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Review Article

New and active role of the interstitium in control of interstitial fluid pressure: potential therapeutic consequences

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Here we present recent data indicating that the present view of the interstitium as a passive fluid reservoir has to be revised. The connective tissue cells and extracellular matrix have a role in the control of P_{if} and a fundamental role in the rapid development of edema in burns and in the initial swelling in inflammation by generating a lowering of interstitial fluid pressure. In this process, the β_1-integrin system seems to provide a common pathway by which the cells can lower as well as raise P_{if}. Inflammatory swelling can be reversed by endo- and exogenous substances, thereby suggesting that the connective tissue can serve as a novel target for pharmacological intervention. Furthermore, the new knowledge in interstitial physiology on means to reduce interstitial fluid pressure may be of importance for drug delivery into solid tumors, where a high P_{if} limits the uptake of therapeutic agents.

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The interstitium has been assigned a rather passive role for transcapillary fluid balance. This role is supposed to be exerted via changes in interstitial hydrostatic and colloid osmotic pressure counteracting changes in capillary filtration, resulting in 'autoregulation' of interstitial fluid volume. In this review we will summarize experiments showing a more active role of the connective tissue cells and the extracellular matrix in transcapillary fluid exchange and thereby in body fluid homeostasis. This role is fundamental for the rapid development of edema in burns and the initial swelling in inflammation. Moreover, we will present data showing that swelling can be reversed by endogenous and exogenous substances. A combination of classical physiological methods and techniques from cellular and molecular biology have provided new insight into control of interstitial fluid pressure by connective tissue cells and the adhesion receptors that anchor them to structural connective tissue components. β_1-integrin adhesion receptors seem to provide a common pathway by which cells can raise as well as lower interstitial fluid pressure. α-Trinositol and platelet-derived growth factor BB can counteract the induced inflammatory swelling, suggesting that the connective tissue can serve as a novel target for pharmacological intervention in inflammation. Furthermore, the new knowledge on interstitial physiology have important implications for drug delivery into solid tumors where a high interstitial fluid pressure limits the uptake of therapeutic agents

Traditionally, the interstitium is defined as the extracellular fluid compartment located between blood vessels and cells. All organs have interstitium, although at a variable amount. The structure and composition of the interstitium differs greatly depending on the mother organ, but may also be similar, like the particular interstitia surrounding peripheral blood vessels or the loose connective tissue underlying basement membranes of glandular structures. Interstitial water and solutes, the interstitial fluid volume, serves as transport medium for nutrients and waste products between cells and capillary blood. The interstitial fluid volume (V_i) is kept fairly constant under normal conditions, at approximately 20% of body weight, by several interstitial buffering

mechanisms (1), including structural changes, adjustment of forces acting across the capillary wall and lymph flow.

This traditional concept of the interstitium does not include a role for cells (1, 2). In this review we will focus on the interstitium in inflammation, based on available data we will present a model showing an active role of the interstitium in inflammation, and for this model we will need to include cells in the term 'interstitium'. The typical connective tissue cells in this context are those that are not organ specific but are an integral part of the extracellular matrix.

Fluid transport across the capillary wall can be described quantitatively according to the so called Starling hypothesis (Fig. 1):

$$J_v = K_f[(P_c - P_{if}) - \sigma(COP_c - COP_{if})],$$

where J_v is net capillary fluid filtration, K_f is the capillary filtration coefficient, P_c and P_{if} are the hydrostatic pressures in capillary and interstitium, respectively, COP_c and COP_{if} are the corresponding colloid osmotic pressures, and σ is the capillary reflection coefficient for proteins. Here we will focus on more recent data on P_{if} and how this pressure participates in control of V_i and how it is affected by the connective tissue cells. For a broader description of transcapillary fluid exchange the reader is referred to recent reviews on transcapillary fluid exchange and interstitial fluid volume regulation (1–6). Furthermore, the methods for P_{if} measurements have been described extensively in recent reviews (1, 7).

Normal transcapillary exchange

Previously, the interstitium has been assigned a rather passive role for fluid balance. As shown by the Starling hypothesis, the transcapillary fluid flux is the product of the pressure imbalance across the capillary wall, i.e. the net filtration pressure, and the capillary filtration coefficient, K_f, again a product of the available area for exchange and the hydraulic conductivity of the capillary ('water permeability'). The colloid osmotic pressure in plasma and interstitial fluid in experimental animals is approximately 20 and 12 mmHg, respectively, with corresponding pressures of approximately 26 and 15 mmHg in man (1, 2). Of the other interstitial forces, interstitial fluid hydrostatic pressure is approximately −1 mmHg in skin and close to ambient pressure in skeletal muscle (1, 7). By use of these numbers we observe that the capillary hydrostatic pressure required to have zero net capillary filtration pressure will be approximately 10 mmHg. Although the net capillary filtration pressure can not be measured, it can be estimated as the ratio between lymph flow (i.e. net fluid filtration) and the capillary filtration coefficient (1, 2, 8). Thus, under normal conditions the pressure imbalance across the capillary is in the order of 0.5–1 mmHg in skin and

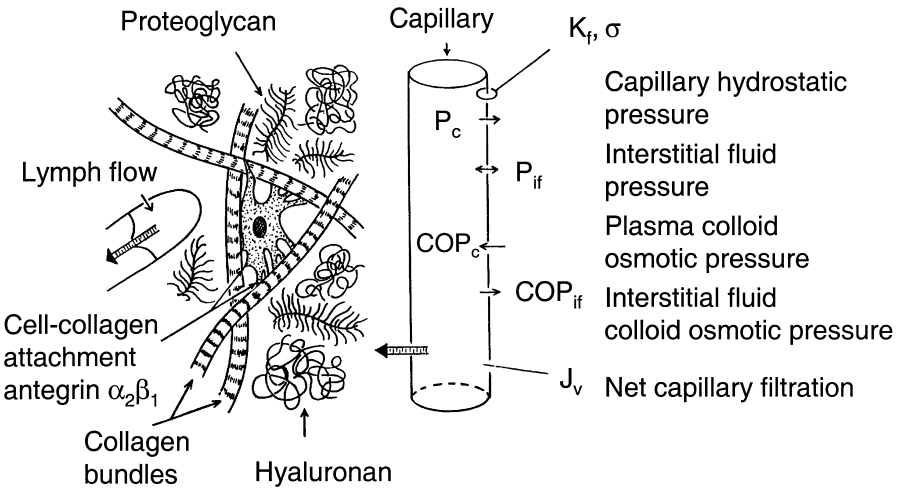

Fig. 1. Overview for the transcapillary-interstitial fluid exchange system. The transcapillary hydrostatic (P) and colloid osmotic pressure (COP) determining capillary fluid flux. Subscripts 'p' and 'if' denote plasma and interstitial fluid, respectively. K_f and σ are capillary hydraulic conductivity and capillary reflection coefficient, respectively. The capillary net filtration pressure (ΔP), is normally 0.5–1 mmHg and results in a net fluid filtration (J_v) that is removed by lymph flow. Collagen and hyaluronan are abundant structural components of loose connective tissues. Redrawn from Wiig (7).

skeletal muscle (1, 2). This net filtration pressure is sufficient to create a net fluid filtration, which will turn over the interstitial fluid in 12–24 h in rat skin whereas a somewhat longer time is needed in man (1).

Normal control of interstitial fluid volume is obtained via changes in interstitial hydrostatic and colloid osmotic pressures, which will counteract changes in capillary fluid filtration and aim at restoring normal filtration, a phenomenon that may be termed 'autoregulation' of interstitial fluid volume (1, 2). To illustrate this mechanism, let us consider a situation with increased capillary filtration brought about by an increased capillary hydrostatic pressure or lowering of the plasma colloid osmotic pressure. These conditions will both cause increased fluid flux with lowered protein concentration and result in a reduction of plasma protein concentration in the capillary filtrate (3, 6). The enhanced capillary fluid filtration will increase interstitial fluid volume and P_{if}, but at the same time also reduce the interstitial fluid colloid osmotic pressure.

As for P_{if}, the rise in pressure will be determined by the interstitial compliance (C_i), defined as the ratio between the change in interstitial fluid volume (ΔV_i) and corresponding change in interstitial fluid pressure, i.e. $C_i = \Delta V_i / \Delta P_{if}$. The normal relationship between volume and pressure is shown in Fig. 2. We observe that the volume-pressure relationship is linear (i.e. compliance is constant) in dehydration and in the initial stage of overhydration, and in this area compliance is 14% $mmHg^{-1}$, meaning that a change of V_i of 14% will lead to a change in P_{if} of 1 mmHg. When V_i increases above control volume, the volume-pressure curve levels off implying that compliance increases. At overhydration above 150–200% of V_i, compliance is virtually infinite because there is no increase in P_{if} as V_i increases. At excessively high volumes compliance may again decrease because of restraints offered by fascias, capsules, etc.

If we return to the situation with increased fluid filtration, the interstitial fluid volume and thereby P_{if} will increase to a level given by the compliance relationship, thus counteracting further fluid filtration. In dehydration, opposite changes in P_{if} will occur. In organs with low compliance, e.g. encapsulated organs like the brain, an increased V_i will cause a large increase in P_{if} with a corresponding greater importance of P_{if} in maintaining constant interstitial fluid volume (9). As with P_{if}, changes in interstitial fluid colloid osmotic pressure resulting from altered capillary filtration will counteract the primary changes in V_i. An increased filtration will result in reduced protein concentration in the filtrate which will consequently reduce the interstitial fluid protein concentration and the interstitial fluid colloid osmotic pressure (COP_{if}). The lowered interstitial colloid osmotic pressure will in turn decrease the net capillary filtration pressure and return the net capillary filtration towards normal, thereby limiting changes in V_i. When interstitial compliance is ~14%, the changes in P_{if} and COP resulting from the altered interstitial fluid volume are of similar magnitude and therefore of equal importance in maintaining constant V_i.

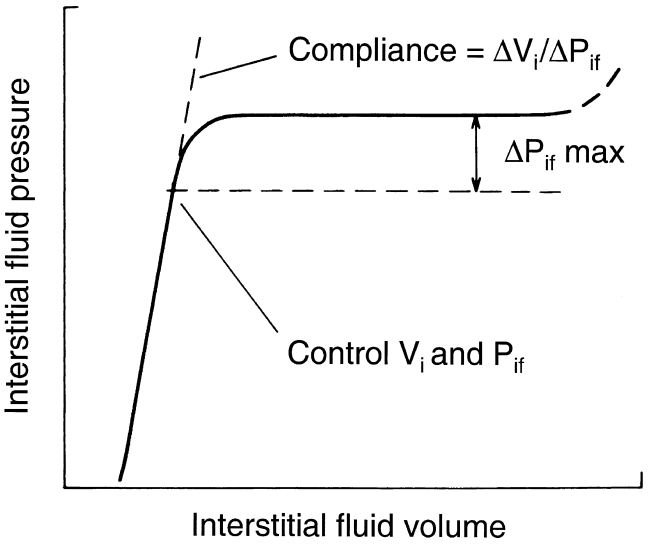

Fig. 2. Interstitial fluid volume (V_i)—pressure (P_{if}) relationship in skin and muscle. Note that the volume-pressure relationship is linear in dehydration and in the initial phase of overhydration. Overhydration corresponding to an increase in interstitial fluid volume 50–100% above control level results in increased compliance ($\Delta V_i/\Delta P_{if}$). ΔP_{if} max is the maximal counter-pressure acting against an increased filtration. Redrawn from Aukland and Reed (1).

The final factor contributing to maintenance of interstitial fluid volume is lymph flow, which will increase and decrease with P_{if} and V_i. Filling of the initial lymphatics requires a pressure gradient, and although the mechanisms for formation of lymph are still not fully understood (1), P_{if} is one of the two pressures determining the pressure gradient from the interstitium to the initial lymphatic. P_{if} is considered a filling pressure for the initial lymphatics, and will thereby contribute to maintain a constant V_i. To summarize, the resulting changes in interstitial fluid hydrostatic and colloid osmotic pressure as well as lymph flow to changes in capillary filtration are such that alterations in interstitial fluid volume will be limited, a phenomenon that may be described as 'autoregulation' of V_i.

Tissue injury and inflammation

We have considered normal interstitial fluid balance where the interstitium has a rather passive role in fluid balance, but we will now consider tissue injury and inflammation, and demonstrate that under certain circumstances this tissue may attain an 'active' role in enhancing transcapillary fluid flux: the first experimental observation to indicate that a primary reduction in P_{if} could enhance transcapillary fluid flux stem from burn injury studies (10). In burn injuries, visible edema develops very rapidly (i.e. within 5–10 min). A visible edema requires a doubling of interstitial fluid volume (2), and as the normal turnover of V_i is 12–24 h in experimental animals and even longer in humans (see above), this rapidity of edema generation means that the capillary filtration must have increased several hundred times above normal.

If we consider the Starling hypothesis, the increased capillary filtration may result from an increase in hydraulic conductivity ('water permeability') or net filtration pressure. Arturson and Mellander (11) measured edema formation and K_f in the paws of anesthetized cats, and found that K_f was 2–3-fold normal in burned skin, a value for this parameter that has been verified in other studies (12, 13). If this was the only change occurring at the capillary–interstitial barrier in this situation, the time required for the doubling of V_i provided unchanged lymph flow would be reduced from 12 to 24 h to 6–12 h. Given the modest rise in K_f in burn injuries, an increase in net capillary filtration pressure would have to be the explanation for the rapid edema generation. Based on the rate of edema generation and the measured K_f in burn injury, Arturson and Mellander (11) calculated a net filtration pressure of 200–300 mmHg, which is far beyond what can be obtained through increased capillary pressure. They attributed the observed rise in filtration pressure to small solute osmolarity. The edema in this situation should therefore not be characterized as a 'permeability' edema because the explanation for the rapid edema generation is a dramatic increase in transcapillary filtration rather than an increase in water permeability.

In the search for an explanation for the rise in net filtration pressure, Lund et al. (10) measured interstitial fluid pressure in full thickness burn injury induced in rats. Somewhat surprisingly, they found that P_{if} did not rise to positive values during edema generation but actually decreased from −1 to −150 mmHg (Fig. 3). The most negative P_{if} was observed when the transcapillary fluid transport into the skin was blocked by circulatory arrest, preventing

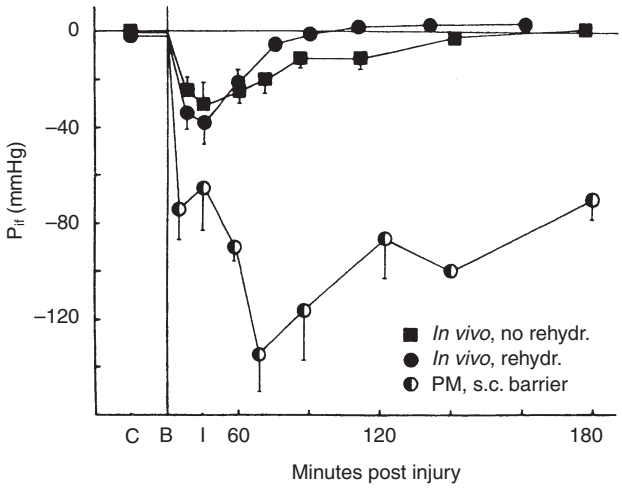

Fig. 3. Interstitial fluid pressure (P_{if}) in rat dermis measured with micropipettes after induction of burn injury and influence of fluid availability. In vivo burn injury without fluid therapy (●), with fluid therapy (■) and with subcutaneous (s.c.) plastic barrier and induction of injury post mortem (PM, half-filled circles). C: pre-burn control measurement; B: burn injury, I: start infusion. Values are mean ± SE. Error bars contained in symbols in control situation. Reprinted from Lund et al. (10) with permission.

transport of fluid from underlying muscle by interposing a water impermeable membrane. If these measures were not taken, P_{if} would rise to positive values (see Fig. 3). The lowering of P_{if} explains the rise in capillary net filtration made by Arturson and Mellander (11) 25 years earlier. However, the principal importance of this observation was for the first time to assign an 'active' role to the interstitium in transcapillary exchange in the sense that the interstitium enhanced rather than prevented transcapillary fluid flux. The increased negativity of P_{if} in burn injury could at least partly be attributed to heat denaturation of collagen to gelatin, i.e. conversion of fibrillar collagen from water insoluble to random-coil denatured collagen and water-soluble α-chains and degradation products (14). Later it was shown that it was possible to attenuate the lowering of P_{if} and edema generation by α-trinositol (54). In a second-degree injury the phenomenon also occurs and lowers P_{if}, although not as profoundly as in third-degree injury, but in this case it is attenuated by large doses of vitamin C (15). The lowering of P_{if} in burns has later been confirmed in other studies (16, 17).

Acute inflammation

As for burn injury, one of the clinical hallmarks of other types of acute inflammation is rapid edema formation, and spurred investigations on whether

the mechanisms behind the swelling were similar to those of the former condition. In parallel with burn injuries, the reported K_f in other types of acute inflammation is 2–3-fold that of controls (18). In analogy to burn injuries this suggests that rapid edema generation requires a dramatic increase in net capillary filtration pressure to explain the rate at which edema is formed in other types of acute inflammation. A similar observed negativity in P_{if} has been observed for several acute inflammatory reactions, as summarized in Table 1. These observations suggest that collagen denaturation is not the only mechanism capable of generating negative tissue pressure. As an example, the anaphylactic reaction in rats caused by dextran results in edema formation localized to the paw and nose. Intravenous administration of dextran in this species resulted in a fall in P_{if} from -1 to between -6 and -10 mmHg (19). As for burn injuries, P_{if} returned to the control level and became positive when edema was allowed to develop when the circulation was intact. Although P_{if} in the studies included in Table 1 did not reach the negative values reported in burn injuries, these studies are important because they show that the increased negativity of P_{if} occurs in situations closer to 'normal physiology' than those represented by burns. Two series of studies included in Table 1 will be described in some detail later, namely those including neurogenic inflammation and the reaction caused by subdermal injection of β_1-integrin antibody, the latter showing a likely link to a common molecular mechanism through which the inflammatory reactions may affect P_{if}.

Neurogenic inflammation

Neurogenic inflammation is an inflammatory reaction that is characterized by edema formation and plasma protein extravasation in response to stimulation of sensory fibers of the vagal nerve (20–22) and is the result of the release of substance P (SP), calcitonin gene-related peptide (CGRP) and neurokinin A. Release of these mediators also results in morphological changes in airway capillaries, as studied extensively by McDonald and coworkers (23, 24). They have demonstrated the formation of gaps up to 1.5 μm in diameter between the endothelial cells, appearing most frequently in venules 15–50 μm in diameter, but also appearing in smaller venules (23).

The normal transcapillary pressures in trachea and the bronchial circulation has been addressed in a limited number of studies (25–27). As for other types of

Table 1

Interstitial fluid pressure (P_{if}) in different tissues and species following administration of various inflammatory agents.

Tissue	Experimental model (species)	P_{if} (mean ± SD) mmHg Control	Experiment	Remarks	Reference
Skin	Burn injury (rat)	-1.1 ± 0.4	-95 to -135	p.m.	(10)
		-1.5	-90 to -170	p.m.	(14)
		-1 to -2	-46.8 ± 10.1	*in vivo*	(15)
		-1 to -2	-40.9 ± 7	*in vivo*	(17)
	Burn injury (sheep)	-2	-11	*in vivo*	(16)
	Xylene (rat)	-1.3 ± 0.6	-7.5	p.m.	(73)
	Carrageenan (rat)	-0.4 ± 0.1	-4.8 ± 0.6	p.m.	(74)
	Zymosan (rat)	0.4 ± 0.1	-2.5 ± 3.1	p.m.	(75)
	Integrins (rat)				
	Anti-β_1	-0.6 ± 0.8	-4 to -6	p.m.	(49)
	Fab anti-β_1	-0.3 ± 0.3	-3.1 ± 0.4	p.m.	(55)
	Anti-$\alpha_1\beta_1$	No effect		p.m.	(49)
	Anti-$\alpha_2\beta_1$	-0.8 ± 0.3	-3.6 ± 0.3	p.m.	(50)
	Prostaglandins (rat)				
	PGE_1	-0.8 ± 0.4	-3.0 ± 0.4	p.m.	(69)
	PGI_2	-0.8 ± 0.4	-3.7 ± 0.9	p.m.	(69)
	Cytochalasin D (rat)	-0.8 ± 0.5	-2.8 ± 0.7	p.m.	(51)
Trachea					
	Dextran (rat)	-2.5 ± 0.4	-10.3 ± 2.6	p.m.	(76)
	C48/80 (rat)	-1.1 ± 0.2	-8.9 ± 2.5	p.m.	(77)
	Polymyxin B (rat)	-1.1 ± 0.2	-4.2 ± 0.9	p.m.	(77)
	Experimental asthma (rat)	-1.3 ± 0.3	-5.8 ± 0.5	p.m.	(59)
	Neurogenic inflammation (rat)	-1.4 ± 1.1	-10.6 ± 3.4	p.m.	(28)
	After capsaicin treatment	-1.2 ± 0.3	-0.7 ± 0.2	p.m.	(28)
Synovium					
	Cytochalasin D (rabbit)	-0.7 ± 0.3	no effect	p.m.	(53)

acute inflammation, the edema generation in neurogenic inflammation is rapid, raising the question of driving forces in this process. In analogy with the studies on P_{if} and inflammation mentioned earlier, Woie and coworkers measured pressure in neurogenic inflammation induced by electrical stimulation of the vagal nerve (28). Under these conditions they found that P_{if} fell from a control level of -1 mmHg to between -5 and -10 mmHg, starting within a few seconds after the onset of electrical stimulation and levelling off within 5 min. Capsaicin treatment, which depletes neuropeptides, abolished this reaction, showing the dependency on neuropeptides. Recent studies have suggested a complicated interplay between the sensory nerves, mast cells and loose connective tissue, suggesting an important role for the mast cell in eliciting at least some of the biological responses induced by stimulation of sensory nerves (29), which is in line with the results obtained during mast cell degranulation in anaphylaxis mentioned earlier (19).

Mechanisms involved in edema formation: role of β_1-integrins

The observation that lowering of P_{if} is a general mechanism participating in the development of inflammatory edema, spurred the interest for the cell biological and molecular events associated with the edema process. This led us to perform experiments using anti β_1-integrin antibodies, based on the following reasoning. Loose connective tissues have an inherent tendency to expand when given free access to saline. A piece of loose connective tissue will thus double its volume in 24–48 h with the major part of the swelling taking part early in this period (30, 31). This tendency to swell is at least in part caused by hyaluronan, as it was abolished after hyaluronan digestion. Moreover, based on a series of enzymatic digestion experiments, Meyer et al. concluded that the tendency of a tissue to swell was balanced by the collagen network and a fibril network that was physically restraining the swelling hyaluronan gel. The connective tissue cells could thus exert a tension on the fiber networks of the tissue via the β-integrin receptors, thereby affecting the swelling characteristics of the tissue and P_{if}. The force needed to exert this tension would be generated intracellularly and relayed to the fiber network by means of β_1-integrin receptors. The role of β_1-integrins in development of inflammatory edema in experimental animals has been explored by the use of the cell-mediated contraction of three-dimensional collagen gels *in vitro* (32, 33) in parallel with *in vivo* experiments. Much focus has been on the role of integrins in the vasculature, where there are presently two known physiological functions of integrin activation: integrins are involved in blood clotting or more specifically in adherence and aggregation of blood platelets and in adherence and extravasation of white blood cells through capillary walls during inflammation (34). Here, however, we will consider the role of integrins for transmission of tension in the extracellular matrix.

Integrins are transmembrane heterodimeric glycoproteins composed of non-covalently associated α- and β-subunits. Integrins mediate intracellular as well as cell-extracellular matrix adhesion (34–36). The eight β- and 18 α- presently characterized integrin subunits combine into the 24 integrin heterodimers identified so far. Four integrins are known to bind triple helical interstitial collagens, namely the $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_{10}\beta_1$ and $\alpha_{11}\beta_1$ integrins (36, 37).

Integrins, or their complexes, function as mechanoreceptors, which sense tension exerted on cells by ECM structures (38–40). Fibroblasts cultured in three-dimensional collagen lattice contract the lattice (41, 42) by forces most likely related to the 'traction forces' characteristically generated by cultured cells on the underlying matrix (43, 44). The contraction process is stimulated by PDGF-BB (33, 45) and depends on β_1-integrins (33), particularly the collagen-binding integrin $\alpha_2\beta_1$ (46, 47). Thus, integrins both convey mechano-receptor signals and are active in force transmission from the cell interior to the ECM. The dependence of β_1-integrins in the lattice contraction can be shown by adding antibodies, which slow or completely attenuate the contraction (33). When the antibody to β_1-integrins is removed from the medium, the contraction process resumes, showing that the antibody is not toxic nor permanently damages the cells (33).

The cell-mediated contraction of three-dimensional collagen gels *in vitro* can be used as an *in vitro* model for connective tissue cell-generated tension on the extracellular matrix fibers (32, 33). At optimal amounts, the fibroblasts will contract the collagen gel to 10–20% of its original volume in 24 h. The decrease in gel volume is believed to result from fibroblasts migrating and sending out processes through the collagen gel inducing traction forces (48). In support of this assumption is the blocking of the gel contraction by cytochalasin D, which disrupts the actinomyosin contractile apparatus. The rate of gel contraction is affected by several growth factors and cytokines, platelet derived growth factor (PDGF) and endothelin enhance the contraction process, whereas interleukin-1

and tumor necrosis factor-α will slow the rate of contraction (48). Furthermore, increased levels of cAMP will inhibit the contraction process. Taken together, these observations suggest a role for the actinomyosin contractile apparatus of the cell in collagen gel contraction, providing the connective tissue with a contractile force, which when reversed or diminished would allow the tissue to swell. That connective tissues actually will swell when soaked in saline has been shown by Meyer and coworkers, as discussed earlier (30, 31).

The *in vitro* experiments using the cell-mediated contraction of three-dimensional collagen gels *in vitro* formed the basis for studies on the effect of anti β_1-integrin antibodies on P_{if} and edema formation. In the rat paw, a polyclonal anti β_1-integrin IgG, which inhibits fibroblast-mediated collagen adhesion *in vitro*, lowered P_{if} from −1 to approximately −5 mmHg (49). The reduction in pressure (illustrated in Fig. 4) occurred concomitantly with edema formation, having a time course and magnitude similar to that seen in several inflammatory reactions. Polyclonal antirat fibronectin IgG and the fibronectin receptor binding protein Arg-Gly-Asp (RGD) and its control peptide Arg-Gly-Glu (RGE) were without effect, showing that this effect was not mediated by fibronectin. These experiments demonstrated that blockage and/or perturbation of the β_1-integrins could induce a reaction similar to that seen in several of the acute inflammatory reactions discussed earlier. Later studies using monoclonal function blocking antirat antibodies towards $\alpha_1\beta_1$- and $\alpha_2\beta_1$-integrins have shown no effect of the former whereas the latter induced increased negativity of P_{if}, strongly suggesting that the collagen- and laminin-binding $\alpha_2\beta_1$ is the integrin involved in creating the more negative P_{if} during inflammation (50). The role of the cytoskeleton in the process of controlling P_{if}, or more specifically the intracellular actin filament system, was shown in recent experiments using the F-actin disrupting agent cytochalasin D, which generated a negative P_{if} in a dose-dependent manner (51). These results are supported by recent studies showing that treatment with phalloidin, an agent that fixes the actin filament within the cell and thereby blocks the link between the intra- and extracellular phases in the interstitium, abolished the reduction in P_{if} observed in dextran-induced edema (52).

A system of this type, with elements in and links between the intra- and extracellular compartments would allow for many and diverse inflammatory reactions to act via a final common end-point, resulting in the same response in interstitial fluid pressure. Although such a mechanism can explain the observations in skin and trachea using various inflammatory agents, it may not apply to all tissues and species. In a recent paper on fluid transport in rabbit joints, Poli and coworkers were unable to demonstrate an effect of cytochalasin D on P_{if} in the synovium, even though the substance led to a marked filtration into the joint mimicking an inflammatory reaction (53).

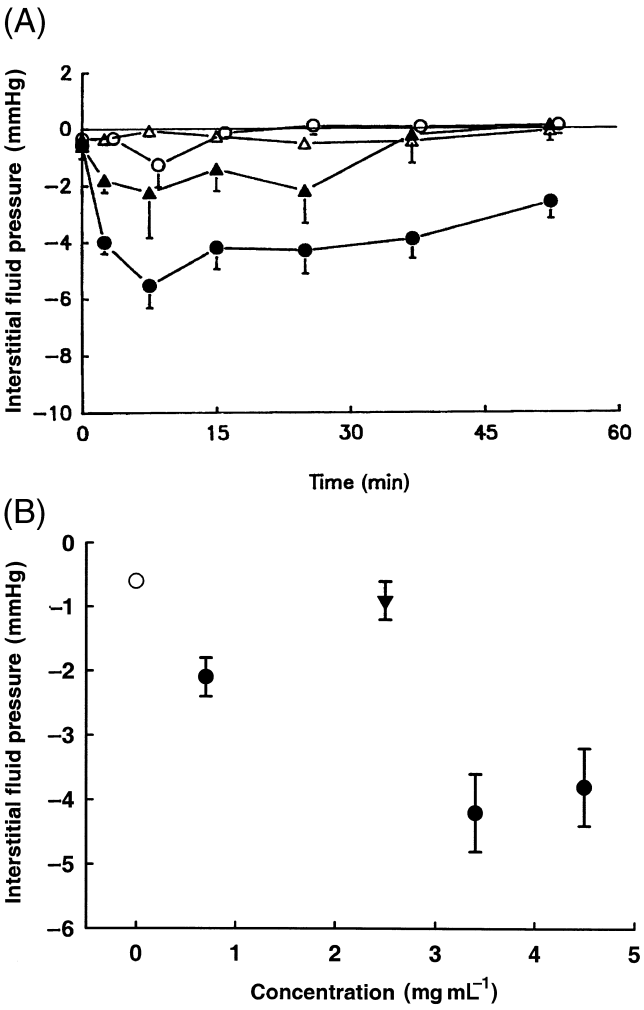

Fig. 4. (A) Effect on interstitial fluid pressure (P_{if}) of subdermal injection of anti-β_1-integrin IgG (3.4–4.5 mg/ml) (●) and preimmune IgG (2.5 mg/ml) (○) obtained from the same rabbit. Also shown is the effect of anti-fibronectin IgG (2.5 mg/ml) (△) and peptide with RGD-sequence (6 mg/ml) (▲). Pressures are mean ± 1 SE. (B) Interstitial fluid pressure as a function of concentration of anti-β_1-integrin IgG (●). Also shown saline control (○) and preimmune IgG (▼). Mean ± SE. Redrawn from Reed et al. (49).

Attenuation of the reduction in interstitial fluid pressure

The inflammatory reactions discussed earlier (except the synovium) all resulted in lowering of P_{if}. In

analogy with the collagen gel contraction assay we have tested substances known to enhance the collagen gel contraction rate to see if it was possible to reverse or abolish a lowering of P_{if} induced by inflammatory reactions and antibodies against β_1-integrin.

The first substance shown to have an effect in this respect was the experimental anti-inflammatory drug α-trinositol (D-myo-inositol 1,2,6 triphosphate) (54, 55). This drug acts via an intracellular mechanism that is not fully elucidated, but involves intracellular calcium channels (56). α-Trinositol given before as well as after onset of an inflammatory response will abolish or strongly attenuate the fall in P_{if} and edema formation in burns (54), dextran anaphylaxis (57), blockade of β_1-integrins (55) and local frostbite injury (58). Moreover, the same substance has a similar effect in the trachea in conditions like experimental asthma (59), dextran anaphylaxis (57) and neurogenic inflammation (60). These experiments show that α-trinositol may become an important new pharmacological tool in preventing edema in vital tissues in the early phase of inflammation.

Another potent stimulator of collagen gel contraction *in vitro* is the BB isoform of platelet-derived growth factor (PDGF-BB), and accordingly this substance was tested for effect *in vivo*. In experiments where PDGF-BB was injected subdermally after dextran anaphylaxis had induced lowering of P_{if} in the rat paw, the substance actually brought pressure back to the normal level as shown in Fig. 5. There are two important implications of these observations. Firstly, they show that the lowering of P_{if} can be reversed by an endogenous substance. Secondly, these data suggest that the interstitial matrix is in a dynamic state in the sense that the tension on the connective tissue fibers, i.e. mostly collagen, can be increased and decreased within a few minutes to modify P_{if}. A proposed schematic model of the events leading to changes in compaction of the loose connective tissue and thereby influencing P_{if} is shown in Fig. 6.

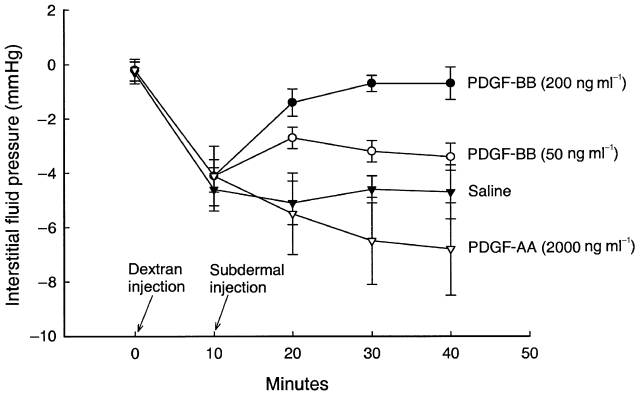

Fig. 5. Interstitial fluid pressure as a function of time after systemic dextran injection and subdermal injection of test substances. Mean ± SE. Redrawn from Rodt et al. (50).

The importance of the PDGF-system for the control of P_{if} has been explored further on the molecular level. In mice, PDGF β-receptors were specifically mutated in tyrosine residues, which binds and activates phosphatidylinositol-3′-kinase when phosphorylated on the ligand-activated receptor (61). In animals homozygous for the mutated PDGF β-receptor, PDGF BB, a reduction in P_{if} brought about by the mast cell degranulating agent C48/80 could not be restored. These specific mutation experiments show that the PDGF β-receptor via the phosphatidylinositol-3′-kinase has a role in control of tissue fluid homeostasis *in vivo* via the effect on interstitial fluid pressure.

Potential therapeutic implications of reduction in P_{if}

We have discussed the effect of α-trinositol and PDGF BB to counteract the lowering in P_{if} during generation of inflammatory edema. In these conditions the 'clinically' significant effect is the reduction in tissue edema, not the reduction in P_{if} per se, an effect that may turn out to be of fundamental importance in treatment of neurogenic inflammation and other conditions with rapidly developing edema in the airways.

Another area where the present new knowledge on P_{if} and the active role of the interstitium may become useful is cancer therapy. It is well established that interstitial fluid pressure in tumors is elevated (62), and this phenomenon may severely influence the delivery of chemical agents as well molecular medicine to tumors (63, 64). Several agents have been shown to be able to reduce tumor P_{if}, including nicotinamide (65), tumor necrosis factor-α (66) and dexamethasone (67), but whether the observed lowering in P_{if} resulted in increased uptake of substances was not established.

In some recent studies different principles have been used to influence the net filtration into the tumor interstitium and thereby increase the uptake of potential therapeutic agents. Netti and coworkers (68) infused angiotensin II chronically or periodically in severely combined immuno-deficient mice with subcutaneous tumors, and measured the tumor P_{if} and uptake of specific and unspecific antibodies. As expected, angiotensin II infusion led to increased blood pressure, but also to increased P_{if} in the

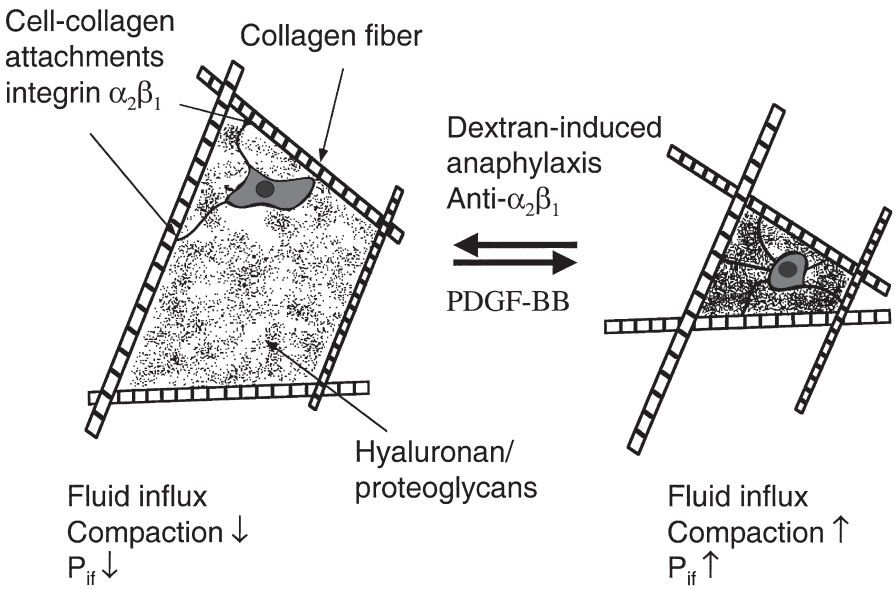

Fig. 6. Proposed model of events leading to decreased compaction of the loose connective tissue with subsequent reduction in interstitial fluid pressure. Dextran anaphylaxis releases or reduces the tension from fibroblasts on the connective tissue fibers, platelet derived growth factor-BB (PDGF-BB) reverses the effect of the dextran and restores normal tissue compaction. Redrawn from Reed et al. (72).

tumor. In spite of the observed rise in P_{if}, which per se will counteract filtration, the tumor uptake of the specific antibody increased by 40% within 4 h of its administration, whereas the uptake of the unspecific antibody was unchanged. Their interpretation was that the increased blood pressure led to an increased capillary ('microvascular') pressure that more than compensated for the rise in P_{if} and thereby to an increased net filtration of antibody.

If we assume that the delivery of macromolecular probes and drugs is dependent on the pressure gradient for filtration into the tumor [microvascular pressure: P_{if} (68)], another approach to enhance tumor uptake would be to reduce P_{if}. The knowledge on the changes in P_{if} generated in studies on edema formation has been used in such a context. Berg and coworkers (69) showed that prostaglandins E_1 and I_2 resulted in a reduction in P_{if} when injected subdermally in rat skin. The potential therapeutic consequence of this observation was explored by Rubin and coworkers, who instilled prostaglandin E_1 in the area around the tumor and measured the resulting tumor P_{if} and uptake of the extracellular tracer ^{51}Cr-EDTA (70). As in normal skin, prostaglandin E_1 led to a lowering of P_{if} in the tumor, but importantly resulted in an increased uptake of tracer as well. Further knowledge gained from studies on P_{if} in inflammation has also been used in a therapeutic context. As discussed earlier, PDGF BB will normalize P_{if} in skin during anaphylactic conditions or after blocking of $\alpha_2\beta_1$-integrins. Rubin et al. used a specific protein tyrosine kinase inhibitor (STI 571) that inhibits PDGF receptor kinase, thereby blocking the PDGF signalling pathway (71). STI 571 treatment resulted in a reduction in tumor P_{if}, showing interference with the PDGF-receptors, and a doubling of the uptake of ^{51}Cr-EDTA.

Agreement exists that the high P_{if} in tumors represents a major obstacle in chemotherapy of solid neoplasms. The approaches described earlier to increase tumor uptake of substances by increasing net filtration pressure, using agents to increase microvascular pressure, or reducing P_{if} are novel and may represent a new strategy in tumor therapy.

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