, Uppsala, Sweden

Received 11 March 2010; revised 26 April 2010; accepted 10 May 2010; online publish-ahead-of-print 13 May 2010

This review will summarize current knowledge on the role of the extracellular matrix (ECM) in general and on the interstitial fluid pressure (P_{if}) in particular with regard to their importance in transcapillary exchange. The fluid volume in the interstitial space is normally regulated within narrow limits by automatic re-adjustment of the interstitial hydrostatic and colloid osmotic pressures in response to perturbations in capillary filtration and by the lymphatics. Contrary to this commonly accepted view, P_{if} can become an active force and create a fluid flux across the capillaries in several inflammatory reactions and trauma situations rather than limit the changes occurring. The molecular mechanisms involved in the lowering of P_{if} include the release of cellular tension exerted on the collagen and microfibril networks in the connective tissue via the collagen-binding β_1-integrins, thereby allowing the glycosaminoglycan ground substance, which is normally underhydrated, to expand and take up fluid. Several growth factors and cytokines, including the platelet-derived growth factor BB, are able to reverse a lowering of P_{if} and restore the normal compaction of the ECM. The magnitude of the lowering of P_{if} varies with the inflammatory response. In several inflammatory reactions, a lowering of P_{if} to −5 to −10 mmHg is seen, which will increase capillary filtration by 10–20 times since the normal capillary filtration pressure is usually 0.5–1 mmHg (skin and skeletal muscle). Unless this lowering of P_{if} is taken into account, the enhanced solute flux resulting from an inflammatory response will be ascribed to an increased capillary permeability.

Keywords Connective tissue • β-Integrin receptors • Glycosaminoglycans • Inflammation

This article is part of the Spotlight Issue on: Microvascular Permeability

1. Introduction

Loose connective tissues are an integral part of all organs. They harbour the vast majority of the interstitial fluid volume and surround peripheral blood vessels. The traditional view on the control of the interstitial fluid volume is that it is regulated within narrow limits, and acts as a sink or storage space for the fluid filtered across the capillary wall.[1] We will summarize the current knowledge that (in part) contradicts the traditional view on the role of the loose connective tissues in transcapillary fluid exchange. We will focus on conditions where these tissues can take on an 'active' role in filtering fluid across the microcirculation via modulations of the interstitial fluid pressure (P_{if}). P_{if} is the pressure that can be measured in a fluid-filled catheter inserted into the tissue and connected to a pressure transducer and acts as filling pressure for the initial lymphatics[1] in the absence of other external forces such as muscle contractions. P_{if} is one of the four pressures determining transcapillary fluid flux and as such, it also contributes to determine transcapillary solute flux via its influence on this fluid flux.

Our concept for the control of P_{if} places a dynamic and active pressure on the outside of the capillary and includes dynamic cellular control of the tension exerted on the extracellular collagen and microfibrillar networks[2,3] via collagen-binding β_1-integrins.[4] This is in addition to P_{if} being a function of the interstitial volume as described by interstitial compliance.[1] In this review, we discuss situations where increased transcapillary fluid flux originates from lowered P_{if}, and how the lowering of P_{if} may contribute to other factors, in particular an apparent altered capillary permeability, since the increased fluid flux will increase convective solute transport across the capillary wall. Therefore, this review should in particular be seen in the context of the review of Levick and Michel[5] on transcapillary fluid flux, that of Curry and Adamson[6] on the regulation of capillary filtration coefficient, and that of Bates[7] on effects of growth factors [or VEGF (vascular endothelial growth factor)] on transcapillary permeability and exchange.

A recent review by us[4] was one of several that had a focus on cardiac function and fibrosis.[8] The present review has a general focus on the normal physiology of transcapillary exchange and how

* Corresponding author. Tel: +47 55586389; fax: +47 55586360, Email: rolf.reed@biomed.uib.no

Published on behalf of the European Society of Cardiology. All rights reserved. © The Author 2010. For permissions please email: journals.permissions@oxfordjournals.org.

Downloaded from https://academic.oup.com/cardiovascres/article-abstract/87/2/211/447690 by Karolinska Institutet University Library user on 28 June 2019

cellular interstitial cells and their signalling can modify transcapillary exchange of fluid and protein across the microcirculation.

2. The loose connective tissues: the composition of the extracellular matrix

Loose connective tissues are an integral part of all organs of the body. Typically, an organ consists of parenchymal cells, the circulatory system (including the lymphatic system), and the loose connective tissues that constitute the framework in which the circulatory system is embedded. Although principally similar in all organs, there are distinct differences in the composition and relative amount of the different constituents between tissues. Finally, pathological conditions such as chronic inflammation or a cancer can alter the composition and function of the circulatory system and the loose connective tissue within an organ.

Typically, the extracellular matrix (ECM) of loose connective tissues consists of four principal components. First, there is the fibrous collagen network creating the scaffold that organizes the three-dimensional structure in which the blood vessels of the organ are embedded and that forms a scaffold for the basal membranes to which parenchymal cells are attached. Second, there is the microfibril-elastin fibrous system.[2] Third, there is the ground substance formed from proteoglycans, composed of glycosaminoglycans (GAGs) covalently linked to a core protein and the free glycosaminoglycan hyaluronan. GAGs are long unbranched amino-sugar polymers, which interact with the fibrous components of the ECM but may also occur freely and unbound in interstitial fluid. The ECM provides the route of transport for nutrients and waste products and determines the physical transport properties (e.g. hydraulic conductivity, compliance) of the tissue.[9] Finally, the interstitial fluid is an ultrafiltrate of plasma with a total protein concentration of ∼50–60% of that in plasma and with an electrolyte composition similar to that of plasma. The composition of the interstitial fluid with respect to proteins in plasma is a function of the fluid flux across the capillary, the size and charge of the molecule in question, as well as the barrier properties of the capillary wall.[10,11]

Several of the structural components of the ECM can be 'washed out' from the interstitium by raising the fluid flux. In the synovium,[12] the concentrations of chondroitin sulfate and heparan sulfate content fell to ∼70% of control with increasing fluid flux, whereas that of hyaluronan remained constant. Furthermore, in the intestine, similar observations have been made for hyaluronan.[13] In these two tissues, the data collectively suggest that hyaluronan synthesis is increased with increased removal to keep the interstitial concentration constant. Likewise, in the renal medulla, the concentration and content of hyaluronan is subject to large and rapid changes (hours) where waterloading in normal rats nearly doubled hyaluronan in medulla, but not in cortex.[14] This increase in hyaluronan was not seen in experimental diabetes with waterloading.[15] Thus, rather than being a structure with a constant composition that is turning over at a slow rate, at least hyaluronan turns over rapidly and synthesis rate can be changed in a relatively short time. This occurs either as a function of the enhanced fluid flux and by 'washing out' the individual matrix components, or by rapidly occurring *de novo* synthesis as a response to altered interstitial fluid flux or concentration. As is evident in the renal medulla, which is likely studied in the most detail from an integrated perspective, the enhanced fluid flux sets off enhanced synthesis, most likely via hyaluronan synthase 2.[16]

3. Biophysical properties of the ECM

A comprehensive review on the physiological properties of ECM was written by Levick more than 20 years ago.[9] This review provides an extensive comparison of available data towards modelling predictions, to a large extent based on Darcy's law. The field has not gained much with regard to new data on interstitial transport properties since then, and it seems that much benefit could have been made from the progress made in molecular biology and the large number of transgenic animal models with altered ECM. One example on how such an approach may give new insight is the recent study of Schwartz and coworkers[17] in which the authors measured hydraulic conductivity based on infusion of saline into the tissue in two mouse models of primary lymphoedema, the Chy mouse, and the K14-VEGF-3-Ig mouse. The K14-VEGF-3-Ig mouse had greatly increased dermal conductivity, but neither their controls nor the Chy mice displayed such an increase.

With regard to mathematical modelling for understanding interstitial transport, several detailed models of transport properties have been developed. As one example, Yao and Gu[18] have modelled complex transport phenomena in the ECM of cartilage as a charged porous fibrous material including diffusive and convective solute fluxes in porous fibrous media and a continuum mixture theory. They included the effect of mechanical loading on solute diffusion under unconfined compression with a frictionless boundary condition using numeric analysis. As another example, Olsen *et al.*[19] developed modelling frameworks for studying dynamic anistropy in connective tissue based on partial differential equations operating on a macroscopic scale and including cells. The model has been expanded to two matrix types and the results are robust and consistent with experiments representing cells as discrete objects in a continuum of ECM. It is the latter type of modelling that would seem to be required to account for the stresses that, in our mechanistic model, contribute to the 'active' control of P_{if}.

4. Normal exchange across the capillary wall

The normal exchange across the capillaries, which is determined by the transcapillary hydrostatic and colloid osmotic pressures, is the focus of the review by Levick and Michel[5] in this issue. The skin and skeletal muscle jointly contain around two-thirds of the extracellular fluid. In a human with an extracellular fluid volume of 15 L, the plasma volume is 3 L and the total lymphatic return is ∼3–5 L per day.[1,5] It is generally accepted that a volume nearly double that may be filtered across the microcirculation and absorbed into the initial lymphatics, but about half the amount entering the initial lymphatics is absorbed in the lymph nodes.[1,5] Furthermore, the contribution to the overall lymphatic return is larger from the intestinal organs than from skin and skeletal muscle. Taken together, the available evidence suggests that the interstitial fluid in skin and skeletal muscle is turned over in ∼24–48 h.[1]

Downloaded from https://academic.oup.com/cardiovascres/article-abstract/87/2/211/447690 by Karolinska Institutet University Library user on 28 June 2019

The fluid flux across the microcirculation (J_v) is described according to Starling's hypothesis as:

$$J_v = CFC(P_c - P_{if} - \sigma(COP_p - COP_{if})) = CFC * \Delta P, \quad (1)$$

where CFC is the capillary filtration coefficient, ΔP is the product of the net filtration pressure, and P and COP are hydrostatic and colloid osmotic pressures, respectively. Furthermore, the subscripts c, p, and if denote capillary, plasma, and interstitial fluid, respectively, and σ is the reflection coefficient for plasma proteins across the capillary. P_{if} in skin and skeletal muscle is around ambient pressure or slightly sub-atmospheric, i.e. around −1 mmHg.[1]

In man, plasma colloid osmotic pressure is 25–28 mmHg and ~60% of this value in the interstitial fluid of skin and skeletal muscle. In experimental animals, the COP_p is ~20 mmHg and again the COP_{if} is ~60% of that in plasma in skin and skeletal muscle in rats and mice.[20,21] Levick and Michel[5] show that colloid osmotic pressure immediately distal to the primary endothelial barrier (e.g. the filtrate beneath the glycocalyx) may not equal interstitial osmotic pressure. In the steady state, this difference may be small but it may be large during rapid filtration.

The review by Levick and Michel[5] argues that, in steady state, there is only filtration in the microcirculation, as opposed to filtration at the arterial end and not re-absorption at the venous end. The important extension of this discussion for the focus of this review is not whether re-absorption actually takes place normally, but rather that the trans-capillary net filtration gradient required to generate the normal net capillary filtration is ~0.5–1 mmHg. This pressure is usually calculated as pre-nodal lymph flow divided by CFC since in a steady state, the net capillary filtration (J_v) will equal the pre-nodal lymph flow (J_l) and then Eq. (1) can be rewritten as:

$$\Delta P = \frac{J_v}{CFC} = \frac{J_l}{CFC} \quad \text{since } J_v = J_l \quad (2)$$

or

$$CFC = \frac{J_v}{\Delta P} = \frac{J_l}{\Delta P}.$$

From this equation, it becomes clear that if CFC is estimated from lymph flow or capillary filtration, most commonly measured as increased weight (using isogravimetric methods) and estimating the alterations in ΔP from the events in the circulation alone, an overestimation of the change in CFC will result since ΔP will be underestimated unless also accounting for a lowering of P_{if}. The clearest example of how complex this situation may be is that a net capillary pressure of ~250–300 mmHg was calculated by Artursson and Mellander[22] to be required in addition to an independently measured rise in CFC of about two to three times above control to account for the fluid accumulation in burn injury.[22–24] If both factors are not considered simultaneously to account for the oedema formation, one may well be misled to ascribe events in the loose connective tissues to changes taking place at the capillary wall. The large increase in net filtration pressure in burn injury was subsequently explained from a lowering of P_{if}.[25,26]

5. Normal control of interstitial fluid volume vs. oedema

The interstitial fluid volume is normally kept within tight boundaries by automatic re-adjustments of the transcapillary fluid flux when the capillary filtration is increased or decreased. When capillary filtration is increased, the protein concentration in the capillary filtrate is lowered due to the properties of the capillary wall.[10,11] Specifically, the filtrate concentration is the ratio of the solute flux (J_s) to fluid flux (J_v) and

$$J_s = J_v * C_p * (1 - \sigma) + PS * (C_p - C_{if}), \quad (3)$$

where C is the concentration of the protein in question, PS is the permeability surface area product, and p and if denote plasma and interstitial, respectively. Eq. (3) takes into account convection and diffusion as separate processes. An increased filtration (J_v) causes increased interstitial volume, but, at the same time, a lowered COP_{if} (resulting from lowered C_{if}) will limit the raised capillary filtration [cf. Eq. (1)]. If a lowered P_{if} contributes to increase J_v, but if only changes in intravascular pressures are considered, the solute flux [cf. Eq. (3)] and increased solute accumulation in the tissue will be ascribed to increased capillary permeability [increased PS and/or lowered σ].

6. Long-term control of interstitial volume: compliance, 'autoregulation', lymphatic function, and interstitial volume exclusion

There are four factors and issues that are important in the long-term control of the interstitial volume, i.e. hours and beyond. First, there is the interstitial compliance as defined in Eq. (4) linking interstitial volume (IFV) and pressure:

$$\text{Compliance} = \frac{\Delta P_{if}}{\Delta IFV}. \quad (4)$$

Compliance around control is ~14% in skin and skeletal muscle that are not enclosed in fascias, i.e. an increase in the interstitial volume of 14% will raise P_{if} by 1 mmHg. When interstitial volume increases above 50% of control, compliance increases dramatically so that even with a large increase (>2–300% of control) and long-standing oedema, P_{if} is only a few mmHg. However, interstitial compliance may not be constant. In acute oedema formation following freeze injury, there can be substantially higher P_{if} as seen from an increase in P_{if} from −1 to 25 mmHg in 60 min as oedema developed to 2–300% above control.[27] After placing a tourniquet and limiting further fluid accumulation, P_{if} fell in the subsequent hour to below 10 mmHg even when maintaining interstitial volume, implying that the compliance must increase simply due to overstretching by the increased interstitial volume.[27]

Second, at a compliance of 14%, an increased capillary filtration will cause an increase in P_{if} and a fall in COP_{if}, which are of similar magnitudes and in turn act across the capillary wall to limit further filtration. Similar but opposite changes are seen upon re-absorption. These automatic re-adjustments of P_{if} and COP_{if} have been called 'auto-regulation' of capillary filtration by the interstitial pressures.[1]

Third, P_{if} and/or interstitial volume act as the filing pressure for the initial lymphatics to increase lymph flow and limit oedema formation. Fourth, interstitial exclusion, i.e. the property that the plasma proteins do not have access to the full interstitial volume due to steric exclusion by the structural macromolecules,[28] will influence the time to

Downloaded from https://academic.oup.com/cardiovascres/article-abstract/87/2/211/447690 by Karolinska Institutet University Library user on 28 June 2019

reach a new steady state, not the steady state itself. Thus, a higher exclusion results in reduced time to reach a new steady state since there are less plasma proteins in the loose connective tissue.[1]

On the road to a new steady state resulting from increased filtration, lymph flow will increase until a steady state is achieved where lymph flow and protein flux again equal the corresponding net capillary fluxes and there will be a relatively larger flux of protein out of the tissue compared with the influx across the capillary. The reason being that the interstitial fluid protein concentration in the new steady state is lower and the enhanced removal of protein with lymph 'washdown' reduces the protein content, not merely dilutes it. In turn this allows the new steady state to be achieved by less expansion of the interstitial volume than would otherwise be the case.

7. Control of total extracellular fluid volume

The control of the interstitial volume is normally linked to the renal control of the total extracellular volume. Control via the kidney and the central nervous system seems sufficient to control the extracellular volume since the transcapillary control of microvascular exchange exhibits such strong ability to 'auto-regulate' the interstitial volume.

However, a novel concept of connective tissue control of the interstitial fluid volume and sodium uncoupled from total extracellular volume has been suggested for tissue macrophages in skin through accumulating sodium in salt-sensitive hypertension, and without being accompanied by the accumulation of water.[29] A high-salt diet (HSD) in rats has been demonstrated to cause interstitial hypertonic sodium accumulation in skin with increased density and hyperplasia of the initial lymphatic capillary network, with sodium likely binding to GAGs.[29]

The mechanisms involved include the activation of tonicity-responsive enhancer-binding protein (TonEBP) in the mononuclear phagocyte system (MPS) cells in the loose connective tissues. The TonEBP binds the promoter of the gene encoding VEGF-C to induce VEGF-C secretion by macrophages. A depletion of the MPS or trapping VEGF-C is suggested to increase interstitial hypertonic volume retention and furthermore decrease endothelial nitric oxide synthase expression resulting in increased blood pressure in response to the HSD. It is suggested that VEGF-C acts as control for osmosensitive and hypertonicity control in the regulation of blood pressure.[29] An increased amount of sodium would have to be bound to the negatively charged GAGs or to be localized intracellularly. However, the former would require either an increased amount of negative charge on the GAGs or altered electrolyte composition in interstitial fluid, and thereby also plasma. A more likely explanation would then seem to be increased intracellular content of sodium in the connective tissue cells. The principal importance of this study is nevertheless to point towards a novel role for the connective tissue and its cells in the handling of sodium. This mechanism points towards the control of sodium balance as being, at least in part and under certain circumstances, uncoupled from renal control of extracellular fluid volume.

8. Fluid exchange in acute inflammation

Acute inflammatory reactions are frequently associated with rapid and large changes in the transcapillary exchange of fluid and protein fluxes. When oedema in skin associated with acute inflammation occurs in <10 min, there has been at least a doubling of the interstitial volume during the same time period. Since it normally takes 1–2 days to filter this amount of fluid across the capillary, the fluid flux in the most extreme cases as seen in a burn injury must have increased by ∼200–300 times above the normal control. CFC is measured to increase by two to three times above normal,[22,23] leaving the net filtration pressure to explain the largest part of the increase in capillary filtration. The increase in net filtration pressure is calculated at 250–300 mmHg and is higher than what can be generated from the circulatory system. Surprisingly, a lowering of P_{if} was seen concomitant with oedema formation rather than an increase as commonly assumed when oedema forms. Lowering of P_{if} to as much as −150 mmHg was observed in these initial studies and was associated with physical denaturation of the collagen.[24–26,30]

Several inflammatory agents, cytokines, and chemokines have been studied for the same effect on P_{if}, and a lowering, rather than an increase in P_{if}, has been seen for a large number of substances as oedema developed. Although the most pronounced lowering was seen after freezing injury[27] and burn injury,[26] both inflicting a physical injury to the tissue, several inflammatory mediators and pathological conditions associated with oedema formation have been demonstrated to have a lowering of P_{if} as part of the pathophysiology. These conditions included, among others, anaphylaxis induced by degranulating mast cells with compound 48/80 (C48/80),[31–33] platelet-activating factor,[34] acute asthma induced by antigen exposure to rats sensitized to egg albumin,[35] neurogenic inflammation following the stimulation of the vagal nerve[36] and also by LPS[37] and tumour necrosis factor-α (TNF-α).[38] Taken together, the range of conditions where a lowering of P_{if} occurred as part of the pathological processes suggested that several mediators that induced a local inflammation could also elicit a lowering of P_{if}. Some of the mediators tested include prostaglandins E_1, E_2, and I_2,[39] IL-1β and IL-6[38], and the phosphatidyl inositol 3-kinase inhibitors wortmannin and LY294002.[40]

Each case demonstrates that the lowering of P_{if} is important as a phenomenon by itself as well as demonstrating that the loose connective tissue can 'actively' enhance the capillary filtration.

It should be emphasized that P_{if} will contribute to the transcapillary fluid flux in the acute part of an inflammation. As soon as a new steady state is achieved between the tension in the fibre networks, the swelling ground substance and P_{if}, the oedema, and increased capillary filtration will have to be maintained by increased capillary hydrostatic pressure and increased capillary permeability (increased CFC and lowered capillary reflection coefficient).

9. Maintenance of an inflammatory oedema. The capillary reflection coefficient

The lowering of P_{if} will contribute in the initial phase of the inflammatory reaction to the rapid and initial flux across the microcirculation to establish the inflammatory oedema. It should be noted [cf. Eq. (1)] that a lowering of σ contributes to the increased capillary filtration by lowering the total colloid osmotic pressure gradient acting across the capillary not only during the acute phase but also at later stages of the inflammation. As the oedema develops, P_{if} will attain positive values and the maintenance of the oedema depends

Downloaded from https://academic.oup.com/cardiovascres/article-abstract/87/2/211/447690 by Karolinska Institutet University Library user on 28 June 2019

upon an increased capillary pressure (from the vasodilation) and a lowered σ.

10. Integrins and P_{if}: antibodies to β_1- and $\alpha_2\beta_1$-integrins

Insight into the molecular mechanisms involved in the control of P_{if} was obtained by parallel studies using the collagen gel contraction assay and *in vivo* experiments. In this assay, typically 100 000 fibroblasts are mixed with soluble native collagen at neutral pH, and gels are formed at 37°C. The rate at which the cells compact the collagen gel is a function of the force by which the cells act on the collagen through integrins that anchor cells to the collagen fibres.[41] The integrins are transmembrane heterodimeric adhesion receptors mediating cell–cell and cell–matrix adhesion and are built from an α- and a β-subunit and capable of inside-out an outside-in signalling.[42] There are four collagen-binding integrins $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_{10}\beta_1$, and $\alpha_{11}\beta_1$ with $\alpha_2\beta_1$ and $\alpha_{11}\beta_1$ that bind native fibrillar collagen.[33,42] A role for the $\alpha_2\beta_1$-integrin in rats in the lowering of P_{if} was established in early studies since subdermal injection of a monoclonal antibody to this integrin will lower P_{if} and cause oedema formation while a control IgG was without effect.[43] There have been *in vivo* studies in parallel with *in vitro* experiments using the collagen gel contraction assay. Thus, once an agent has been shown to lower the rate of collagen gel contraction *in vitro*, it has also been shown to cause lowering of P_{if} and oedema *in vivo*. Recently, it has also been established that the $\alpha_{11}\beta_1$-integrin also has a role similar to that previously described for the $\alpha_2\beta_1$-integrin.[33]

Having established that the $\alpha_2\beta_1$-integrin was involved in lowering P_{if}, Lidén *et al.*[32] demonstrated that the reversal of a lowered P_{if} by platelet-derived growth factor (PDGF)-BB[44] (see what follows) exerted its effect via the $\alpha_V\beta_3$-integrin. This conclusion and the novel function of the $\alpha_V\beta_3$-integrin were based on two lines of evidence. First, specific anti-β_3-integrin IgG blocked PDGF-BB-induced collagen gel contraction in cells lacking functional collagen-binding β_1-integrins or when the latter were perturbed. Second, this observation was confirmed *in vivo* since PDGF-BB had no effect in β_3-integrin null mice, i.e. the growth factor was not able to normalize P_{if} in the dermis of such animals. Thus, we therefore suggest that under normal conditions, tension from the cells to the dermal fibres is maintained by β_1-integrin-mediated contraction. Pro-inflammatory mediators will release the β_1-integrins to result in lowering of P_{if} and oedema formation, whereas PDGF-BB will counteract the tendencies to oedema through stimulating the activity of the $\alpha_V\beta_3$-integrin.

11. Reversal of an already lowered P_{if}

In the collagen gel compaction assay, several agents will raise the speed of compaction. In particular, the effect of PDGF has been studied and was typically administered subcutaneously after an anaphylactic reaction or other inflammatory reaction had been induced by i.v. dextran. PDGF-BB raised P_{if} in a dose-dependent manner, whereas both PDGF-AA, which specifically binds and stimulates the α-receptor, and fibroblast growth factor were without effect, clearly demonstrating specificity.[44] Subsequently, insulin,[45,46] prostaglandin F2α,[39] and α-trinositol[27] have also been demonstrated to reverse a lowering of P_{if}. In addition, large doses of vitamin C[47] and α-trinositol[48] attenuated the lowering of P_{if} in burn. Furthermore, corticotropin-releasing factor and mystixins have well-documented effects in limiting the oedema associated with the inflammatory response towards several inflammatory agents and do this at least in part via attenuating the lowering of P_{if} occurring as part of the inflammatory response.[49]

12. Involvement of the cytoskeleton

In relation to microvascular exchange, the cytoskeleton will influence both the function of the endothelial cells and P_{if}. Cytochalasin D disrupts actin; it lowered P_{if} and caused oedema,[50] whereas both nocodazole (depolymerizes microtubules) and colchicine (inhibits tubulin polymerization) were without effect on P_{if} and oedema formation.[50] Phalloidin caused fixation of the actin and had no effect on P_{if} and oedema formation but attenuated the effects of taxanes paclitaxel and docetaxel, which both caused fixation of microtubule, and when given alone lowered P_{if} and caused oedema.[51] These observations are in agreement with the model outlined in what follows where the expanding glycoasminglycan gel is restrained by cellular tension and the release of this tension allows the tissue to expand, in turn lowering P_{if}. Furthermore, the lowering of P_{if} in dextran-induced anaphylaxis was abolished by phalloidin.[52] Altogether, the cytoskeleton can modify P_{if}, and thereby attenuate or enhance an inflammatory oedema.

Phalloidin will also attenuate oedema formation in other models of inflammation. As two examples, phalloidin prevented bradykinin-induced leucocyte/endothelial cell-adhesive interactions and leucocyte migration,[53] and oedema induced by ischemia-reperfusion is prevented by treatment with phalloidin.[54] The referenced studies point to complex effects on several cell types after pharmacological interference with the cytoskeleton. This, in turn, requires that both interstitial changes in pressures and endothelial alterations are analysed separately when the overall effects is to be outlined.

13. Mechanistic model for dynamic control of P_{if}

We have suggested the following mechanistic model for the cellular control of P_{if} based on experiments with agents that induce a lowering of P_{if} or attenuation of the lowering of P_{if} as well as the studies on integrins, growth factors, cytokines, and chemokines. First, when loose connective tissues are excised and immersed in isotonic saline, they will swell due to the properties of the ground substance made up from hyaluronan and other GAGs.[3] Normally, the swelling is restrained by the fibre networks in the tissues, and when these are enzymatcially digested, the tissues will swell even further.[3] This is interpreted to indicate that the tissues are normally underhydrated and kept under tension by the fibre networks. A cellular tension has been added to this purely mechanical model so that the connective tissue cells provide tension to the fibre networks via the β_1-integrin system. The cells can release this strain, as in acute inflammation, with the result that the ground substance is allowed to expand until a new balance is reached between the swelling ground substance, the tension in the fibre networks, and a lowered P_{if} since there is no fluid initially available to hydrate the expanding ground substance. The lowered P_{if} initially provides an increased driving force for

Downloaded from https://academic.oup.com/cardiovascres/article-abstract/87/2/211/447690 by Karolinska Institutet University Library user on 28 June 2019

capillary filtration until a steady state is reached between the forces in the fibre networks, the ground substance, and P_{if} where the interstitial volume again is constant and capillary filtration again equals lymph flow.

14. Intracellular signalling pathways

An important issue for the studies of intracellular signalling has been to compare the *in vivo* studies in mice and rats and the *in vitro* studies using fibroblasts in the collagen gel compaction assay. The studies underlying the mechanistic model for cellular control of P_{if} clearly demonstrate that the cytoskeleton can influence P_{if} and have been based on a combination of the *in vitro* collagen gel contraction assay and *in vivo* animal studies. This same approach was used in studies where we demonstrated that under normal homeostasis the collagen-binding integrin $\alpha_2\beta_1$ is of particular importance for the control of P_{if} in rat dermis as well as to mediate contraction of collagen gels by cultured cells.[43,55]

Principally, two different classes of substances have been tested. First, those that like PDGF induced contraction of fibroblasts in the collagen gel compaction assay. The signalling by PDGF-BB involves signalling via phosphatidylinositol 3′-kinase activity. Furthermore, the migratory response induced by PDGF-BB induces reorganization of the actin cytoskeleton and has been abolished or attenuated by inhibitors of phosphoinositide-3-kinase and protein kinase C.[56] The involvement of PDGF-BB has been demonstrated in several *in vivo* studies, including the reversal of an already lowered P_{if} in anaphylaxis.[56] Based on *in vivo* experiments in transgenic mice and *in vitro* experiments, we have demonstrated that the reversal of a lowering of P_{if} by PDGF-BB[44] is dependent on phosphatidylinositol 3-kinase (PI3K) since transgenic mice having mutated PDGF β-receptors that cannot activate PI3K after ligand stimulation with PDGF-BB were unable to restore P_{if} in response to PDGF-BB.[31]

The second group is pro-inflammatory substances that attenuate the contraction rate *in vitro* and also lower P_{if} *in vivo*. The involvement and also the specificity of the prostaglandins have been demonstrated where prostaglandins E_1, E_2, and I_2 will attenuate the collagen gel compaction, whereas prostaglandin F2α will enhance it.[39] The inhibition of collagen gel compaction seems to result from signalling pathway(s), resulting in intracellular concentrations of cAMP.[57] This is in line with the observation that intradermal injection of cAMP alone will attenuate a lowering of P_{if}.[55]

Also, pro-inflammatory cytokines, e.g. IL-1 and TNF-α and IL-1, will inhibit fibroblast-mediated collagen gel contraction.[57] Prostaglandins have been demonstrated to act as second messengers for the inhibitory effects of IL-1 and TNF-α on contraction,[58] and prostaglandins E_1, E_2, and I_2 inhibit fibroblast-mediated collagen gel contraction.[39] cAMP has been suggested to interfere with collagen gel compaction via phosphorylation of myosin light chain kinase.[57,59]

The intracellular signalling pathways involved in the control of P_{if} are complex as outlined earlier, with the signalling via phosphatidylinositol 3′-kinase activity and PDGF-BB as central and important focal points.[31]

15. Future directions

The loose connective tissues can ‘actively’ alter transcapillary fluid flux via active cellular tension on the collagen and microfibril networks. Inflammatory cells and soluble mediators such as IL-1, TNF-α, and prostaglandins E_1, E_2, and I_2 can initiate an oedema response which likely is an intrinsic part of the innate immune system directly responsible for the tumour component of the classical signs of an inflammation. The oedema response in inflammation would be functionally relevant in that it would enable an increased diffusion and convection of defence plasma proteins and migration of egressed inflammatory cells into the inflamed tissue. At the same time, a process is initiated that will balance the oedema response. Our data suggest that these processes involve the PDGF system that acts upstream of the $\alpha_V\beta_3$-integrin. In addition, a wound-healing response that, if the inflammation is persistent, will lead to fibrosis should follow the oedema response. Knowledge of molecular mechanisms involved in the transition from an initial oedema response to fibrosis is important to elucidate in order to be able to intervene in the processes and reduce the risk for sustained damage and functional derangement. In order to define these mechanisms, a combination of *in vitro* systems such as collagen gel contraction and tissue has to be made.

Conflict of interest: none declared.

Funding

Financial support was received from the Research Council of Norway, The Swedish Research Council, and The Swedish Cancer Foundation.

References

1. Aukland K, Reed RK. Interstitial-lymphatic mechanisms in the control of extracellular fluid volume. *Physiol Rev* 1993;**73**:1–78.
2. Ushiki T. Collagen fibers, reticular fibers and elastic fibers. A comprehensive understanding from a morphological viewpoint. *Arch Histol Cytol* 2002;**65**:109–126.
3. Meyer FA, Laver-Rudich Z, Tanenbaum R. Evidence for a mechanical coupling of glycoprotein microfibrils with collagen fibrils in Wharton's jelly. *Biochim Biophys Acta* 1983;**755**:376–387.
4. Reed RK, Lidén Å, Rubin K. Edema and fluid dynamics in connective tissue remodelling. *J Mol Cell Cardiol* 2010;**48**:518–523.
5. Levick J, Michel C. Microvascular fluid exchange and the revised Starling principle. *Cardiovasc Res* 2010;**87**:198–210.
6. Curry FE, Adamson RH. Vascular permeability modulation at the cell, microvessel, or whole organ level: towards closing gaps in our knowledge. *Cardiovasc Res* 2010;**87**: 218–229.
7. Bates DO. Vascular endothelial growth factors and vascular permeability. *Cardiovasc Res* 2010;**87**:262–271.
8. Lindsey ML, Borg TK. Understanding the role of the extracellular matrix in cardiovascular development and disease: where do we go from here? *J Mol Cell Cardiol* 2010;**48**: 431–432.
9. Levick JR. Flow through interstitium and other fibrous matrices. *Q J Exp Physiol* 1987; **72**:409–437.
10. Taylor A, Granger D. Exchange of macromolecules across the microcirculation. In: Renkin EM, Michel CC, eds. *Handbook of Physiology The Cardiovascular System Volume IV: Microcirculation, Part 1*. American Physiological Society 1984. p467–520.
11. Michel CC, Curry FE. Microvascular Permeability. *Physiol Rev* 1999;**79**:703–761.
12. Price FM, Levick JR, Mason RM. Changes in glycosaminoglycan concentration and synovial permeability at raised intra-articular pressure in rabbit knees. *J Physiol* 1996;**495**:821–833.
13. Ostgaard G, Reed RK. Increased lymphatic hyaluronan output and preserved hyaluronan content of the rat small intestine in prolonged hypoproteinaemia. *Acta Physiol Scand* 1994;**152**:51–56.
14. Rügheimer L, Johnsson C, Maric C, Hansell P. Hormonal regulation of renomedullary hyaluronan. *Acta Physiol (Oxf)* 2008;**193**:191–198.
15. Rügheimer L, Carlsson C, Johnsson C, Hansell P. Renal hyaluronan content during experimental uncontrolled diabetes in rats. *J Physiol Pharmacol* 2008;**59**:115–128.
16. Rügheimer L, Olerud J, Johnsson C, Takahashi T, Shimizu K, Hansell P. Hyaluronan synthases and hyaluronidases in the kidney during changes in hydration status. *Matrix Biol* 2009;**28**:390–395.
17. Rutkowski JM, Markhus CE, Gyenge CC, Alitalo K, Wiig H, Swartz MA. Dermal collagen and lipid deposition correlate with tissue swelling and hydraulic conductivity in murine primary lymphedema. *Am J Pathol* 2010;**176**:1122–1129.
18. Yao H, Gu WY. Three-dimensional inhomogeneous triphasic finite-element analysis of physical signals and solute transport in human intervertebral disc under axial compression. *J Biomech* 2007;**40**:2071–2077.

Downloaded from https://academic.oup.com/cardiovascres/article-abstract/87/2/211/447690 by Karolinska Institutet University Library user on 28 June 2019

19. Olsen L, Maini PK, Sherratt JA, Dallon J. Mathematical modelling of anisotropy in fibrous connective tissue. *Math Biosci* 1999;**158**:145–170.
20. Markhus CE, Wiig H. Isolation of interstitial fluid from skeletal muscle and subcutis in mice using a wick method. *Am J Physiol Heart Circ Physiol* 2004;**287**:H2085–H2090.
21. Karlsen TV, Bletsa A, Gjerde EA, Reed RK. Lowering of interstitial fluid pressure after neurogenic inflammation in mouse skin is partly dependent on mast cells. *Am J Physiol Heart Circ Physiol* 2007;**292**:H1821–H1827.
22. Arturson G, Mellander S. Acute changes in capillary filtration and diffusion in experimental burn injury. *Acta Physiol Scand* 1964;**62**:457–463.
23. Dyess DL, Ardell JL, Townsley MI, Taylor AE, Ferrara JJ. Effects of hypertonic saline and dextran 70 resuscitation on microvascular permeability after burn. *Am J Physiol* 1992;**262**:H1832–H1837.
24. Demling RHMD. The burn edema process: current concepts. *J Burn Care Rehab* 2005; **26**:207–227.
25. Lund T, Wiig H, Reed RK. Acute postburn edema: role of strongly negative interstitial fluid pressure. *Am J Physiol* 1988;**255**:H1069–H1074.
26. Lund T, Onarheim H, Wiig H, Reed RK. Mechanisms behind increased dermal imbibition pressure in acute burn edema. *Am J Physiol* 1989;**256**:H940–H948.
27. Berg A, Aas P, Gustafsson T, Reed RK. Effect of α-trinositol on interstitial fluid pressure, oedema generation and albumin extravasation in experimental frostbite in the rat. *Br J Pharmacol* 1999;**126**:1367–1374.
28. Wiig H, Gyenge C, Iversen P, Gullberg D, Tenstad O. The role of the extracellular matrix in tissue distribution of macromolecules in normal and pathologic tissues: potential therapeutic consequences. *Microcirculation* 2008;**15**:283–296.
29. Machnik A, Neuhofer W, Jantsch J, Dahlmann A, Tammela T, Machura K *et al*. Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. *Nat Med* 2009;**15**:545–552.
30. Kinsky MP, Guha SC, Button BM, Kramer GC. The role of interstitial Starling forces in the pathogenesis of burn edema. *J Burn Care Rehabil* 1998;**19**:1–9.
31. Heuchel R, Berg A, Tallquist M, Ahlen K, Reed RK, Rubin K *et al*. Platelet-derived growth factor β receptor regulates interstitial fluid homeostasis through phosphatidylinositol-3′ kinase signaling. *Proc Natl Acad Sci USA* 1999;**96**:11410–11415.
32. Lidén A, Berg A, Nedrebø T, Reed RK, Rubin K. Platelet-derived growth factor BB-mediated normalization of dermal interstitial fluid pressure after mast cell degranulation depends on β3 but not β1 integrins. *Circ Res* 2006;**98**:635–641.
33. Svendsen OS, Barczyk MM, Popova SN, Lidén A, Gullberg D, Wiig H. The α11β1 integrin has a mechanistic role in control of interstitial fluid pressure and edema formation in inflammation. *Arterioscler Thromb Vasc Biol* 2009;**29**:1864–1870.
34. Iversen VV, Nedrebø T, Borge BA, Salvesen GS, Reed RK. Platelet activating factor (PAF) increases plasma protein extravasation and induces lowering of interstitial fluid pressure (P) in rat skin. *Acta Physiol Scand* 2005;**185**:5–12.
35. Woie K, Westerberg E, Reed RK. Lowering of interstitial fluid pressure will enhance edema in trachea of albumin-sensitized rats. *Am J Respir Crit Care Med* 1996;**153**: 1347–1352.
36. Woie K, Koller ME, Heyeraas KJ, Reed RK. Neurogenic inflammation in rat trachea is accompanied by increased negativity of interstitial fluid pressure. *Circ Res* 1993;**73**: 839–845.
37. Nedrebø T, Reed RK. Different serotypes of endotoxin (lipopolysaccharide) cause different increases in albumin extravasation in rats. *Shock* 2002;**18**:138–141.
38. Nedrebø T, Berg A, Reed RK. Effect of tumor necrosis factor-α, IL-1β, and IL-6 on interstitial fluid pressure in rat skin. *Am J Physiol* 1999;**277**:H1857–H1862.
39. Berg A, Ekwall AK, Rubin K, Stjernschantz J, Reed RK. Effect of PGE_1, PGI_2, and $PGF_2\alpha$ analogs on collagen gel compaction *in vitro* and interstitial pressure *in vivo*. *Am J Physiol* 1998;**274**:H663–H671.
40. Åhlen K, Berg A, Stiger F, Tengholm A, Siegbahn A, Gylfe E *et al*. Cell interactions with collagen matrices *in vivo* and *in vitro* depend on phosphatidylinositol 3-kinase and free cytoplasmic calcium. *Cell Adhes Commun* 1998;**5**:461–473.
41. Gullberg D, Gehlsen KR, Turner DC, Åhlen K, Zijenah LS, Barnes MJ *et al*. Analysis of $\alpha_1\beta_1$, $\alpha_2\beta_1$ and $\alpha_3\beta_1$ integrins in cell–collagen interactions: identification of conformation dependent $\alpha_1\beta_1$ binding sites in collagen type I. *EMBO J* 1992;**11**: 3865–3873.
42. Hynes RO. Integrins: bidirectional, allosteric signaling machines. *Cell* 2002;**110**: 673–687.
43. Reed RK, Rubin K, Wiig H, Rodt SÅ. Blockade of β_1-integrins in skin causes edema through lowering of interstitial fluid pressure. *Circ Res* 1992;**71**:978–983.
44. Rodt SÅ, Åhlen K, Berg A, Rubin K, Reed RK. A novel physiological function for platelet-derived growth factor-BB in rat dermis. *J Physiol* 1996;**495**:193–200.
45. Svendsen OS, Lidén Å, Nedrebø T, Rubin K, Reed RK. Integrin $\alpha_v\beta_3$ acts downstream of insulin in normalization of interstitial fluid pressure in sepsis and in cell-mediated collagen gel contraction. *Am J Physiol Heart Circ Physiol* 2008;**295**:H555–H560.
46. Nedrebø T, Karlsen TV, Salvesen GS, Reed RK. A novel function of insulin in rat dermis. *J Physiol* 2004;**559**:583–591.
47. Tanaka H, Lund T, Wiig H, Reed RK, Yukioka T, Matsuda H *et al*. High dose vitamin C counteracts the negative interstitial fluid hydrostatic pressure and early edema generation in thermally injured rats. *Burns* 1999;**25**:569–574.
48. Lund T, Reed RK. Alpha-trinositol inhibits edema generation and albumin extravasation in thermally injured skin. *J Trauma* 1994;**36**:761–765.
49. Gjerde EA, Woie K, Wei ET, Reed RK. Corticotropin-releasing hormone inhibits lowering of interstitial pressure in rat trachea after neurogenic inflammation. *Eur J Pharmacol* 1998;**352**:99–102.
50. Berg A, Rubin K, Reed RK. Cytochalasin D induces edema formation and lowering of interstitial fluid pressure in rat dermis. *Am J Physiol Heart Circ Physiol* 2001;**281**: H7–H13.
51. Brønstad A, Berg A, Reed RK. Effects of the taxanes paclitaxel and docetaxel on edema formation and interstitial fluid pressure. *Am J Physiol Heart Circ Physiol* 2004; **287**:H963–H968.
52. Brønstad ARA, Berg A, Reed RK. Effect of the cytoskeletal fixation agent phalloidin on transcapillary albumin transport and interstitial fluid pressure in anaphylaxis in the wistar rats. *Microcirculation* 2002;**9**:197–205.
53. Jerome SN, Dore M, Paulson JC, Smith CW, Korthuis RJ. P-selectin and ICAM-1-dependent adherence reactions: role in the genesis of postischemic no-reflow. *Am J Physiol Heart Circ Physiol* 1994;**266**:H1316–H1321.
54. Shigematsu S, Ishida S, Gute DC, Korthuis RJ. Bradykinin-induced proinflammatory signaling mechanisms. *Am J Physiol Heart Circ Physiol* 2002;**283**:H2676–H2686.
55. Rodt SÅ, Reed RK, Ljungström M, Gustafsson TO, Rubin K. The anti-inflammatory agent alpha-trinositol exerts its edema-preventing effects through modulation of β_1 integrin function. *Circ Res* 1994;**75**:942–948.
56. Rodt SÅ, Åhlén K, Berg A, Rubin K, Reed RK. A novel physiological function for platelet-derived growth factor-BB in rat dermis. *J Physiol* 1996;**495**:193–200.
57. van Wieringen T, Kimani SG, Hultgård-Ekwall A-K, Forsberg J, Reyhani V, Engström Å *et al*. Opposite effects of PDGF-BB and prostaglandin E1 on cell-motility related processes are paralleled by modifications of distinct actin-binding proteins. *Exp Cell Res* 2009;**315**:1745–1758.
58. Zhu JIE, Majumdar S, Qiu Y, Ansari T, Oliva A, Kips JC *et al*. Interleukin-4 and interleukin-5 gene expression and inflammation in the mucus-secreting glands and subepithelial tissue of smokers with chronic bronchitis. Lack of relationship with CD8+ cells. *Am J Respir Crit Care Med* 2001;**164**:2220–2228.
59. Ehrlich HP, Rockwell WB, Cornwell TL, Rajaratnam JB. Demonstration of a direct role for myosin light chain kinase in fibroblast-populated collagen lattice contraction. *J Cell Physiol* 1991;**146**:1–7.

Downloaded from https://academic.oup.com/cardiovascres/article-abstract/87/2/211/447690 by Karolinska Institutet University Library user on 28 June 2019