Review

Tumor Interstitial Fluid as Modulator of Cancer Inflammation, Thrombosis, Immunity and Angiogenesis

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Abstract. *Tumor interstitial fluid (TIF) is a watery phase that accumulates inside the tumor interstitium. Its genesis and fate depend on various factors, namely tumor type, metabolic state of the tumor, expression of vascular endothelial growth factor, and absence of lymphatic system. For almost 30 years TIF remained a neglected entity until it was demonstrated that TIF, and in particular its high pressure, constitutes an important obstacle to drug delivery and immunotherapy. The present review not only summarizes the abundant literature on the processes of TIF genesis and on its effects on therapy but it also presents data that, in our opinion, point towards what is perhaps the real physiological purpose of TIF: a primitive means of providing nourishment, oxygen, cytokines and matrikines to tumor cells that furthermore promotes the invasion of the normal surrounding tissue and passive metastatization through lymphatics. It is also an inducer of inflammation through increased osmolarity due to albumin loss. Recently, a role for TIF as a possible source of biomarkers has also been suggested.*

Genesis of Tumor Interstitial Fluid (TIF)

The microcirculatory unit is a highly specialized anatomical entity, which plays a key role in many physiological and pathological processes. One of its main functions is the regulation of the extravasation of nutrients, solutes, hormones and leukocytes (1, 2). In normal tissues, molecular exchange of gases (O_2 and CO_2), water, small molecules such as salts and sugars, and only small amounts of plasma proteins, takes place primarily in capillaries (3).

The liquid normally extravasated towards the interstitium is later reabsorbed along the capillary length (3). The driving forces responsible for fluid exchange through the normal capillary wall are essentially two: capillary hydrostatic pressure and plasma colloid osmotic pressure. The magnitude of fluid movement on the both sides of the endothelium (luminal and abluminal side) has been mathematically described by the Starling equation (4, 5):

$$J_v=(L_p\ S)[(P_c-P_i) - \sigma\ (\pi_c-\pi_i)] \quad \text{[eq.1]}$$

where J_v is volume flux of fluid (ml/min); L_p is hydraulic conductivity (cm min^{-1} $mmHg^{-1}$); s is capillary surface area (cm^2); P_c and P_i are capillary and interstitial fluid hydrostatic pressures, respectively (mmHg); π_c and π_i are capillary and interstitial colloid (oncotic) pressures, respectively (mmHg); and σ is the osmotic reflection coefficient of the vessel wall (σ 0 if the membrane is fully permeable to transport molecular species and σ 1 if the membrane is impermeable). Basically thus, the exchange of solutes and ions between the luminal and abluminal compartments of the circulation is critically dependent on the permeability of the vascular endothelium (6,7).

As recently reviewed by Nagy *et al.* (3), the tumor vasculature is exposed acutely to a number of vascular permeabilizing factors [*e.g.* vascular permeability factor (VPF)/vascular endothelial growth factor (VEGF), histamine, serotonin, platelet-activating factor]. These sustained stimuli

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greatly increase the quantity and composition of extravasated fluid with respect to the plasma filtrate typical of basal conditions. By contrast, the fluid that extravasates in acute vascular hypermeability (AVP), called exudate, is rich in plasma proteins, approaching the levels found in plasma. Fibrinogen and various members of the clotting cascade are among the extravasated plasma proteins. In contact with tissue factor (thromboplastin), the clotting system is activated and the exudate clots to deposit fibrin (3, 7). Fibrin forms a gel that traps water and other solutes, restraining their clearance by lymphatics or capillaries and causing tissue swelling, or edema (8). Fibrin also acts as a provisional stroma that supports the migration and growth of macrophages, fibroblasts and endothelial cells that will support tumor growth (7, 8).

A further characteristic of AVP is that vascular leakage takes place not from capillaries but, as first demonstrated by Majno and Joris (9), from post-capillary venules. Compared to normal endothelium, endothelial cells originating from tumor vessels do not form a normal monolayer but are irregularly shaped and disorganized, and some of them overlap one another (10, 11). These cells have loose interconnections and focal intercellular openings. The size of the openings as determined by electron microscopy is generally less than 2 μm in diameter (11, 12). These openings, called vesiculo-vacuolar organelles (VVOs) by Dvorak *et al*. (12-14), are grape-like clusters of uncoated, largely para-junctional, cytoplasmic vesicles and vacuoles that traverse endothelial cytoplasm from lumen to ablumen. The individual vesicles and vacuoles that comprise VVOs are linked to each other, and to the luminal and abluminal plasma membranes, by stomata that are normally closed by thin diaphragms. Vasoactive mediators, such VPF/VEGF, cause these stomata to open, providing a pathway for plasma and plasma protein extravasation in contrast to interendothelial cell junctions, which remain tightly closed. Pericytes, which play an important role in the regulation of vascular formation, stabilization, remodelling and function, play different roles in tumor vessels (15-17). They show diverse alterations, such as increased perivascular deposition of extracellular matrix (ECM) components, expression of marker proteins, loose association with endothelial cells and extension of their cytoplasmatic processes deep in the tumor tissue. They also seem to play a role in vessel sprout growth and metastatization (17, 18).

As a consequence, in the tumor tissue, several factors of Starling's equation are not met. In fact, the osmotic reflection and hydraulic conductivity coefficients, and capillary hydrostatic pressure forces and plasma colloid osmotic pressures behave differently, with several consequences on interstitial fluid exchange and hence on therapy (see below). Permeability of tumor vessels is much higher compared to normal endothelium and is mainly due to increased secretion of VEGF and other vasoactive substances by hypoxic cells (7, 19). Degranulation of mast cells, which are plentiful in the inflammatory reaction at the tumor periphery (7, 20), also contributes to the increased plasma extravasation. However, on a molecular basis, VPF/VEGF was shown to have a potency for increasing vascular permeability of about 50,000 times that of histamine (7, 21, 22).

Hypoxia is a common feature of solid tumors (23). It results from an imbalance between the O_2 consumption rate and the O_2 supply to the tumor cells. It can be caused by several factors, such as inadequate blood flow, increase in diffusion distances with tumor expansion, reduced O_2 transport capacity of the blood subsequent to tumor-associated or therapy-induced anemia (23-25), and increased viscosity of blood (26) due to the sustained loss of plasma from hyperpermeable tumour blood vessels and to the acidic tumor microenvironment (27). Once developed, the hypoxic/ischemic condition triggers a vicious cycle that progressively worsens the situation (26).

Since hypoxia in tumors does not occur abruptly but rather progressively, poorly oxygenated (pO_2 <7 mmHg) tumor cells have the time to develop strategies to become adapted or overcome the O_2 and nutrient-deprived condition and to survive in or to escape from such a hostile environment (23-27). In particular, they increase the expression of genes for erythropoietin, for VPF/VEGF (8, 23-25), transferrin receptors, and other proteins allowing for the development of a more effective O_2 (and nutrient) supply (23). VPF, exhaustively studied by Dvorak and collaborators (8, 22), was indeed later shown also to be a potent angiogenic molecule and since then is often described as VPF/VEGF. A further group of genes involved in this adaptive response controls metabolic pathways that can meet the cellular energy requirements (*e.g*. those of glycolytic enzymes and glucose transporters) (23, 28). The major regulator of tumor cell adaptation to hypoxia is the transcription factor hypoxia-inducible factor 1 (HIF-1) (23, 28).

The nature of TIF compared to fluid found in normal tissues is different in several aspects: volume, composition, interstitial fluid pressure (29).

TIF volume. Regarding the volume, Gullino *et al*. used two methods for measuring the vascular space: one was based on mannitol and dextran as a marker, the second was morphometric analysis. Both methods suffer limitations in measuring the vascular space in several animal models (29, 30). Mannitol was used to measure the extracellular space, whereas dextran 500 was used as a marker of the vascular space. The volume was obtained by subtracting vascular space from the extracellular water. They found that TIF volumes in tumors were 36-53% greater than the total tumor volume compared to 14-34% in normal tissue (27, 29, 30).

TIF composition. TIF differs with respect to aortic plasma in terms of: glucose, lactate, cholesterol and lipid phosphorus content, the concentration of protein was lower (peculiarly

fibrinogen was lacking and never coagulated) and that of free amino acids higher in the TIF than in aortic plasma. The higher levels of hyaluronidase activity in TIF compared to subcutaneous areas distant from the tumor was presumed to contribute to the low concentration of hyaluronic acid in TIF. The pH of TIF was 0.2 to 0.4 units lower, the pCO_2 16 to 39 mmHg higher, the dissolved CO_2 about 1 mM higher, and bicarbonate concentration 4 to 6 mM liter higher, as compared to plasma of the blood afferent to the tumor (27, 29-32). Furthermore, Sylven and Bois (33), obtained TIF by inserting glass capillaries (0.1-0.6 mm in thickness), in different parts of tumor and reported high levels of proteolytic and lysosomal activity, particularly in necrotic areas. The different techniques used by Sylven and Bois (33) and Gullino (30) to obtain TIF can explain the inequalities in proteolytic enzymes quantities found between the two groups of researchers.

Gullino *et al*. reported a higher concentration of prostaglandins (especially of E_1 type) and of gangliosides (GAG) and of GT_{1b} (trisialoganglioside) in the TIF, and showed their importance in angiogenesis (34, 37). Association of the ratio between the ganglioside GM1 and GD1b and Gt1b, with antiangiogenic activity has been observed by these researchers (35, 36). They also demonstrated that copper was essential in the process of angiogenesis (34). Another important component of TIF is collagen (38). Normally collagen and GAG confer the properties of hydrogel on the extracellular space (39, 40). The importance of connective tissue as a morphoregulator, and as support, *in vivo*, of the vascular system has been noted (39, 40). Gullino *et al*., supposing the importance of collagen, studied its content in several lines of carcinoma (hepatoma, fibrosarcoma, Walker carcinoma 256, and lymphosarcoma R-2788) using the isolated organ method (38). All transplanted tumors showed an increase in collagen content proportionally to the increase of tumor mass, but dependent on the tissue of origin. In this sense, hepatomas comprised more collagen than did the normal liver.

A step further in the research of TIF composition has been recently taken, by Celis *et al*. and Alexander *et al*. (41, 42). Using proteonomic analysis, western immunoblotting and cytokine-specific antibody arrays, a tissue lysate in the case of Celis *et al*. (41) and a breast nipple aspirate by Alexander *et al*. were studied (42). Quantitative information on TIF was gained and some of them in the near future could potentially be disease markers (43). There are differences in the two techniques and also with the method of collection of TIF by Gullino. Experimentally, the method of Gullino is more complex and requires more time to obtain an isolated organ tumor entity, but remains, in our opinion, the best way to see differences between luminal and abluminal compartments.

TIF pressure. Interstitial fluid pressure (IFP) in most normal tissues is subatmospheric in value or near zero (4). IFP in human and animal tumor tissues is generally elevated and can reach value in the range of 10 to 100 mmHg (43-61). Recent studies by Milosevic *et al*. and Lunt *et al*., have clearly demonstrated a correlation between TIF pressure and clinical outcome (54, 55). In fact TIF pressure, studied by invasive needle-based assessments, is an independent predictor of disease recurrence in patients with cervical cancer treated with radiotherapy (54). No correlations between TIF pressure, oxygen, carbonic anhydrase-IX and pelvic metastases have been found, however a correlation between TIF pressure and disease recurrence has been demonstrated (54). The authors did not provide explanations for the biologic mechanisms that may regulate the interrelationship between elevation of TIF pressure and tumor recurrence. A possible explanation, has been hypothesized by recent studies by the Healey group (56). It seems that TIF pressure may regulate some angiogenic factors at least in osteosarcoma. A certain variability, however, on TIF pressure measurements has been demonstrated by Lunt *et al*. (55). These authors measured TIF pressure values in a selection of murine and xenograft models, spontaneously arising or transplanted either intramuscularly or orthotopically and analyzed their relationship to tumour vascularity and metastatic spread. They demonstrated a significant variation in TIF pressure between individual tumors growing in the same mouse, and found no correlation between donor and recipient tumour TIF pressure values (55). Another explanation correlating TIF pressure with angiogenesis, come from studies that outline the importance of IFP on capillary morphogenesis (55-57). Generally TIF pressure is high in the center of a tumor, but not homogeneously so. The outward efflux of liquid from the tumor center *versus* the tumor periphery block the convection of all therapeutic agents [see below; (58-63)].

TIF and Therapy

TIF and its pressure have been implicated as an important factor that impairs the delivery of chemotherapy and cell therapy to tumors and may influence the regulation and distribution of cytokines and growth factors (58, 64). In fact, the route followed by every drug to reach cancer cells in solid tumors is the following: agents must enter tumor blood vessels, cross the vessel wall and migrate through the interstitium. In general, small lipid-soluble molecules such as carbon dioxide and oxygen move freely across vascular barrier by diffusion, whereas, water-soluble molecules and macromolecules cannot passively pass through endothelial walls and are dependent on membrane permeability. The rate of tumor transvascular transport is characterized by the microvascular permeability coefficient (59, 63, 65, 66). Permeability is a measure of the property of capillary endothelium that allows for the selective exchange of substances or whole cells (*e.g*. lymphocytes) between the

blood and surrounding tissues. Increased vascular permeability has been demonstrated in both physiological and pathological angiogenesis. A different vision and explanation of microvascular permeability is offered by physiologists and vascular biologists as outlined by Nagy *et al*. (3). Normally, endothelial cells are joined each other by complex structures formed by different adhesion molecules. When these tight junctions (TJ) and adherens junctions (AJ) are stimulated, a series of pores are formed. These pores are channels that selectively restrict passage of macromolecules depending on their size, shape, and electrical charge (65, 66, 69, 70, 71). However these predicted specific pores (channels) in the cell membrane have only recently been characterized. In some anatomical areas, such as brain, pleura and endothelia involved in fluid transport a water channels called aquaporins have been demonstrated. These water channels are a family of small hydrophobic integral membrane proteins that transport water and in some cases also small solutes (67, 68). Aquaporins are now recognized to play an important role in several forms of cancer (68). According to pore theory normal endothelium has a cutoff pore size of 20 nm, whereas in tumors, pore sizes are heterogeneous and can be up to 2 μm in diameter (63, 66). Tumor permeability and pore cutoff change with tumor microenvironment were demonstrated by Jain and his co-workers, who analyzed transport pathways by implanting human tumors subcutaneously or intracranially (69). Notwithstanding a higher permeability, favoring drug release, several other factors contribute to decrease drug uptake by tumors. These are: TIF, transvascular transport, drug distribution in the interstitium, interstitial composition and drug physicochemical characteristics (65, 66, 72, 73). The relationship between molecular weight (MW) and vascular permeability has been studied and found to have a negative inverse relationship, but a positive relationship exists between MW and plasma half-life concentration (74). Swabb *et al*. (73), Jain (58, 59) and Netti and Jain (74) tried to describe the behavior of macromolecules and cells in the interstitium as disturbances in physiological mechanisms and have clearly demonstrated that TIF pressure is a major factor in preventing optimal tumor concentrations of systemically administered chemotherapeutic agents. Furthermore, other authors have also demonstrated that chemotherapy drug uptakes are influenced by TIF pressure (59, 62, 65, 75, 76). In fact, Salnikov *et al*. (62), using prostaglandin E1-methyl ester (PGE 1) which is known transiently to reduce IFP, has shown that 5-fluorouracil (5-FU) caused significant growth inhibition on two experimental tumors in rats, but only after administration of PGE 1. Stuhr *et al*. (75) evaluated the effects of dexamethasone (DXM) alone or in combination with 5-FU on dimethyl-alpha-benzanthracene (DMBA)-induced mammary tumors in rats. They analyzed TIF pressure with the wick-in-the needle technique, tumor growth by external size measurements and vessel density and inflammatory cell infiltration of tumor tissue were by immunohistochemistry. Treatment with a combination of DXM and 5-FU reduced tumor size significantly more than any of the agents alone ($p<0.01$-0.001). This reduction was associated with TIF pressure decrease by DXM. Similar results have been obtained by Navalitloha *et al*. (76) in subcutaneous RG-2 tumors. These results clearly demonstrate that the combination of certain drugs with chemotherapy can augment drug uptake and probably drug effect.

Another important effect linked to increased permeability is the enhanced permeability and retention effect (EPR) described by Maeda *et al*. EPR refers to a long retention of macromolecular drugs in tumor tissue due to a leaky endothelium and lack of effective tumor lymphatic drainage (19). TIF composition and its interaction with tumor stroma, as outlined by Wiig *et al*. (71), Netti *et al*. (74) and Oldberg *et al*. (78), can be associated with elevated TIF pressure, creating another important transport barrier between tumor tissue and blood. In fact, tumor stroma is characterized by distorted blood vessels and activated connective tissue cells producing a collagen-rich matrix (78), which can increase hydraulic conductivity of IgG as demonstrated by Netti *et al*. (74). These researchers concluded that contrary to previous studies, these functional properties are correlated with total tissue content of collagen, not glycosaminoglycan (74). Oldberg *et al*. (78) showed that the collagen-binding proteoglycan fibromodulin controls stroma structure and fluid balance in experimental carcinoma. Jacobson *et al*. investigated the role of hyaluronan, the major water-binding polysaccharide of the extracellular matrix, for the generation of a high TIF pressure. They studied a human anaplastic thyroid carcinoma (KAT-4) xenografted in athymic mice and a syngeneic rat colon carcinoma (PROb), and concluded that hyaluronan content is not a major pathogenetic mechanism for the generation of high TIF pressure in malignant carcinoma (79). Notwithstanding these studies, several factors acting on TIF and stroma have been demonstrated to effectively increase drug uptake [for a complete review see Heldin *et al*. (80)].

TIF and Nutritive Aspects

As suggested by various studies, TIF can be considered a source of energy for many cancer and normal cells not adjacent to nutritive cells. The source of these substances is double, one part comes from plasma filtration and another part from the discharge of normal cancer cells actively proliferating or dying from apoptosis. An example comes from the abundant presence of lactic acid that can be reconverted to glucose becoming an important source of energy for non- hypoxic cancer cells (81). Recent studies by Sonveaux *et al*. (82) has clearly demonstrated this aspect of recycling of lactate. Lactate generally considered a waste

product is the prominent fuel for oxidative metabolism of oxygenated cells. In this manner, hypoxic cells may be considered to sustain oxygenated cells and TIF may have a role as a store rather than as a collection of rubbish. This aspect may also be true for plasma albumin, as demonstrated by Stehle *et al*. in cancer cachexia (83).

TIF as Inducer of Inflammation and as a Source of Exosomes

Roberts and Palade clearly demonstrated that chronic exposure to VEGF can induce fenestrations in nonfenestrated endothelium similar to the fenestrated endothelium found in tumor vessels (84). Therefore, the fenestrated neovascular endothelium is more permeable to larger solutes such as albumin as also shown by several authors (84-88). Albumin is the most abundant protein in plasma and its principal function is to provide a stable plasma pH (89), to maintain an antioxidant effect (90) and to control the osmotic pressure of plasma itself (91). Osmotic pressure increase has recently been demonstrated by Schwartz *et al*. to trigger inflammation and inflammatory cytokines such as: interleukin-1β, interleukin-6, and tumor necrosis factor-α (92). Furthermore, exposure of macrophages to long-term hyperosmotic culture can extend their half life from 44 days to 102 days and can alter the expression of p53, BCL-2 and BAX (93). These oncogene derived proteins exert important control on apoptotic machinery (93), and their altered expression in the inflammatory action of albumin can be crucial for controlling life and death of cancer cells.

Associated to albumin loss, tumor endothelium may permit the outflow of other macromolecules such as matrikines, exosomes and microparticles towards the TIF. Matrikines are peptides liberated by partial proteolysis of extracellular matrix macromolecules which are able to regulate cell activities (94). They are derived from interstitial elastin and basement membrane collagens, and may play a significant role in physiological or pathological processes such as wound healing, tumor invasion and angiogenesis (94, 95).

Exosomes are intracellular luminal vesicles (50–90 nm diameter) originating from endosomes. They fuse with plasma membranes and are released into blood circulation under normal physiologic conditions and in many biological fluids and exudates such as ascites and pleural effusions of cancer patients (96-100). Microparticles, as described by Aharon and Brenner (96) are membrane vesicles (≈1 mm in diameter) shed from the cell surface into the local milieu and the blood circulation following chemical or physical triggers, and are often a hallmark of cell apoptosis. Initially, considered artefacts with no physiological function, microparticles are in fact now considered a nanoscale messenger with important functions in thrombosis and immunity (101, 102, 103). Exosomes and microparticles are generally lost by platelets, monocytes, endothelial cells and cancer cells. Under the presence of reactive oxygen species, they become aggregates and probably accumulate in the TIF. Following extrusion at the tumor external boundaries, following the flux of TIF from the tumor center towards the periphery, they concentrate and, through lymphatics, are returned into the blood stream. The increased concentration of these agglomerates at sites of endothelial injury, intensify the recruitment of tissue factor through p-selectin/p-selectin glycoprotein ligand-1 interaction, they accumulate in the TIF, are then released into the blood stream, and can trigger coaugulation (103). Further to this procoagulant activity by exosomes and microparticles, recent studies seem to indicate that tumor-derived exosomes containing the tetraspanin Tspan8 can efficiently induce angiogenesis in tumors and tumor-free tissues (104). In fact, endothelial cell uptake of Tspan8 CD49d complex-containing exosomes was accompanied by enhanced endothelial cells (EC) proliferation, migration, sprouting, and maturation of endothelial cells progenitors (104). Other authors have demonstrated that angiogenesis and the consequent promotion of tumor growth and metastatization is induced by microvescicles containing sphingomyelin (105), or vesicles containing activated epidermal growth factor receptors (106). Recent studies have demonstrated that exosomes stimulate the angiogenic process in a dose-dependent manner and elicit paracrine endothelial signaling by regulating inflammatory cytokines of endothelial origin (107). Tumor immunity is able to restrain tumor spreading, but in the majority of cases it fails (108, 109). Recently, the active release by tumor cells of exosomes has been recognized to have immunosuppressive activity (110). This immunosuppressive activity can be accomplished by exosomes containing prostaglandins of E2 type and transforming growth factor beta and by promoting bone marrow myeloid cells as demonstrated by Xiang *et al*. (111). New studies indicate that exosomes induce immune evasion by enhancing the expansion of T-regulatory cells (112). Other mechanisms used by exosomes for immune evasion are the expression of natural killer cell lectin-like receptor 2D ligands (NKG2D), decreasing so the proportion of NKG2D-positive effector cells (113), or expressing FAS ligands (114). Other authors have outlined that exosomes can work both as immunosuppressant and immune activators depending on the secretion modalities and manipulation (115-116). However, the *in vivo* mechanisms remain unclear.

TIF Cancer Cell Shedding and Metastatization

Metastatization is the worst event for a cancer patient. Butler and Gullino tried utilizing the isolated organ technique to elucidate the mechanisms of shedding and to quantify cells leaving the tumor mass that can metastasize (117). These authors found that tumor (MTW9 rat mammary carcinoma) shed almost 10^6 cells per 24 per gram of tissue in the efferent

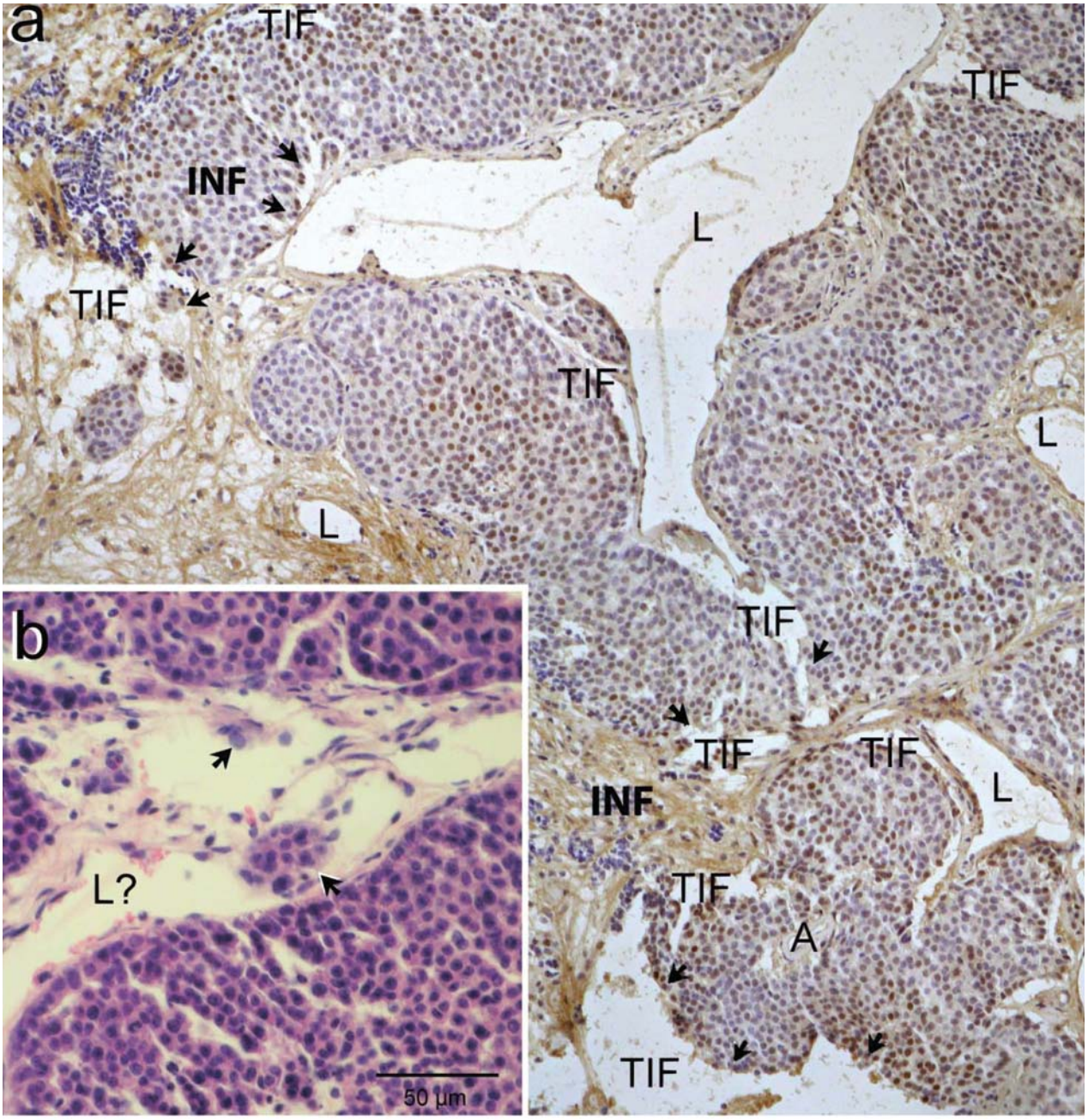

Figure 1. *Drop-out of tumor cells mediated by tumour interstitial fluid, the likely initial phase of lymphatic dissemination in a mammary tumor developed in a MMTV-neu (erbB-2) transgenic mouse. a: Immunoperoxidase demonstration of hypoxia-inducible factor-1alpha, in p-formaldehyde-fixed, Paraplast-embedded section. b: Hematoxylin and eosin; staining of p-formaldehydel fixed, Paraplast-embedded section. Arrow heads: Tumor cells in contact with or in clusters in the TIF and in lymphatic vessels; L: lymphatic vessel; L?: vessel with morphological features similar to lymphatic vessels but containing a few erythrocytes. A: Artery; INF: inflammatory cells.*

venous blood. Using their own words "a 2 g MTW9 carcinoma pours enough cells into the host circulation to transplant the tumor every 24 h" (117). As shown by Freitas *et al*. (20), tumor cells at various stages of differentiation can leave the center of tumor and move towards the periphery, along connective sheaths of nerves and muscles (see Figure 1). These are zones of less resistance and can facilitate, in our opinion, the shedding of cells. It seems also that the risk of local and systemic lymphatic metastasis of a tumor increases with the size of the malignant neoplasia (118). TIF pressure, like

lymphatic metastases, increases with increasing tumor mass and seems implicated in lymphangiogenesis (119). As outlined by these authors, lymphatic vessel formation is initiated along pre-established routes of fluid flow. Given these premises that no lymphatics are present or functioning inside the tumor mass (31, 120), we anticipate that a reorganization of lymphatic channels or tumor-adjacent lymph node takes place to prepare the right environment for the establishment of metastasis. This aspect has been demonstrated in the sentinel lymph node by Qian *et al*. (121). Associated with such morphogen gradient created by TIF flow from the tumor mass, interstitial fluid, being rich in proteolytic enzymes probably permits the degradation of the extracellular matrix and the expression of VEGF that is generally stored inside it (122). In association with the subtle that permit lymphatic capillary morphogenesis, TIF may cooperate to release active and to complete the capillary structure (123, 124). In fact, as demonstrated by Helm *et al*. (124) both VEGF and biophysical forces are necessary for a complete formation of lymphatic structure (57). Another factor that we would like to outline is the nutritive aspect of TIF in lymphatic metastatization. As reported by Cao (125), unlike blood vessels, lymphatics do not provide oxygen or nutrients that are essential for tumor growth, so cancer cells would appear to derive their nutrients from TIF. The paucity of oxygen may also favor infiltration by hypoxic cells, which in the search for better 'soil', are just ready for this hostile environment, and are not only transported to lymphatics by TIF flow but are also nourished by it.

Conclusion

In recent years with the advent of proteonomic methodologies for ascertaining individual biochemical markers of cancer, TIF is regaining its importance (43). Notwithstanding its recognized usefulness, the role of TIF in the maintenance and progression of tumor remain somewhat unclear. As suggested by Schwartz *et al*. (92), a simple osmolarity increase may, for example, explain the peritumoral inflammatory process, and, in this sense, the loss of albumin is obligatory for triggering and sustaining the inflammatory reaction.

Acknowledgements

This article is dedicated to our dear friend P. M. Gullino.

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