Extracellular matrix moieties, cytokines, and enzymes: dynamic effects on immune cell behavior and inflammation

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Abstract: Tissue injury caused by infection or physical damage evokes inflammatory reactions and events that are necessary for regaining homeostasis. Central to these events is the translocation of leukocytes, including monocytes, neutrophils, and T lymphocytes, from the vascular system, through endothelium, and into the extracellular matrix (ECM) surrounding the injured tissue. This transition from the vasculature into the site of inflammation elicits remarkable changes in leukocyte behavior as cells adhere to and migrate across ECM before carrying out their effector functions. Growing evidence suggests that, through its interactions with cytokines and degradative enzymes, the ECM microenvironment has a specialized role in providing intrinsic signals for coordinating leukocyte actions. Recent advances also reveal that enzymatic modifications to ECM moieties and cytokines induce distinctive cellular responses, and are likely part of the mechanism regulating the perpetuation or arrest of inflammation. This article reviews the findings that have elucidated the dynamic relationships among these factors and how they communicate with immune cells during inflammation. *J. Leukoc. Biol.* 67: 149–159; 2000.

Key Words: *lymphocyte · leukocyte · proteinase · heparanase*

INTRODUCTION

Inflammatory responses to tissue injury or infection require the emigration of certain leukocyte subsets from the vasculature into inflamed sites. Thus, the immune system must coordinate a vast array of cellular and molecular mechanisms to efficiently abate injury, resolve inflammation, and sustain homeostasis. To accomplish this task, leukocytes maintain a constant immune surveillance of tissues to facilitate a rapid and efficient response during tissue insult. One aspect of immune surveillance involves the trafficking and recirculation of lymphocytes to and from lymphoid organs, as well as anatomical sites that routinely require immune effector cells. Although naive lymphocytes normally migrate into secondary lymphoid tissues where antigens are presented to invoke differentiation, mature memory and effector lymphocytes distinctly home to certain sites in the likelihood of encountering their specific antigen or context for effector function. Subsequently, these mature lymphocytes, primarily memory T cells, extravasate across tissue barriers into inflamed loci [1–3].

Upon extravasation into the subendothelial compartment, leukocytes encounter the extracellular matrix (ECM), which functions as a scaffold for cell adhesion and tissue architecture [4]. However, in addition to its structural importance, it is becoming increasingly clear that the ECM also serves as a specialized reservoir of factors that promote cell proliferation, differentiation, activation, and migration. These factors, which may include macromolecules (i.e., collagen type IV, fibronectin, laminin, heparan sulfate proteoglycans), proteases and their inhibitors, and growth factors and cytokines, can greatly influence the outcome of the inflammatory response [4, 5]. Because the nature of a particular inflammatory stimulus demands the extravasation of certain immune cell subtypes, the ECM often undergoes compositional changes from its latent form to its activated form, dynamically altering its contextual information to signal the appropriate cellular responses; these modifications to ECM foster the activation, adhesion, and migration of leukocytes [6]. Migratory leukocytes themselves actively participate in governing ECM modifications by secreting cytokines, chemokines, and degradative enzymes [7, 8]. Accumulating evidence suggests that the presence of these molecules and their derivatives in ECM may act reciprocally with migratory leukocytes, communicating in highly organized, regulatory circuitries to either promote or diminish inflammatory reactions. In this review, we discuss recent contributions and new perspectives of this concept, with special emphasis on its significance in T lymphocyte function. This concept will likely continue in developing a better understanding of how immune cells modify their activation status and migratory behavior, features that are particularly important in homeostatic and pathological conditions.

LYMPHOCYTE-ECM INTERACTIONS

The specific response of leukocytes in general, and lymphocytes in particular, recruited into inflamed areas is modulated via activated ECM by expression and engagement of surface membrane receptors with adhesive ligands that mediate cell-

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cell and cell-matrix interactions. This engagement, which is enhanced by lymphocyte activation, represents a critical step in extravasation, namely the adhesion of cells to ECM moieties. Activation via cytokines, chemokines, or chemical agents, such as phorbol esters, results in integrin transition from low- to high-affinity ligand binding states. This feature of integrins significantly regulates the rapid and reversible adhesiveness to ECM substrates during cell migration [9–11]. The β1 integrin family of membrane receptors, comprised of a common β chain (CD29) and one of several α chains, is the major transducer of signals from the extracellular milieu into immunocytes, including lymphocytes. Intracellular tyrosine phosphorylation ensues via integrin-cytoskeleton interactions, resulting in expression of several genes, particularly proinflammatory cytokines and chemokines [4]. Recent data from our laboratory and others have provided evidence that mediators such as tumor necrosis factor α (TNF-α) [12, 13], interleukin-7 (IL-7) [14], IL-2 [15], RANTES, and macrophage inflammatory protein-1β (MIP-1β) [16], as well as the growth factors basic fibroblast growth factor (bFGF) [17, 18] and transforming growth factor β (TGF-β) [19, 20], bind to ECM constituents (**Table 1**). Thus, the release of these soluble mediators into the ECM by migrating lymphocytes or surrounding cells, such as fibroblasts, epithelial cells, and keratinocytes, may present pleiotropic effects when in the context of the ECM. Their biological activities as chemoattractants and as regulators of cell physiology may indeed augment inflammatory processes in concert with the ECM environment, thereby fine-tuning the immune response (i.e. duration, intensity) as needed.

CYTOKINE-ECM INTERACTIONS

A clear advantage of ECM-bound cytokines is their sequestration in inflamed tissue sites, with the ECM serving as a storage depot for cytokines to act on newly recruited, proximal leukocytes. A chemoattractant gradient may also be created in this context, further attracting and activating incoming leukocytes. Recent studies on one model of cytokine-ECM protein interactions have yielded the conclusion that such complexes cause striking alterations in cell behavior. TNF-α, a key mediator of immune responses, binds avidly to the major ECM constituents fibronectin [12] and laminin [13]. TNF-α binds with high affinity to immobilized fibronectin at its 30-kDa amino-terminal domain, at a site distinct from the cell-adhesive and the heparin- and fibrin-binding domains [12]. This TNF-α-fibronectin association augments β_1 integrin-dependent adhesion of activated $CD4^+$ T cells by increasing cellular protein tyrosine phosphorylation [21]. It is interesting that activated lymphocytes adhere to a fibronectin-TNF-α substrate more avidly than cells exposed to low concentrations of soluble TNF-α and immobilized fibronectin substrate. Similar pro-adhesive effects were also observed in laminin-TNF-α interactions [13]. If TNF-α-ECM interactions represents a paradigm, ECM macromolecules and cytokines appear to act synergistically: the ECM provides a substrate for cytokines to bind, while the bound cytokines consequently enhance cell-matrix interactions. There is another putative biological advantage for cytokine-ECM interactions: the association of pro-inflammatory mediators with molecules in the ECM microenvironment also limits their bioavailability and activity to target foci of inflammation and ensures the localization of the responding immune cells to restricted sites.

For well over the last decade, the chemokine superfamily of inflammatory mediators has drawn much attention, with a wide scope of significance in research topics, including hematopoiesis, tumor immunology, angiogenesis, HIV infection, and immune cell chemotaxis and trafficking [22–24]. Indeed, chemokine interactions with glycosaminoglycans may serve as significant modulators of leukocyte transition through blood vessel endothelium and the ECM, considering their capacity to bind to highly abundant, cell- or ECM-associated heparan sulfate (HS) molecules [25, 26]. Chemokines have potent effects on T cell adhesion to activated endothelium [27] and ECM components (i.e., fibronectin, laminin) by enhancing β_1- and β_2-integrin-ligand binding [28, 29]. Chemokines also cause activated T cells and neutrophils to preferentially migrate, demonstrating that these molecules operate as chemoattractants during haptotaxis [27, 30, 31]. These findings are further complimented by evidence that the chemokines RANTES and MIP-1β, complexed to intact endothelial cell-derived ECM, augment $CD4^+$ T cell adhesion [16]. Thus, the potency of chemokines is effective whether they are in solution, immobilized on endothelial ligands, or in the context of ECM [16, 27, 31]. The capacity for inflammatory mediators, such as TNF-α, RANTES, and MIP-1β to be effective in such different states clearly emphasizes their versatility in vascular, as well as extravascular compartments during inflammation.

TABLE 1. Inflammatory Mediators that Interact with ECM Moieties

Mediator	Specific affinities	Primary target cells	Selected references
bFGF	HS, fibrinogen	Fb, Ec, keratinocytes	[17, 18, 130]
TGF-β	Decorin	Tc, Bc, Mo, Fb	[19]
IFN-γ	HS	Tc, Bc, Ec, Mo, PMN	[131]
MIP-1β	HS	Mo, Tc, dendritic, NK, hematopoietic progenitors	[16]
RANTES	HS	Memory Tc, eosinophils, basophils, NK, dendritic	[16]
IL-4	HS	Bc	[132]
IL-7	Co-IV, LN, FN, HS	Lymphocytes, hematopoietic stem cells	[14]
IL-2	FN, LN, Co-IV	Tc, Bc	[15]
TNF-α	FN, LN	Tc, Bc, Mo, Ec, PMN, Fb, tumor, and somatic cells	[12, 13]

Abbreviations used: Tc, T cells; Bc, B cells; Fb, fibroblasts; Ec, endothelial cells; PMN, polymorphonuclear cells; NK, natural killer cells; Mo, monocyte or macrophage; HS, heparan sulfate; Co-IV, collagen; FN, fibronectin; LN, laminin.

ENZYME-ECM INTERACTIONS

The ECM context that lymphocytes and other inflammatory cells encounter is inherently dynamic. Changes in ECM

composition probably owe to the important role the ECM plays in transducing regulatory signals into cells to coordinate their behavior [32]. Several components are structurally and physiologically fundamental, including fibronectin, collagen, and HS proteoglycans. However, the natural dynamics of ECM remodeling during inflammation and wound repair involve changes in the balance of these components, as well as the deposition of other adhesive molecules not usually present in ECM in ample quantities [33, 34]. The degradation of matrix components, such as fibronectin, is a major determinant in ECM remodeling, evoking cellular responses and signaling the direction of the inflammatory response. Chemotactic fibronectin fragments, among other distinct biological activities, may selectively promote the recruitment of monocytes and other leukocytes into inflamed tissues [35, 36]. Thus, peptide derivatives of fibronectin have been investigated for their therapeutic efficacy in suppressing inflammation *in vivo* [37–39]. Fragments of laminin [40], collagen type XIV [41], and fibrin [42] also have biological roles in modulating inflammatory cell infiltration and cell proliferation. Such activities of ECM derivatives further underscore the importance of matrix-degrading enzymes during inflammation.

Heparanase

An important consideration in the binding of RANTES and MIP-1β to intact ECM is that treatment with the enzyme heparanase dissociates their binding [16]. Several blood-borne cells, including macrophages, neutrophils, activated $CD4^+$ T cells, mast cells, and platelets, exhibit heparanase activity [43]. By virtue of its ability to degrade HS, heparanase can alter cell-surface makeup and ECM composition. These HS molecules interact with the major ECM proteins collagen, laminin, and fibronectin, implicating their role in ECM self-assembly, turnover, and insolubility [44]. The degradation of HS moieties during inflammation by heparanase may facilitate passage of lymphocytes into tissues by altering the composition and insolubility of extracellular matrices [45]. The resultant disruption of MIP-1β- and RANTES-ECM binding may thus be attributed to the probable HS proteoglycan substrates in the ECM. Similarly, the implications of enzymatically removing heparin-binding growth factors and proteases, including bFGF [18] and plasminogen activators (PAs) [46, 47], from their HS-ECM depot have been previously suggested. These findings support the notion that enzymatic removal of bound mediators may either perpetuate or curtail inflammatory reactions by altering the context of ECM, and consequently altering inflammatory cell behavior. Years after the first report of HS-degrading, endoglycosidase activity, only recently has mammalian heparanase been cloned and the recombinant proteins purified for functional studies [48–50]. Considering the efficacy of heparanase-inhibiting molecules in reducing inflammatory disease [51], the molecular tools likely to follow will certainly be of major interest in the development of anti-inflammatory drugs to target immune cell heparanase activity, as well as in the prevention of heparanase-facilitated tumor metastasis.

Memory T cells release heparanase from preformed pools immediately after activation, suggesting its early responsive role in the proceeding inflammatory processes [45]. In fact, inhibiting T lymphocyte heparanase with low doses of heparin, a substrate that is chemically related to HS, hinders enzymatic activity both *in vitro* [52] and in T cell-mediated immunity in rat models of inflammation [51]. These findings underscore the multifaceted, clinical applications of heparin and its sulfated saccharide derivatives, which have distinct roles in preventing coagulation, acute inflammation, and angiogenesis. Implementation of heparin or heparin-/heparan-like derivatives in controlling T lymphocyte heparanase activity thus may have clinical applications in modulating inflammation.

Heparanase possesses other diverse attributes in its capacity to modify, degrade, and adapt to ECM compartments. A previous study conducted in our laboratory focused attention on the pH-dependency of heparanase biological and enzymatic activity [53]. Although mammalian heparanase is enzymatically quiescent at the physiological pH of 7.2, its activity is optimal at relatively acidic pH conditions (6.4–6.8). This characteristic suggests an effective mechanism for restricting heparanase activity to anatomical sites harboring acidic conditions, such as sites of inflammation and tumor growth. At physiological pH, heparanase does, however, retain its capacity to bind to ECM, emphasizing the stability of the enzyme to exist and adapt to varying pH environments. Moreover, at pH 7.2, heparanase promotes $CD4^+$ T cell adhesion to ECM, which is partially inhibited by antibodies directed to β_1 integrins. A previous report by Ratner [54] demonstrated an increased locomotory activity of IL-2-activated T cells at pH 6.7 compared to pH 7.1 in ECM gels, implying that increased migration through ECM may largely correlate with enhanced release of heparanase or other degradative enzymes. These data suggest that heparanase binding to ECM may function in much the same fashion as cytokine- or chemokine-binding to ECM, serving to sequester the enzyme for its pro-adhesiveness, or for its enzymatic activity when inflammatory stimuli arise.

The repertoire of methods by which heparanase modifies the ECM milieu during cell infiltration is perhaps best illustrated by the recent findings that delineated the degradative potential of connective tissue-activating peptide-III (CTAP-III) [55, 56]. CTAP-III and its shorter derivative, neutrophil-activating peptide-2 (NAP-2), are N'-truncated derivatives of leukocyte-derived growth factor and are members of the C-X-C chemokine subfamily [57]. The ability of CTAP-III to induce histamine release from basophils [58] and plasminogen activator activity [59], and its detection in wound fluid [60] and the sera of rheumatoid arthritis patients [61], collectively suggest that it may be a mediator of inflammation. CTAP-III is also capable of desensitizing CXC chemokine receptors on neutrophils, perhaps as a mode of regulating cell activation [62]. In addition to these characteristics, it was recently reported that CTAP-III derived from platelets [55], as well as T cells and neutrophils [56], exhibits heparanase activity at slightly acidic pH conditions, similar to the lower-pH dependency for placental heparanase activity previously reported [53]. The results of the study examining polymorphonuclear neutrophil (PMN)- and T cell-derived CTAP-III suggest that this chemokine may function as at least one of the heparanases that facilitate immune cell migration into ECM during inflammation. Therefore, it may be conceivable that CTAP-III, and perhaps other heparanases, are

released by cells of the immune system to serve as utility factors with multiple capacities to modify the ECM and inflammatory cell behavior. What are the ramifications of these capacities of CTAP-III and other heparanases? These factors may exhibit chemokinetic activities, whereby immune cells sense, migrate toward, and bind to ECM to which heparanases are bound. Moreover, alterations in the microenvironment may signal heparanases to degrade HS proteoglycans in ECM, disrupting the composition and generating a path for migratory cells.

In addition to disrupting the ECM for immune cell passage, the resultant degradation products of heparanase-degraded substrates may actually potentiate other pro- or anti-inflammatory processes. Our laboratory found that heparanase action on intact ECM generates a trisulfated disaccharide with the capacity to inhibit T cell secretion of biologically active TNF-α upon cell activation. This ability to alter this inflammatory response by T cells was further investigated *in vivo*. T cell-mediated inflammation could be suppressed in a mouse model of delayed-type hypersensitivity by administering the ECM-derived disaccharide, likely a derivative of HS components [63]. It was also found that derivatives of heparin, a molecule chemically related to HS glycosaminoglycans, produced by the action of heparinase inhibits TNF-α secretion by macrophages. Furthermore, these disaccharides effectively inhibited delayed-type hypersensitivity and arrested progression of adjuvant arthritis in *in vivo* animal models [64]. Other oligosaccharides have proven effectiveness in relieving diseases characterized by excessive heparanase activity [65–67]. Therefore, it is plausible that HS or heparin disaccharides or similar moieties inherently affect inflammatory cell behavior and have promising therapeutic, anti-inflammatory properties. Such moieties of ECM HS proteoglycans, its related molecules, or other ECM byproducts generated by enzymatic action may represent yet another fundamental loop in the regulation of inflammatory events.

Proteinases

The complexity of ECM composition and the necessity for several cell types with differing immunological roles to penetrate the ECM during inflammatory cell infiltration are dealt with adequately via the release of numerous other degradative enzymes, such as proteinases [68]. It is conceivable that infiltrating immune cells respond to soluble and ECM-complexed cytokines and chemokines by secreting ECM-modifying enzymes that cleave multiple substrate specificities [69]. Thus, a migratory immune cell can actively orchestrate its own fate as it traverses into extravascular spaces by tailoring ECM composition to facilitate its passage and its response to a given inflammatory stimulus. Primarily two subgroups of proteinases have been implicated in immune cell-directed ECM remodeling and degradation: serine proteinases and matrix metalloproteinases (MMPs). Although the number of members in these families is large and still growing, only a select few have been well described in T cells [8]. These include urokinase plasminogen activator (uPA) [70], gelatinase A (or MMP-2) and gelatinase B (or MMP-9) [71–73], and human leukocyte elastase [74]. Herein, we describe recent contributions about these enzymes and their regulatory inhibitors as they relate to T cell-ECM interactions. Such specificity of enzymatic activity in certain cell types likely occurs as a mechanism for regulating ECM organization and immune cell translocation into tissues. Surrounding endothelial, epithelial, fibroblast, and stromal cells may subsequently provide additional enzymes that are necessary to remodel ECM as inflammation resolves (**Table 2**).

uPA

PAs, their specific inhibitors (PAIs), and their membrane-bound receptor (uPAR), are considered key participants in the enzymatic cascades that govern matrix production, remodeling, and turnover [75, 76]. The zymogen plasminogen is the preferred substrate of uPA, and the outcome of plasminogen activation is the generation of plasmin, a serine proteinase with critical roles in fibrinolysis and ECM degradation. Binding between uPA and its membrane-bound receptor, uPAR, impacts several cellular processes that are fundamental in immune cell interactions with ECM. For example, this association accelerates proteolytic activity, and PAI-1/PAI-2 regulate this activity by inducing internalization and recycling of uPAR. Differentiation or proliferation of myeloid and other cell types can also be induced by uPA-uPAR binding [77]. The significance of the uPAR-uPA-PAI complex is further exemplified by interactions between PAI-1-vitronectin, uPAR-vitronectin [75], PAI-heparin, and PA-heparin [78]. These ECM components may therefore be important determinants in promoting activation and de-activation of uPA-mediated proteolysis by sequestering these molecules and providing substrates for cell adhesion. Vitronectin may also greatly influence immune cell adhesion and migration, considering the novel finding that it binds directly to uPAR, as the receptor also complexes with membrane integrins, blocking native integrin function [79–81]. Thus, the nonintegrin receptor uPAR not only mediates the complex system of proteolysis, but it also exhibits the ability to

TABLE 2. Versatile Functions of Enzymes Putatively Involved in Immune Cell Migration Through ECM

Enzyme	Function
Plasminogen activator (uPA)	Converts plasminogen into plasmin, which degrades ECM components (fibrin, fibronectin, laminin, proteoglycans) and activates proMMPs [75]
Human leukocyte elastase (HLE)	Degrades ECM components (elastin, fibronectin, laminin, collagen, proteoglycans) [88]; cleaves inflammatory mediators (IL-2, IL-1β, TNF-α, IL-8) [15, 101–103]; cleaves membrane molecules (TNFr, CD4, CD8, CD2) [93, 104]; lymphocyte and platelet activation [91]; binds to Mac-1 (CD11b/CD18) [92]; induces cytokine secretion [89]
Metalloproteinases (e.g., MMPs, ADAMs)	MMP family collectively cleaves all ECM components [106]; cleaves inflammatory mediators (TNF, IL-1β, TGF-α) [119–121, 124]; cleaves membrane molecules (IL-6r, CD44, L-selectin) [121, 126, 127]
Heparanase	cleaves HS [43]; releases HS-bound proinflammatory mediators [16]; interacts with ECM [53]; yields HS products that modulate inflammation [63, 64]

interact with activated integrins, disrupt integrin-ligand binding, and regulate cell adhesion to an ECM constituent.

Plasminogen is found in fibrin clots, as well as cell-bound via its binding to the plasminogen receptor. Thus, upon dissociation of PAI-1/PAI-2 from the complex, uPA-mediated proteolysis may take place entirely within the vicinity of a given cell, resulting in the generation of plasmin [76]. This localization may be an important factor in immune cell migration, considering that uPA-uPAR binding can also induce a conformational change in uPAR to expose a chemotactic epitope on the receptor, which in turn induces signal transduction [82, 83]. Indeed, chemotaxis induced by uPA has been reported in activated leukocytes, endothelial cells, fibroblasts, and cancer cells [82]. Ligand binding to uPAR thus variably functions as a proteolytic site, as well as a chemokinetic site, able to affect the same and proximal cells.

Substantial evidence suggests a role of the uPA system in matrix degradation and migration by T lymphocytes (Table 2). Binding of uPA-uPAR facilitates invasion into a fibrin matrix by Jurkat T lymphoma cells [84]. Upon phorbol myristate acetate stimulation, HUT 78 lymphoid cells secrete a modulator that induces uPA activity [85]. uPAR has been established as an activation antigen on peripheral blood T lymphocytes both *in vivo* and *in vitro* [86]. T cells from healthy versus HIV-positive donors differ significantly in uPAR expression; no significant expression is detected in cells from healthy individuals, whereas up to 80% of cells from infected donors are uPAR-positive. *In vitro* activation of healthy donor T cells by phorbol ester (PMA), mitogens (phytohemagglutinin, concanavalin A), and cytokines (IL-2, IL-4, IL-7) up-regulates uPAR expression. Similarly, uPAR expression increases during co-clustering of the TCR complex and β_1 or β_2 integrins via treatment with specific antibodies; subsequently, plasminogen activation by these cells augments their invasion in an ECM gel [86]. Demonstration that tumor-infiltrating T cells within primary tumors express uPAR further accentuates the importance of the uPA system in lymphocyte extravasation [87]. A recent study by Gundersen et al. [70] reported the ability of T cell-derived uPA to yield plasmin-degraded products of tenascin C, an anti-adhesive ECM protein that inhibits T cell activation. Plasmin-mediated degradation of tenascin C has no effect on activation, but does convert the ECM protein from anti- to pro-adhesive. The notion that lymphocyte-mediated proteolysis of ECM components reciprocally alters cell behavior by generating potent neo-modulators heralds a novel perspective on cell-matrix interactions.

Elastase

A primary example of the context-dependent, versatile functions of ECM-specific enzymes in immune cell migration is human leukocyte elastase (HLE). HLE is a serine proteinase with a broad spectrum of matrix substrates, such as elastin, collagen, fibronectin, and heparin [88]. The biological activities of HLE also include the induction of cytokine secretion [89], degradation of clotting factors [90], and cell activation [91] (Table 2). Elastase may modulate the availability and pro-adhesiveness of the integrin Mac-1 (CD11b/CD18) by its binding on neutrophils and monocytes [92]. Cleavage of the T lymphocyte surface molecules CD2, CD4, and CD8 by elastase can yield a temporal impairment of T cell physiology in inflammatory diseases [93]. Such an immense variety in activities obliges a tight and efficient regulation by the natural inhibitors of serine proteinases, termed serpins, which actually comprise about 10% of total plasma proteins. Although most serine proteinases, as well as MMPs, are secreted as inactive zymogens, active HLE is stored within granules in neutrophils [94], as well as distinct subpopulations of monocytes [95, 96]. The proinflammatory cytokines TNF-α and IL-8 also up-regulate expression of membrane-bound elastase either alone (TNF-α only) or in response to sequential stimulation (TNF-α, then IL-8) [97]. Membrane translocation of elastase and other serine proteinases could be a novel mechanism of ensuring a focused localization of the enzymes' biological activities within specific proximity, perhaps at the leading edges of migrating cells. This concept of migratory cell polarization has been described extensively in motile T lymphocytes, which exhibit a region of high sensitivity to chemokines and antigen at the leading pole of cell migration [98, 99]. Localizing proteolytic enzymes to distinct cellular regions may also be a factor during lymphocyte migration because membrane-bound and soluble forms of T cell elastase have been identified [74, 100]. Therefore, T cells and other immune cells likely concentrate a battery of receptors, adhesion molecules, and enzymes at specific cellular zones to control cell movement through tissues.

A substantial amount of evidence has elucidated the proteolytic actions of elastase on inflammatory cytokines and their receptors, which may lead to modifications in their biological activities. Unlike MMP-mediated inactivation of IL-1β, HLE may be involved in processing the precursor cytokine to its active form [101]. Similarly, HLE proteolysis converts a larger variant of IL-8 (77 amino acids) to more potent, truncated variants, implying that HLE enhances IL-8-mediated inflammatory events [102]. Soluble immunoreactive TNF-α is susceptible to proteolysis and inactivation by HLE, which degrades the cytokine into fragments [103]. Because TNF-α has also been shown to bind ECM constituents, it is possible that HLE can alter the biological activities of the insoluble cytokine that inflammatory cells encounter in the ECM. HLE can also affect cellular responses to TNF-α by shedding the 75-kDa TNF receptor, thereby depleting the potential for the cytokine to bind target cells [104]. Consequently, HLE exhibits an ability to orchestrate the actions of inflammatory mediators, particularly cytokines, by generating either potent or quiescent forms that contribute to the regulation of the inflammatory response.

The issue of how leukocyte-mediated proteolysis of inflammatory mediators can regulate inflammation has recently been addressed at the level of T cell adhesion to ECM. IL-2 has been extensively studied for its abilities to induce T cell activation, differentiation, proliferation, and migration, all of which are critical processes in the inflammatory response [105]. Our laboratory undertook a study to examine the ability of IL-2 to promote T cell adhesion to ECM moieties. It was found that human T cell adherence to major ECM proteins, as well as intact ECM, is induced after short exposure to IL-2 [15]. This finding prompted further investigation of whether proteolytic degradation products of IL-2 could modify T cell adhesion to

ECM. Considering the efficiency of HLE proteolytic activity on other cytokines, it was conceivable that HLE could, in turn, yield degraded products of IL-2 with potential bioactivities. Indeed, elastase-digested human IL-2, and its peptide fragments were found to have differing abilities to inhibit not only IL-2-stimulated T cell adhesion to fibronectin, but also adhesion induced by other pro-inflammatory mediators. Three short peptides of different amino acid sequences and locations in the IL-2 primary sequence strongly inhibited adhesion to multiple ECM components. The possibility that these elastase-generated IL-2 peptides could modulate T cell migration toward the chemoattractants MIP-1β and IL-2 was also explored. Chemotaxis was, in fact, markedly inhibited by the IL-2 degradation products. The role of β_1 integrins in modifying T cell-matrix interactions during this inhibition by IL-2 peptides appeared to prevent the rearrangement of the actin cytoskeleton typically associated with cell adhesion [15]. Taken together, the occurrence of elastase-generated, bioactive IL-2 peptides may be a prototype of other leukocyte-driven enzymatic actions on inflammatory mediators, particularly cytokines. Elastase and other proteinases may degrade the very proteins (cytokines) that stimulate immune cells and drive the inflammatory response toward new directions. Truncated or degraded products of these mediators may still maintain or even enhance immunoreactivity in a mode distinct from the intact molecule; conversely, they may lose their potency, thus terminating their participation in the inflammatory response. In any case, the shift in activities, from the whole molecule before proteolytic cleavage to its products after degradation, is likely to be an intrinsic part of a natural regulatory mechanism governing inflammation.

MMPs

The MMP family of enzymes consists of at least 15 distinct members, 9 of which are expressed by leukocytes. A common structural domain is shared by these enzymes, which collectively can degrade every ECM substrate [106]. Aside from inhibition by the universal proteinase inhibitor, α_2-macroglobulin, MMPs are also regulated by endogenous tissue inhibitors of metalloproteinases (TIMPs), which engage in non-covalent complexes with MMPs [69]. It is noteworthy that TIMP-1 and TIMP-2 are soluble inhibitors of MMPs found in body fluids, whereas TIMP-3 binds to the insoluble ECM. It is believed that the balance between MMP activation and the level of TIMP mediates the level of proteolysis by MMPs. A wide distribution of TIMPs in both fluid and insoluble environments highlights the need to regulate MMPs, whose activities have been implicated in normal tissue remodeling during development, menstruation, and wound repair after tissue injury. Their proteolytic and destructive potential is also of major concern in pathological states such as metastatic cancer, multiple sclerosis, rheumatoid arthritis, and periodontal disease. As major sources of MMPs, leukocytes also rely heavily on these enzymes to mediate their extravasation and penetration into tissues during inflammation [8, 69, 106]. The diverse effects of MMP activities, ranging from beneficial to damaging, are thus dependent on their highly regulated gene transcription [107].

In most cases, MMPs are synthesized *de novo* and rapidly secreted, rather than stored. With the exception of 72-kDa gelatinase (MMP-2), expression of MMPs by migrating leukocytes requires cell stimulation via certain inflammatory cytokines, growth factors, and eicosanoids [107–109]. In contrast, interferon-γ, progesterone, and corticosteroids are among the inflammatory mediators that may suppress MMP synthesis [106, 108]. A recent report by Johnatty et al. [108] also demonstrated that the proinflammatory chemokines RANTES, MIP-1α, and MIP-1β induced MMP-9 secretion by peripheral blood T lymphocytes, whereas TIMP-1 secretion remained constitutive. T cell migration through basement membrane is evoked by these chemoattractants after 24 h, as well as by short exposure to vasoactive intestinal peptide (VIP) and IL-2 [110]. T lymphocyte activation via IL-2 greatly augments MMP-9 and MMP-2 expression, the predominant MMPs of T cells, and thereby, migration across gels of basement membrane [71]. IL-2 also promotes memory T cell migration toward RANTES and other chemokines of the CC subfamily by augmenting CC chemokine receptor expression [111]. Because several of the same cytokines and chemokines that enhance MMP expression may also interact with ECM moieties, their effects on T cell adhesion and MMPs may have a profound impact during cell migration across tissue barriers. Factors governing T cell adhesion to endothelium and ECM may also influence metalloproteinase synthesis. Indeed, adhesion of leukocytes to vascular endothelial cells via VCAM-1 induces expression of 72-kDa gelatinase [112], and cell-matrix interactions via β_1 integrins control MMP-dependent migration through basement membrane [113]. Therefore, MMP expression in immune cells may be intrinsically regulated at every stage of their extravasation from blood into the ECM, including: (1) activation and homing to specific tissues; (2) adhesion to endothelial cells; and (3) adhesion to extracellular matrices.

Further studies should address the question of whether the resultant fragments of ECM proteins and other target substrates of metalloproteinases may act in an immunomodulatory manner, as has been shown with heparanase and elastase. Such studies could determine whether these fragments perpetuate tissue injury by acting as immunoreactive and proinflammatory factors, or conversely, whether these cleaved substrate products may attenuate inflammatory reactions. It has been postulated that MMP activity results in immunogenic segments of normal proteins that may cause autoimmune disease [114]. MMP-mediated degradation of ECM components may also release growth factors and other inflammatory mediators bound to the target substrate [115]. It is interesting that the same cytokines that can induce metalloproteinase production may also be subject to processing by MMPs (Table 2). Metalloproteinases are capable of cleaving and inactivating the proinflammatory cytokine IL-1β [116], and can thereby down-regulate their own activities because IL-1β is an inducer of MMP gene transcription. This insight on MMP regulation may prove to be significant in treating diseases of excessive MMP activity as the inflammatory properties of more degraded cytokine and ECM fragments generated by a variety of enzymes are further revealed.

Recent studies in the field of metalloproteinases have identified enzymes that can inactivate or shed proteins from

cell surfaces, leading to potential modulation of inflammatory reactions [117, 118]. Although cleavage of membrane forms of cytokines and growth factors, such as TNF-α [119, 120], stem cell factor (SCF), and TGF-α [121], can be processed by classical MMPs, it appears that related metalloenzymes with similar catalytic sites (termed sheddases, secretases, or convertases) perform much of the processing. Characterization of a related family of proteins termed 'a disintegrin and metalloprotease' (ADAM) recently emerged with implications in inflammation, fertilization, and other processes [122, 123]. The metalloprotease domains of these proteins enable them to shed or convert cell-surface molecules, whereas their disintegrin domains contain the Arg-Gly-Asp (RGD) or RGD-like motifs characteristic of ligands for integrins. Its members have intriguing potential to alter immune cell behavior [123] by binding integrins involved in cell-cell and cell-matrix interactions. Furthermore, ADAMs function in converting or shedding membrane cytokines, such as precursor TNF-α, into soluble forms [124]. This process has especially significant implications for both the beneficial and pathological effects of the cytokine [124]. The mechanism of such release has been of recent interest in treating inflammatory diseases such as multiple sclerosis, where cocktails of inhibitors to MMPs and sheddases have been shown to have therapeutic effects [125]. Future studies of the versatility of ADAMs and related metalloproteases could reveal many more important roles in their regulation of immune cell-cytokine-ECM interactions during inflammation.

The outcome of an inflammatory response can also be influenced by metalloenzymatic cleavage of cytokine receptors and adhesion molecules from cell membranes by ADAMs or other sheddases. TNF-α receptors liberated from cell surfaces inhibit soluble and membranal TNF, illustrating a sophisticated loop of metalloproteinase activities on an inflammatory molecule and its receptors [124]. In marked contrast, cleavage of the IL-6 receptor stabilizes its proinflammatory ligand on binding, and the complex actually functions as an agonist of IL-6 [121]. Adhesion molecules may also be targets for solubilization. Shedding of CD44 has important implications in modulating tumor cell, and possibly immune cell, invasion [126]. There is evidence that a T cell surface sheddase releases L-selectin and may alter lymphocyte-endothelial cell interactions [127]. This suggests that T cells may employ sheddases or other as yet unidentified metalloenzymes to modulate the context of migration by releasing surface-bound or immobilized molecules (**Fig. 1**). Collectively, these actions of metalloproteinases (Table 2) demonstrate their significance in governing inflammatory responses by not only creating a trail for immune cells to migrate into inflamed tissue, but also by modifying the contextual molecules that affect cell behavior and enzyme secretion itself.

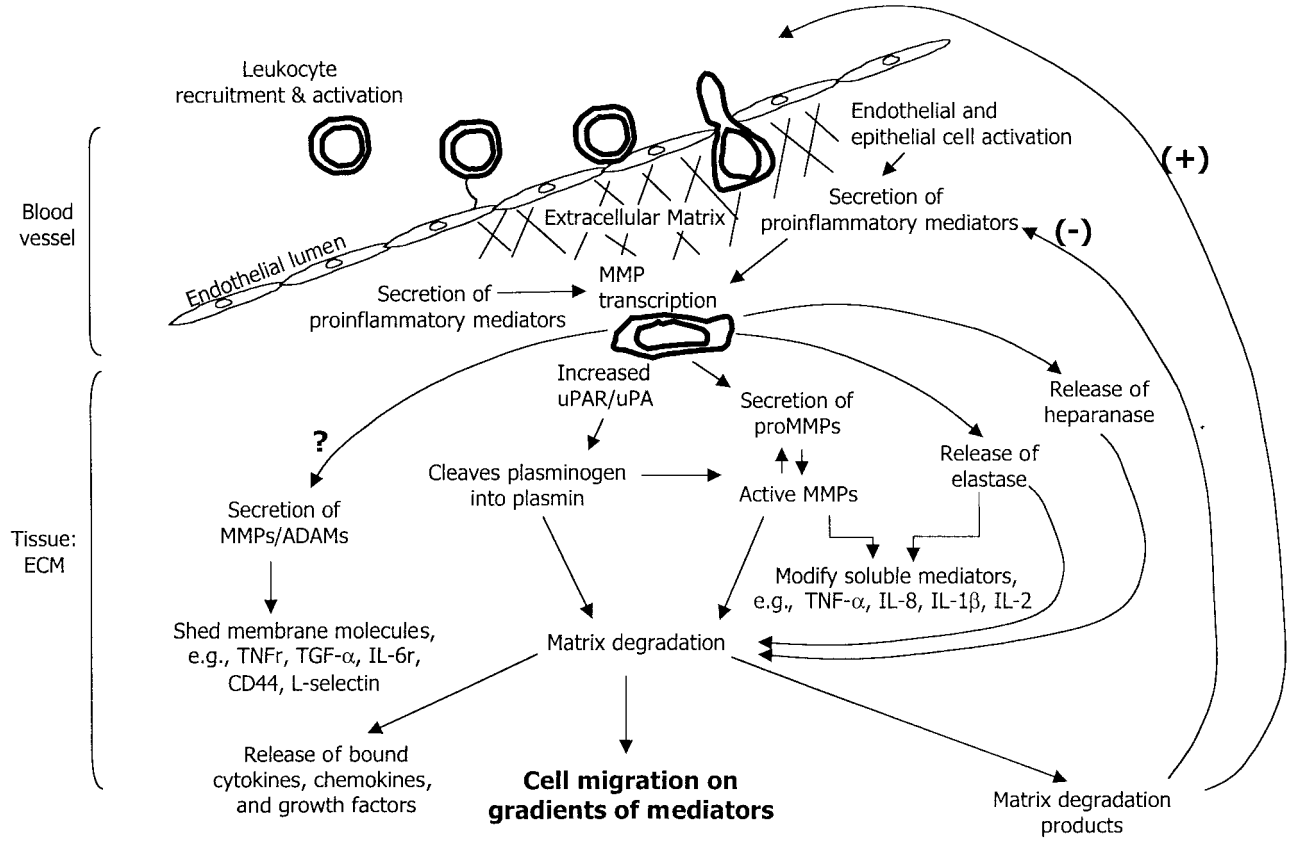

Fig. 1. Schematic representation of immune cell translocation from the vasculature into tissues. Inflammation induces T cell activation and recruitment to specific loci. Cytokine secretion by T cells, as well as surrounding leukocytes, endothelial, and epithelial cells, evokes proMMP expression and secretion. uPA-generated plasmin can activate proMMPs. Elastase and heparanase are also secreted, and all of these enzymes degrade ECM to facilitate cell migration. Cytokines and membrane molecules are modified by such enzymes, altering the molecules' function in the context needed. Matrix degradation products participate in a feedback loop to modulate cytokine secretion or further recruit leukocytes.

CONCLUDING REMARKS

Inflammation requires the translocation of leukocytes from the circulatory system into extravascular compartments [2]. The ECM microenvironment possesses a myriad of signals that cooperate in a dialogue with immune cells, coordinating their behavior as they make their way into inflamed tissues (Fig. 1) [128]. Several lines of evidence have indicated that the crosstalk between immune cells and their surroundings profoundly influence cells to adapt and respond as needed. For example, interactions between integrins and fibronectin stimulate fibroblast expression of metalloproteinases [129] and mediate T cell chemotaxis through basement membranes digested by MMPs [113]. MMPs and other enzymes released by activated inflammatory cells act on and modify the constituents of this milieu through which cells migrate to reach target organs. The action of these enzymes, whether proteinases, heparanases, or other subgroups, creates not only a path for cells to follow, but also a collection of cytokine and ECM substrate fragments possessing distinct activities that may affect the outcome and length of the inflammatory response. Hence, mediators and enzymes secreted from immune cells invading into tissues through ECM may all participate, as part of the entire complex of information, in determining the fate of the inflammatory reaction. Reservoirs of ECM-bound mediators may impose chemotactic gradients on the matrix environment and activate incoming cells, as well as restrict specific subsets of cells to localized inflamed sites. ECM-complexed mediators can also be liberated by enzymes, releasing them as intact, soluble participants in the immune response. Soluble or bound mediators are themselves subject to various enzymatic modifications or degradation into small fragments, altering their inherent bioactivities and thereby demonstrating participation in an elaborate system of modulating inflammation. Collectively, these bilateral cell-matrix signals require efficient regulatory mechanisms, as their dysregulation may perpetuate and prolong tissue injury. Inhibitors of modifying enzymes and cytokines are thus an elemental part of the network that transmits signals between immune cells and their surroundings. It is likely that the string of signals created in the ECM microenvironment to recruit, activate, and regulate immune cells varies from inflammatory episode to episode [128]. Each medley of ECM constituents, cytokines, and enzymes provides a context of information for migrating leukocytes, promoting reciprocal cell responses to ultimately resolve inflammation.

Future studies aimed at understanding the chemical and physical interplay among the molecules secreted by immune cells within ECM during inflammation should provide additional insights into the cells' mode of action, as well as into the mechanisms underlying the termination of immune reactions. The effects and potency of inflammatory mediators, such as cytokines, on immune cell activities when encountered in the ECM context remain to be elucidated. Similarly, the still-growing breadth of research on endoglycosidase and proteolytic enzymes may reveal other breakdown products of tissues and cytokines with novel potential to modulate inflammation. Such studies should indeed indicate whether immune cells within the inflammatory milieu utilize an intrinsic program of chemical signals that alert and activate, and with time, elicit important negative signals to adapt their behavior to their dynamically altered environment.

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