

ORGANIZING A TÊTE-À-TÊTE BETWEEN CELL ADHESION MOLECULES AND EXTRACELLULAR PROTEASES: A RISKY BUSINESS THAT COULD LEAD TO THE SURVIVAL OF TUMOR CELLS

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1. ABSTRACT

Several membrane-bound molecules expressed at the surface of tumor cells have been shown to be released in a soluble form, thereby affecting cell-cell interactions by reduction of ligand densities. Moreover, since the binding domain of the soluble molecules often remains functional, proteolytic cleavage can also reduce the recognition of tumor cells by effector cells bearing the corresponding receptor. Proteolytic cleavage of cell adhesion molecules (CAMs) at the surface of stromal cells, most notably at the surface of vascular endothelial cells, can also limit the recruitment of effector cells at tumor sites. It is noteworthy that, in most cases, the signals that regulate the expression of extracellular proteases are mediated by the same adhesion molecules than those that are targeted by the proteases, suggesting that there is an intimate relationship between extracellular proteases and cell surface adhesion molecules. In this review, we will discuss the functional relationship between CAMs and proteases and how this may lead to tumor evasion.

2. THE ROLE OF CELL ADHESION MOLECULES IN CANCER PROGRESSION

2.1. Recruitment of immune effector cells

One of the major roles of adhesion molecules is to recruit immune effector cells at tumor sites. Although several families of adhesion molecules are involved in this process, the LFA-1/ICAM-1-mediated interactions remains one of the major adhesion pathway by which immune effector cells, including lymphocytes, neutrophils, and monocytes, migrate to tumor sites. ICAM-1 on endothelium plays an important role in migration of

activated) leukocytes to sites of inflammation. The intercellular adhesion molecule (ICAM)-1 is an Ig-like cell adhesion molecule (CAM) expressed by several cell types, including leukocytes and endothelial cells (EC) (1). It can be induced in a cell type-specific manner by several cytokines, for example, TNF- α , or IL-1, that are induced at different steps during tumorigenesis. Its ligands are the integrin receptors lymphocyte function-associated molecule-1 (LFA-1) and Mac-1 expressed on all circulating leukocytes populations. Using ICAM-1-deficient mice, we and others have shown that ICAM-1-plays a crucial role in leukocytes-EC interactions by reducing the rate of emigration of leukocytes into tissues during inflammation (2, 3). In absence of such interactions, these mice are completely resistant to septic shock induced by injection of a lethal dose of lipopolysaccharide (LPS), as their leukocytes are prevented from migrating to tissues and therefore from releasing extracellular proteases as well as toxic metabolites and vasoactive mediators that cause tissue damage and ischemia respectively (3). In vitro experiments with EC derived from ICAM-1-deficient mice have also allowed establishing that ICAM-1 plays an important role both in T cell adhesion to endothelium and in transendothelial migration of T lymphocytes (4). These studies have established that ICAM-1- is critical for allowing leukocytes to emigrate from the blood circulation.

The fact that ICAM-1 and other adhesion molecules of the Ig-family are overexpressed at the surface of tumor-associated blood vessels supports the idea that this molecule also plays an important role in allowing infiltration of activated immune effector cells into cancer

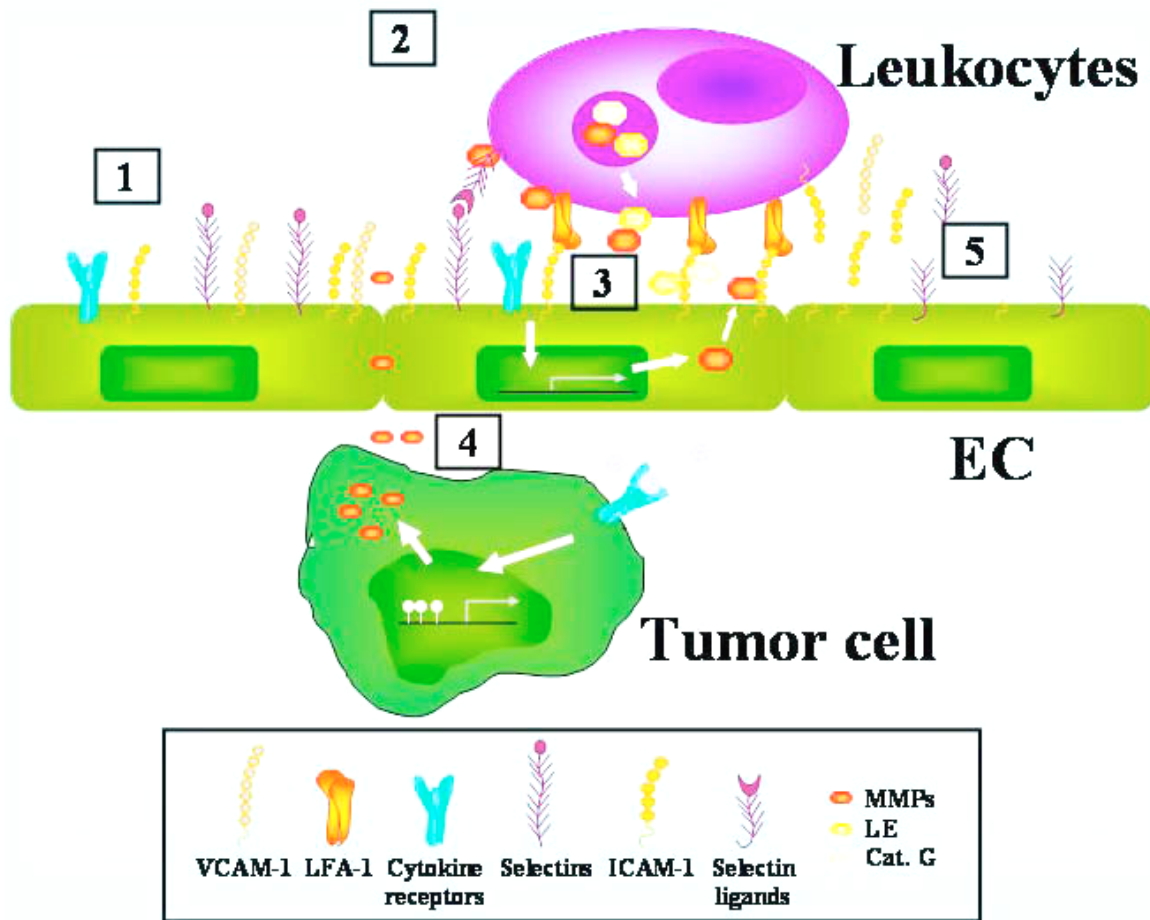


Figure 1. Interactions between adhesion molecules and extracellular proteases at the vascular endothelium interface. Tumor-associated-vascular endothelial cells express a large repertoire of cell surface adhesion molecules and cytokine/chemokine receptors in response to the presence of tumor cells. These cell surface molecules play a significant role in the recruitment and activation of effector cells via a well-coordinated series of interactions implicating low-affinity interactions via selectins and integrin-mediated firm adhesion. Following contact with between leukocytes and endothelial cells, a bi-directional activation process in both cell types implicates adhesion molecules and lead to de novo expression of extracellular proteases, such as MMPs [12, 13]. Release of extracellular proteases can also occur via degranulation of protease-rich endosomal vesicles, such as neutrophil granules, which contain high amounts of MMP-9, leukocyte elastase, proteinase 3, and cathepsin G. Binding of these proteases to cell surface molecules precedes their proteolytic cleavage. For instance, MMP-9 and LE have both been shown to bind ICAM-1, inducing its subsequent release from the cell surface into a soluble form (50). Hypomethylation of the promoter of mmp genes and activation of tumor cells by inflammatory cytokines, such as tumor necrosis factor (TNF)- α and IL-1, also favors the expression and release of specific MMPs, including MMP-9 and stromelysins [78-80]. Extensive cleavage of cell surface adhesion molecules by extracellular proteases negatively regulates recruitment of immune effectors to tumor site while inducing the release of high levels of circulating forms of adhesion molecules.

lesions (5). T-cell migration to tumor tissue is indeed inhibited by treating tumor-bearing animals with LFA-1-blocking antibodies (6). In humans, subcutaneous administration of low-dose recombinant IL-12 in metastatic melanoma patients promotes infiltration of neoplastic tissue by CD8⁺ T cells concomitantly with an increased expression of ligand receptor pairs contributing to the ICAM-1 and selectin adhesion pathways (7). Irradiation with a dose that does not affect tumor growth renders tumor-bearing mice susceptible to lymphocyte infiltration by increasing the expression of ICAM-1, allowing T cells to extravasate and to destroy the tumor (8). In contrast, the ability of certain tumors to secrete high levels

of angiogenic factors, such as vascular epidermal growth factor (VEGF) and fibroblast growth factors (FGF) confers to them the ability to block the entry of immune effector cells by downregulating the expression of ICAM-1 on the surface of vascular EC (9).

2.2. The role of ICAM-1 in the dissemination of tumor cells

The implication of CAMs in neoplastic progression is not limited to its role in leukocyte infiltration. CAMs are also used by tumor cells as adhesion receptors during metastatic spread. Information from the

behaviour of LFA-1-positive lymphoma cells in presence of blocking antibodies to LFA-1 (10) and of adhesion-deficient mice have provided clear evidence that CAMs are involved in the dissemination of tumors, most likely by generating intracellular signals that are critical for the functions of tumor cells. We have also shown that immunocompetent mice bearing mutations in both alleles of the *icam-1* gene were resistant to the dissemination of LFA-1-bearing 164T2 syngeneic lymphoma cells to peripheral organs (11). We found, however, that resistance of these mice was due to a factor other than an inability of lymphoma cells to “home” to the target organ, since they migrated with the same efficiency in both normal and ICAM-1-deficient mice. This finding suggests that ICAM-1 expression on the vascular endothelium is not essential for the migration of tumor cells to target organs. It seems, rather, that it is at stages subsequent to homing that ICAM-1 expression by the host is essential, most likely by promoting bi-directional activation of tumor and stromal cells (Figure 1). Tumor-stromal interactions have been cited as a possible factor in both tumor invasion and tumor metastasis. Our data, showing that binding of LFA-1-bearing tumor cells to ICAM-1 on the host is essential for the delivery of activation signals to both tumor and endothelial cells support this hypothesis (12). These signals play an important role in the expression of metastatic genes, such as MMP-9 and MMP-10, two members of the family of matrix metalloproteases that are overexpressed in aggressive lymphoma cells and confer tumor cells with increased tumorigenicity (12-14). This model is thus consistent with the hypothesis stating that it is *after* homing to the target organ that the ability of lymphoma cells to metastasize is manifested (15-17). Thus, the ability of adhesion molecules to control the expression of MMP genes in tumor cells following adhesion with the vascular endothelium represents an important step in the cascade of events leading to the dissemination of tumor cells in peripheral organs.

2.3. The role of selectins in cancer progression

The participation of CAMs in promoting tumor metastasis has also received strong support from studies that focused on the role of selectins and their ligands. Selectins are a family of adhesion molecules closely involved in the contact between circulating leukocytes and vascular endothelial cells (18). Three members of this family have been cloned. E- and P-selectins are expressed on vascular endothelial cells and, like ICAM-1, their expression is rapidly upregulated upon stimulation; they are involved in the recruitment of circulating leukocytes at inflammation sites. P-selectin is also expressed in α granules of platelets. In contrast, L-selectin is constitutively expressed on circulating leukocytes, and on lymphoma cells. Genes for all three selectins are clustered over a short region of human chromosome 1. All selectins share a high degree of structural homology, and all bind to sialylated, glycosylated, or sulfated glycans on glycoproteins, most notably the tetrasaccharides sialyl Lewis X (sLe^x) and sialyl Lewis A (sLe^a) found on a wide spectrum of cell types (19).

Normally, selectins are responsible for the initial stickiness of circulating leukocytes to vascular

endothelium, while members of the immunoglobulin family of CAMs, like ICAM-1, are responsible for firm adhesion of leukocytes to the vascular endothelial cells. With the availability of combined adhesion-deficient mice (for example, mice deficient for both ICAM-1 and P-selectin), it has been possible to confirm that selectins and ICAM-1-like adhesion molecules recruit leukocytes at the vascular endothelium in a highly coordinated adhesion process that implicates low and high affinity interactions between circulating cells and the vascular endothelium (20). It is thus logical to believe that selectins play a crucial role in tumor progression.

The importance of selectin-mediated interactions in tumor metastasis has been indirectly suggested on experimental systems that focused on selectin ligands. For example, the altered pattern of glycosylation, most notably regarding overexpression of sLe^x/sLe^a epitopes frequently observed at the surface of tumor cells (21), correlates with tumor progression and metastasis in humans. Weston et al (22) have indeed shown that human tumor cells expressing stable antisense nucleotides specific for the α (1,3)fucosyltransferase (FUT) gene are unable to colonize spleens of nude mice. Moreover, the high expression of sLe^{a/x} on tumor cells confers them with the ability to interact with endothelial E-selectin (23). This association between the expression of selectins and the metastatic potential of a given tumor cell is not, however, perfectly obvious given the fact that the repertoire of selectin ligands is rich in terms of numbers and functional overlap. Evidence establishing that selectins are involved in metastasis has therefore mostly been derived from *in vivo* models using genetically engineered selectin-deficient mice. For instance, L-selectin-deficiency has been shown to attenuate metastasis of adenocarcinoma cells expressing functional L-selectin ligands (24). Furthermore, human colon carcinoma cells, which express functional L- and P-selectin ligands via the presence of sialomucins at their surface, are able to interact with platelets under flow conditions in a shear-dependent manner (25), suggesting that selectins may promote platelet-carcinoma emboli with a leukocyte coat, favouring mechanical trapping of the emboli in blood vessels. Alternatively, selectins expression on tumor cells may dictate where tumor cells will migrate and where they will establish secondary tumors. Using a transgenic model where L-selectin expression on highly angiogenic islet cell carcinomas is driven by an insulin promoter, Qian et al., (26) have shown that metastatic cells preferentially migrate to lymph nodes expressing L-selectin ligands. Organ colonization of intravenously injected carcinoma cells is also severely impaired in P-selectin-deficient mice and in mice receiving tumor cells pretreated with O-sialoglycoprotease (which selectively removes functional groups of selectin ligands) (27). These data obtained with experimental models complement those studies showing that overexpression of sialylated fucosylated glycans at the surface of tumor cells is associated with a poor prognosis (28-30). For P-selectin, the mechanism underlying its implication in metastasis has been at least partially elucidated by the demonstration that this selectin favors the formation of platelet coating on cancer cells during the initial phase of the metastatic process (24). Thus, selectins are

Cleavage of adhesion molecules during cancer

mainly involved in tumorigenesis by controlling the migration of tumor cells and immune effector cells. Despite the ability of some selectins or their ligands to mediate intracellular signalling, there is yet no evidence that they are also involved in controlling the expression of metastatic genes in tumor cells, unlike ICAM-1. They could, however, contribute to tumor angiogenesis in the adult by favouring the recruitment of circulating endothelial progenitor cells (31).

3. SOLUBLE FORMS OF ADHESION MOLECULES DURING CANCER

3.1. The presence of circulating forms of adhesion molecules

As our knowledge regarding the role of adhesion molecules during cancer progression was increasing remarkably in recent years, several groups have reported elevated levels of soluble forms of adhesion molecules in the blood of patients with different forms of cancer. Circulating levels of these soluble forms of CAMs can be measured by sensitive enzyme-linked immunoassay techniques. Thus, while circulating forms of adhesion molecules have been described in normal human serum, elevated levels have been found in patients with melanoma, myeloma, non-Hodgkin lymphoma, Hodgkin's disease, and other malignancies (32-37). In most cases, elevated levels of adhesion molecules have been shown to correlate with metastasis, tumor spread, and poor prognosis (32, 33, 35, 38, 39). In other cases, such as in patients with primary extranodal lymphomas, soluble forms of CAMs have been associated with a poorer response to therapy (40). The biologic function of serum CAMs and the mechanism leading to the early dissemination and poor prognosis remains largely unknown. Given the physical constraints that govern the adhesion of circulating cells, it is unlikely that soluble forms of CAMs may inhibit the interactions between tumor cells and EC. However, since soluble forms of adhesion molecules, most notably members of the Ig-family of adhesion molecules which possess a semi-rigid structure, retains their ability to bind specifically to their ligands, it has been postulated that high levels of circulating sCAM may block the attachment of immune effector cells to tumor cells in extravascular spaces. This mechanism could thus lead to escape from the immunosurveillance, thereby promoting cancer dissemination. Alternatively, cleavage of CAMs may compromise the recognition of tumor cells by immune effectors. Indeed, whereas it is clear that presentation of specific antigenic epitopes is mediated by MHC class I and II antigens, several studies have shown that immune effector cells, such as natural killer cells and cytotoxic lymphocytes, react *in vitro* with tumor cells only if these express appropriate adhesion molecules, including ICAM-1 or CD58 (41-45). Moreover, decreased expression of adhesion molecules on malignant cells is associated with a poor host T-cell immune response (46).

3.2. Sensitivity of LFA-1-ICAM-1 interactions with proteolytic cleavage

There is an increasing evidence supporting the idea that the origin of soluble CAMs is the enzymatic cleavage of membrane bound CAMs released from the cell surface of tumor cells. For example, the serum of nude

mice bearing subcutaneous human melanoma tumors expressing ICAM-1 contains soluble ICAM-1 of human origin (47). However, since elevated adhesion molecules are also observed in other physiological processes, such as inflammation, it is likely that the activated vascular endothelium, which express high levels of CAMs and is at the interface of immune effector cells with tumor cells, is also a prime target for proteases. In the case of ICAM-1, leukocyte elastase (LE), cathepsin G, and matrix metalloproteinase-9 (MMP-9) are responsible for the cleavage of the membrane form of ICAM-1 (48-50). This cleavage is likely to occur in situations where ICAM-1 is not bound to LFA-1 since antibodies specific for the LFA-1-binding domain for ICAM-1 inhibit the release of soluble forms of ICAM-1 by preventing the initial binding of LE to its substrate (48). Moreover, the binding of LE to ICAM-1 is reversible and LE released from the cell surface remains proteolytically active (51). In the case of LFA-1, the major ligand for ICAM-1, it seems to be resistant to proteolytic cleavage by most proteases. The inhibitors for metalloproteinases, such as GM6001 and the zinc chelator 1,10-phenanthroline, as well as serine protease inhibitors and various protease inhibitors, do not affect the CD18 down-regulation on neutrophils observed by fluid shear (52). It should be noted that E-64, a cysteine protease inhibitor, is one of the rare protease inhibitors to act on β_2 -integrin expression. Conversely, *in vitro* treatment of neutrophils with cathepsin B, a member of the large family of lysosomal cysteine proteases, slightly reduces the expression level of β_2 . Thus, although cathepsin B has been described as an active extracellular form of this enzyme in some systems (53), it is likely that it is through their action on Ig-like CAMs that extracellular proteases modulate ICAM-1/LFA-1-dependent adhesion during the immune response.

3.3. Alternative splicing regulates the cleavage of adhesion molecules

Generation of ICAM-1-deficient mouse models by genetic engineering, targeting either exon 4 or 5 (2,3), which encode the third and fourth Ig-like extracellular domains respectively, led to the unexpected finding of alternative isoforms of ICAM-1 generated by alternative splicing of exons encoding complete extracellular Ig domains (54). Thus, in addition to the "common form" of ICAM-1, which contains five Ig-like domains, at least five alternative isoforms have been cloned and characterized. All isoforms contain the first and fifth (membrane-proximal) extracellular Ig-like domains, but each contains a distinct repertoire of Ig-like domains. The length of the isoforms thus varies, the shortest of the isoforms expressing only domains 1 (D1) and D5. Since all the alternative ICAM-1 isoforms, except for the exon 2-5 isoform, have been shown to retain the ability to bind LFA-1 in their soluble form (54). We have studied the impact of such molecular diversity on the ability of ICAM-1 to be cleaved by extracellular proteases. We found that all isoforms of ICAM-1 were much more susceptible to cleavage by LE and cathepsin G than the common form of ICAM-1 (50). Surprisingly, we also found that the length of the molecules does not influence their susceptibility to cleavage. Indeed, the shortest of the alternative isoforms, the 2-6 splice variant, which contains only two Ig domains that barely protrude above the glycocalyx, is highly sensitive to

proteolysis. Our results showing that the longest of the ICAM-1 isoforms, the common form, is the most resistant to proteolysis indicate that relative susceptibility of the different adhesion molecules to proteolysis may also be dictated at least in part by their ability to dimerize or to form larger multimeric complexes. Indeed, a portion of ICAM-1 expressed at the surface of activated cells exists as a covalent dimer (55, 56). Thus, the reason why the common form of ICAM-1 is cleaved by only a minor group of proteases might be that only a fraction of the common form of ICAM-1 is found as a monomer at the cell surface. Alternatively, if dimerization requires only the D5, transmembrane and cytoplasmic domains, as recently suggested by Jun et al., (57), the resistance of the common form would be consistent with its unique capacity to form a closed, ring-like structure. Conversion to an "opened", "w-shaped" structure, following interaction with another ICAM-1 dimer, together with some hinge-like motions between D1 and D5, would then allow exposure of putative protease cleavage sites. Although alternative roles for dimerization of ICAM-1 most likely include its ability to properly present the binding epitope to LFA-1, the existence in ICAM-1 of such ring-like dimers suggests ICAM-1-mediated cell-cell interactions might be controlled by proteolysis during cell trafficking according to the ability of different cell types to express a specific repertoire of ICAM-1 structures. A similar diversity in structure and function has also been observed in other adhesion molecules of the Ig superfamily of the adhesion molecules such as VCAM-1 and CD31 (58, 59). These receptors, just like ICAM-1 and its isoforms, have been shown to exist in a soluble form in circulation (60), although the proteases that are responsible for their cleavage remain poorly characterized.

3.4. The cleavage of adhesion molecules by proteases of various sources

Proteases can be produced and secreted by a variety of cells, including those involved in the development of the immune response, such as macrophages, T and B cells, and granulocytes. Proteases can thus play a major role in controlling the intercellular interactions at any steps of the response, and in almost any location where the concentration of active proteases is greater than that of inhibitor molecules. In the sputum of patients with cystic fibrosis, for example, leukocyte elastase and cathepsin G have both been shown to cleave CD2, CD4, and CD8 on peripheral blood T lymphocytes, leading to a significant reduction of cytotoxicity toward target cells and a significant reduction of interleukin-2 (IL-2) and IL-4 secretion (61). Weber et al., (62) have also reported that treatment of monocytic cells with various proteases (trypsin, alpha-chymotrypsin, elastase, papain) also substantially decreased anti-CD43 binding capacity and caused the release of soluble forms into the supernatant. Similarly, CD43, CD44, and CD16 have been previously shown to be enzymatically cleaved from the surface of stimulated leukocytes (63).

The literature indicates that a number of adhesion molecules or receptors involved in the immune response are susceptible to cleavage by non-self proteases. Thus, in addition to host cells, several pathogens also secrete proteases. A unique metalloprotease that is secreted by the

bovine fibrinous pneumonia pathogen *Pasteurella haemolytica* cleaves the glycosylated cell surface receptors CD34, CD43, CD44, and CD45. In contrast, the glycoproteins LFA-1 and Mac-1, CD71 (transferrin receptor), and HLA class I are all resistant to the action of this enzyme, designated *P. haemolytica* glycoprotease (64). In fact, treatment of cells with this enzyme inhibits migration of CD34-positive cells across the vascular endothelium (65). Similarly, elastase from *Pseudomonas aeruginosa* can cleave IL-2. This enzyme, as well as *Vibrio cholerae* proteinase and *thermolysin*, can also indirectly modulate the cleavage of other adhesion molecules by activating self proteases, including proMMP-9 (66). Whether non-self proteases modulate the anti-tumoral response locally and/or favour tumor evasion is currently unknown but remains a real possibility.

4. IMPLICATIONS OF THE PROTEOLYTIC CLEAVAGE OF ADHESION MOLECULES DURING ANTI-TUMORAL IMMUNE RESPONSE

4.1. Modulating the recruitment of effector cells

The circulation of lymphocytes through lymphoid organs via the bloodstream and the lymphatics plays a central role in the initiation of the immune response by favouring encounters with antigen-presenting cells. Thus, it is expected that cleavage of adhesion molecules from the surface of endothelial cells will significantly affect leukocyte recruitment during the anti-tumoral immune response. Through this mechanism a tumor has the opportunity to escape the immune system. Studies using genetically-engineered mice with transgenic mice that express a non-cleavable form of L-selectin on T lymphocytes have shown that the inability to shed L-selectin significantly affect lymphocyte recirculation or homing to lymph nodes (LNs) (67). Rapid removal of ICAM-1 could also be used as a means to modify the type of cytokines produced during interactions between leukocytes and endothelial cells. In general, cytokine-induced expression of ICAM-1 on endothelial cells reaches maximal levels within 6–8 h of cytokine stimulation. This increased expression is maintained for up to 72 h, unless neutrophils are allowed to migrate across EC (68). Since neutrophils contain high concentrations of MMP-9, of leukocyte elastase and of cathepsin G in their cytoplasmic granules, these results suggest that adhesion molecules are proteolytically removed from the cell surface of endothelial cells upon transmigration by leukocytes, rendering the vascular endothelial cell wall refractory to leukocyte recruitment. It is also possible that MMP-9 secreted by EC contributes to cleavage of ICAM-1. We have shown, for instance, that binding of T lymphoma cells upregulate MMP-9 secretion by endothelial cells (12). Sultan et al., (69) have recently shown that the initial upregulation of ICAM-1 at the surface of endothelial cells *in vitro* following shear stress was accompanied by a concomitant expression of MMP-9. They further showed that subsequently endothelial ICAM-1 levels rapidly returned to baseline, with concomitant increase in soluble ICAM-1 (sICAM-1) and induction of MMP-9.

Inclusion of a hydroxamate metalloproteinase inhibitor partially reversed the effects on ICAM-1 and MMP-9, but not on MMP-2, co-immunoprecipitated with ICAM-1. sICAM-1 was processed distally to Arg441, indicating that MMP-9 docking to ICAM-1 contributes to sICAM-1 shedding and attenuation of the shear stress-induced upregulation of ICAM-1. Similarly, synthetic hydroxamic matrix metalloproteinase inhibitors, such as KD-IX-73-4, that block L-selectin shedding, reduces the rolling velocity, increases the number of cells that tether from flow, and increases the transit time through blood vessels in mice (70, 71). Normally, L-Selectin is rapidly down-regulated from the T cell surface within 30–60 min after antigen receptor engagement (72). Further analyses using MMP inhibitors demonstrated that shedding of L-selectins regulated selectin-dependent β_2 -integrin activation and adhesion between leukocyte and EC *in vivo* (73). Cleavage of L-selectin and ICAM-1 is thus an important event in the recruitment of leukocyte at inflammatory sites and may allow certain type of tumor cells to escape immune surveillance by inhibiting infiltration of immune effector cells. Indeed, inhibition of mammary carcinogenesis in transgenic mice expressing rat HER-2/*neu* oncogene by IL-12 treatment has been attributed to increased infiltration via *de novo* expression of cell adhesion molecules on tumor blood vessels, allowing subsequent killing of tumor cells by immune effectors (74).

4.2. Escaping immune surveillance through the absence of co-stimulatory signals

Because of their potential susceptibility to T-cell-mediated cytotoxicity, tumor cells have developed mechanisms to escape immune recognition. Thus, an over-production of proteases by tumor cells will downregulate the immune response by cleaving a relatively large repertoire of molecules essential for mounting an immune response; these molecules include CD4, CD8, and ICAM-1 (48, 61). ICAM-1 also provides costimulatory signals to T cells, thus potentiating, through LFA-1, T-cell proliferation and secretion of T helper 1 (Th1) cytokines. Escape from immune surveillance by favouring the secretion of proteases in the peritumoral environment has in fact received support from several investigations carried out on lung disorders where secretion of extracellular proteases has been shown to reduce the host's immune response against opportunistic infectious agents (75). In cystic fibrosis, for instance, killing of opportunistic pathogens such as *Pseudomonas aeruginosa* is ineffective because a high concentration of LE is released into the extra-cellular space. We have also recently shown that virus infection of the lungs can lead to a transient increase in proteolytic activity that favors the establishment of opportunistic infections (76). In cancer, soluble forms of ICAM-1 may also be released from the cell surface as an indirect consequence of anti-tumoral immune

response or simply as a result of shedding in the form of vesicles occurring during extensive tumor cell growth. This hypothesis is supported by experiments showing the presence of soluble ICAM-1 in culture media from human tumor cell lines (47). Shedding of ICAM-1 by the tumor cells would allow the latter to survive by escaping immune surveillance. We can thus envisage that constitutive expression of extracellular proteases in the vicinity of immune effector, or helper, T cells will prevent proper recognition of tumor cells by the immune system (Figure 2). It is also possible that tumor cells expressing high levels of adhesion molecules may favour a high concentration of sICAM-1 in the peritumoral microenvironment in response to leukocyte invasion, thereby inhibiting LFA-1-mediated conjugate formation between the tumor-infiltrating lymphocytes and the tumor cells and its subsequent MHC-restricted killing. Indeed, as discussed above, it has been well documented that soluble forms of ICAM-1 are elevated in malignant tumors. Further studies are required to determine whether tumor cells expressing a large repertoire of adhesion molecules are more tumorigenic than those expressing a lower level. It could thus be envisaged that genetic engineering of tumor cells expressing protease-resistant forms of adhesion molecules that prevents their release in the peritumoral environment while promoting the recognition of tumor cells and activation of immune effector T cells will prevent tumor evasion and enhance anti-tumoral immune response.

5. CONCLUSIONS

The interaction between extra-cellular proteases and cell adhesion molecules is likely to be more intimate than previously thought. Accumulating evidence show that the signals that induce the expression and release of extra-cellular proteases is in many cases mediated through intercellular adhesion implicating the same adhesion molecules that will eventually be targeted by these proteases. For instance, direct L-selectin ligation down-regulates L-selectin surface expression on T lymphocytes through proteolysis via signals involving phosphorylation of protein tyrosine kinase (77). Elevated levels of ICAM-1 and of other endothelial cell-associated adhesion molecules found in various human malignant diseases suggest that the soluble form of ICAM-1 is released following proteolytic cleavage as an indirect consequence of infiltration and progression of tumor and effector cells through the vascular endothelium. To date, however, emphasis has been put on the importance of cell adhesion molecules on immune effector cells. Since tumor cells express a large repertoire of adhesion molecules, it will be pertinent to determine whether cleavage of these molecules will affect not only the recognition of tumor cells by the immune system, but also if it affects the ability of these molecules to transduce signals in tumor cells. The use of transgenic models where tumor cells do not express adhesion molecules or express proteolytically-resistant forms of CAMs will help to address this issue.

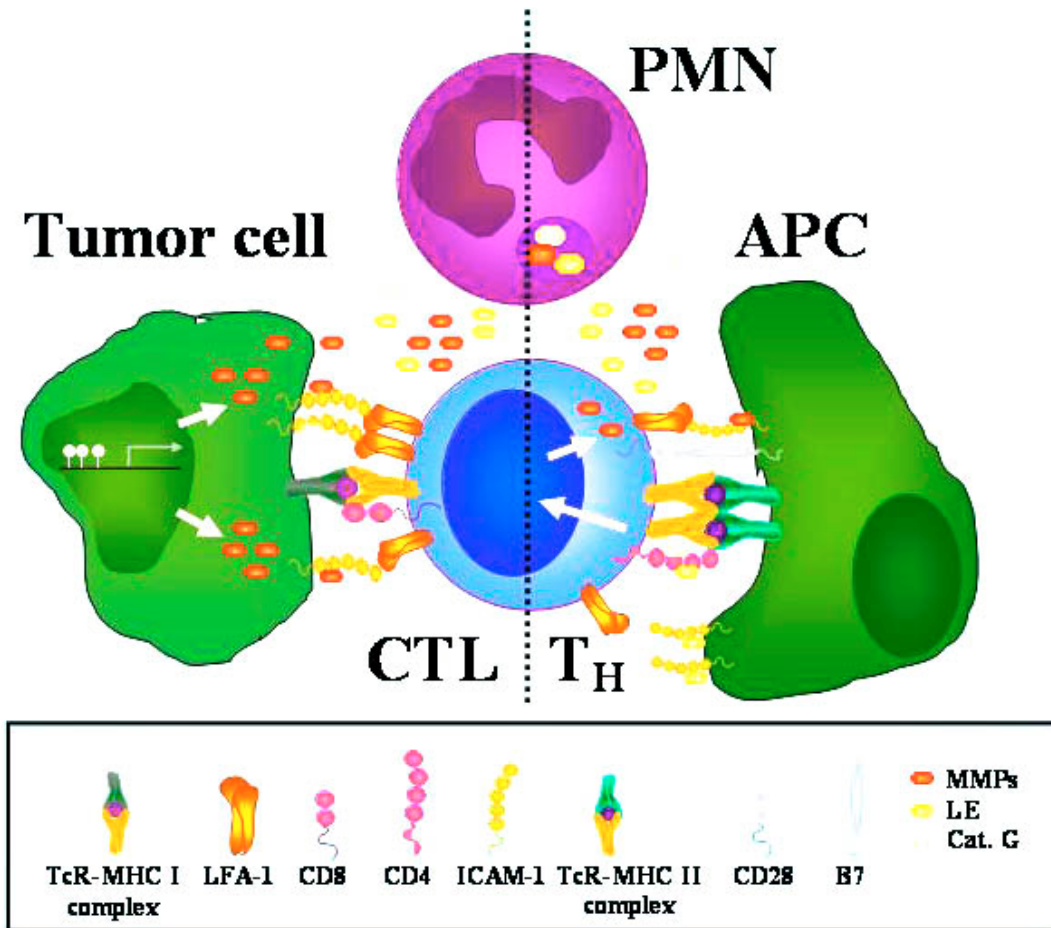


Figure 2. How extracellular proteases may favor tumor evasion. Extracellular proteases play a key role in T cell-mediated interactions. Right: The sensitivity of ICAM-1, CD28-mediated interactions, and CD4 to proteolytic cleavage prevents the formation of immunological synapses and the delivery to T cells of secondary signals that are essential for their activation (48, 61, 81). The delivery of signal 1 alone via the antigen-receptor might in fact favor the induction of tolerance (82). Left: The constitutive expression of MMP-9 favors the cleavage of ICAM-1 and the formation of cognate interactions between effector T cells and tumor cells, thereby reducing the functions of cytotoxic lymphocytes (CTLs). In both cases, the secretion of extracellular proteases by bystander cells, such as neutrophils or tumor-associated fibroblasts, may also contribute to tumor evasion, most notably by favoring the cleavage of ICAM-1 and CD8 by leukocyte elastase, MMP-9, and cathepsin G.

6. ACKNOWLEDGEMENT

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Abbreviations: ECM, extracellular matrix; MMP, matrix metalloproteinase; ICAM-1, intercellular adhesion molecule-1; EC, endothelial cells; LFA-1, lymphocyte-associated molecule-1, CAMs, cell adhesion molecules; IL, interleukin; TNF α , tumor necrosis factor α ; LPS, lipopolysaccharide; VEGF, vascular epidermal growth factor; FGF, fibroblast growth factors; FUT, fucosyltransferase; sialyl Lewis (sLE); LE, leukocyte elastase; LN, lymph nodes; Th1, T helper 1.

Key Words: Cancer, Adhesion Molecule, Icam-1, Protease, Matrix Metalloproteinase, Immunosurveillance, Homing, Gene, Review

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