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Distinct cellular functions mediated by haemopoietic cell-surface proteases

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Introduction

The presence of proteolytic activities on cellular surfaces initiates a variety of essential biological responses. Likewise, haemopoietic cell-surface proteases appear to have multiple effects in a wide variety of physiological and pathophysiological processes. Protein turnover, ontogeny, inflammation, cell migration and tissue invasion are among the many systems which depend upon cell-surface protease control mechanisms.

It is possible to discern two classes of enzymes at the surface of haemopoietic cells: the cell-associated protease family and the integral protease family. The cell-associated proteases are bound to the membrane either through ionic interactions or specific binding sites and they include serine elastase, cathepsin G and the plasminogen activators (PAs). The integral proteases are transmembrane type I or type II molecules and they include a growing number of members with various and partly overlapping specificities.

The structural features and degradative properties of these two classes of haemopoietic cell-surface proteases are summarized in this review. Recently, there has been an increase in the information available about extended functions of ectoproteases and we have tried to provide some insight into the way haemopoietic cell-surface proteases, through multifunctional mechanisms, may influence physiological and pathological events.

Sources and regulation of haemopoietic cell-surface proteases

As summarized in Table 1, some well-characterized proteases are present on the surface of haemopoietic cells. Although initially described as being found in granulocytes, monocytes and alveolar macrophages, serine elastase and cathepsin G are also present on promonocytic cells (Maison *et al.*, 1991; Villiers and Bauvois, unpublished results). Both proteases are the primary enzymes responsible for extracellular matrix (ECM) degradation (reviewed in Janusz and Doherty, 1991). Alpha 1-protease inhibitor (α1-PI) synthesized by myeloid cells blocks the activity of both elastase and cathepsin G (Remold O'Donnell *et al.*, 1989) and the complex formed following the interaction of α1-PI with elastase is chemoattractant for granulocytes (Banda *et al.*, 1988). Monocytes/macrophages synthesize both urokinase [u-PA] and tissue plasminogen activator [t-PA] as well as inhibitors of plasminogen activators (Vassalli *et al.*, 1984; Chapman and Stone, 1985; Hart *et al.*, 1989). In addition, monocytes, macrophages and monocytic cell lines express both u-PA and plasminogen receptors and several agonists, including interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α) and urokinase itself, have the capacity to modulate the expression of u-PA receptors (Kirchheimer *et al.*, 1988; Felez *et al.*, 1990). PAs are chemotactic for granulocytes *in vivo* (Boyle *et al.*, 1987) and they catalyze the proteolytic

Table 1. Major cell-surface proteases expressed by the haemopoietic system

Enzyme	Localization	Degrades
Associated		
Elastase	myeloid lineage cells	elastin, proteoglycan fibronectin, collagen plasminogen activator C3, PDGF
Cathepsin G	myeloid lineage cells	fibronectin, proteoglycan angiotensin I, C3
Plasminogen activator (u-PA)	monocytes, macrophages monocytic cell lines	plasminogen fibronectin
Transmembrane		
Neutral endopeptidase 24.11 (CD10/CALLA)	immature B and T cells granulocytes bone marrow stromal cells	enkephalins endothelins substance P bradykinin IL-1β, IL-6 f-Met–Leu–Phe angiotensin I
Aminopeptidase N (AP-N/CD13)	myeloid lineage cells	enkephalins endorphins
Dipeptidylpeptidase IV (DPP IV/CD26)	activated B, T cells myeloid lineage cells	melittin, cecropins β-casomorphin substance P GH-releasing factor collagen I, TNF-α
Dipeptidylcarboxypeptidase A (ACE/ kininase II)	pre-B, T cells macrophages	enkephalins bradykinin angiotensin I neurotensin
Aminopeptidase A (AP-A)	early B lineage cells	angiotensins I and II

C3, third complement; GH, growth hormone; IL-, interleukin; PDGF, platelet-derived growth factor; TNF-, tumor necrosis factor.

activation of plasminogen to plasmin which activates or degrades extracellular proteins (zymogens, fibrinogen, fibronectin, laminin, collagen IV). Thus, PAs can regulate extracellular proteolysis, fibrin clot lysis, tissue remodelling and such physiological processes as inflammation and metastasis. Secreted u-PA does not significantly contribute to the invasive process unless it is receptor-bound and cell-surface-associated

(Ossowski, 1988). Moreover, the receptor for u-PA polarizes expression of the protease to the leading edge of migrating monocytes suggesting that this receptor is a key determinant in the control of u-PA-catalysed extracellular proteolysis (Estreicher *et al.*, 1990).

CD10 antigen [CALLA (common acute lymphoblastic leukaemia antigen)] is a type II integral membrane protein that is identical to neutral endopeptidase (NEP, EC 3.4.24.11) (Letarte *et al.*, 1988). Originally identified as associated with 80% of cases of childhood acute lymphoblastic leukaemia with a pre-B cell phenotype, CD10/NEP is now identified on B lymphocyte progenitors in bone marrow and foetal liver, suggesting it may play a role in the early stages of B cell development. It is also found on culture bone marrow stromal cells, mature granulocytes and Jurkat T cells (reviewed in Bowes and Kenny, 1987; Kee *et al.*, 1992; Mari *et al.*, 1992) as well as in a large number of nonhaemopoietic tissues. CD10/NEP is a zinc-containing endopeptidase which can hydrolyze numerous small biological peptides such as enkephalins, endothelins, substance P, bradykinin, the pleiotropic cytokines IL-1β and IL-6 (reviewed in Turner *et al.*, 1985; Bauvois *et al.*, 1991). NEP, present on the surface of granulocytes cleaves the chemotactic peptide *N*-formyl-Met–Leu–Phe (f-M–L–P), suggesting that this enzyme can modulate chemotactic responses (Painter *et al.*, 1988). CD13/aminopeptidase-*N* (AP-N, EC 3.4.11.2) is a zinc-containing exopeptidase expressed by acute B lymphoid and myeloid leukaemic cells and cells of the granulocyte-macrophage lineage at all stages of differentiation (Look *et al.*, 1989; reviewed in Bauvois *et al.*, 1991; Naquet and Pierres, 1991). Although CD13 specific antibodies do not bind normal B and T cells, an aminopeptidase-*N* activity which is enhanced upon mitogenic stimulation, is described on human T lymphocytes and differentiating murine thymocytes (Amoscato *et al.*, 1989; Bauvois, 1990). As for CD10, the natural substrates for CD13/AP-N appear to be regulatory peptides including enkephalins and other opioid peptides (reviewed in Turner *et al.*, 1985; Checler, 1991). Both CD10/NEP and CD13/AP-N are found to be increased on myeloid cells stimulated with C5a *in vitro* or obtained from patients undergoing activation of C during haemodialysis (Werfel *et al.*, 1991). CD26/dipeptidyl peptidase IV (DPP IV, EC 3.4.14.5) is a type II serine protease widely distributed in mammalian tissues and in the immune system is expressed by activated B cells, cells of T and granulocyte-macrophage lineages (Schön *et al.*, 1987; Bauvois *et al.*, 1992; Laouar *et al.*, 1993; Bristol *et al.*, 1992). By removing dipeptides of the X-Pro-type from peptides and proteins, DPP IV is involved in the processing of bioactive peptide precursors including cecropins and melittin (Kreil *et al.*, 1980; Boman *et al.*, 1989) as well as in the degradation of bioactive peptides (β-casomorphin, substance P and growth hormone-releasing factor) (Bongers *et al.*, 1992) and denatured collagen I (Bauvois *et al.*, 1991). In our laboratory, we have recently shown that DPP IV of U937 cells participates in the degradation and inactivation of TNF-α (Bauvois *et al.*, 1992). Dipeptidyl carboxypeptidase A (ACE or angiotensin converting enzyme, kininase II, EC 3.4.15.1) is a widely distributed membrane-bound metallopeptidase present on pre-B derived leukaemia cells and cells of T and monocyte-macrophage lineages (reviewed in Checler, 1991; Costerousse *et al.*, 1993). ACE inactivates enkephalins, substance P and bradykinin and converts angiotensin I into angiotensin II (reviewed in Checler, 1991). Inside the haemopoietic system, aminopeptidase A (AP-A) is expressed on a subpopulation of cortical thymic epithelial cells and on pre-B and immature B lineage cells (Witlock *et al.*, 1987; Wu *et al.*, 1991). Such selective expression on pre-

B lymphocytes may reflect a requirement for these cells to hydrolyze (a) regulatory peptide(s) at the stage of lymphopoiesis. Outside the haemopoietic system, AP-A can degrade angiotensins I and II. Moreover, IL-7 preferentially induces AP-A expression together with pre-B cell proliferation (Wu *et al.*, 1991).

A few other cell-surface proteases have been less thoroughly characterized. Among them, a trypsin-like serine protease (TSP-1) on effector T cells is capable of cleaving casein and releasing high-molecular weight products from sulphated proteoglycans (Kramer and Simon, 1987). A membrane-bound arginine-specific serine enzyme detected on B murine spleen cells is synthesized to a higher level following mitogenic stimulation (Ku *et al.*, 1983). Human lymphoid T and B cells possess a trypsin-like protease and a neutral endopeptidase on their surface that both participate in the degradation of the vasoactive intestinal peptide (VIP) released by eosinophils and are shown to inhibit the proliferative responses for murine lymphocytes to mitogens (Goetzl *et al.*, 1989). A neutral serine protease on activated neutrophils may be in part responsible for cytolysis of target cells (Pontremoli *et al.*, 1986). Finally, the low affinity IgE receptor FcεRII/CD23 present on B cells and inducible on monocytes and T cells could be cleaved according to an autocatalytic mechanism (Letellier *et al.*, 1989).

Proteolytic role of haemopoietic cell-surface proteases—scope and diversity

It is readily apparent from Table 1 that the scope of haemopoietic cell-surface proteases is remarkable. This diversity encompasses virtually all types of peptides and proteins grouped by functional criteria. Most of the proteins listed include pleiotropic cytokines (IL-1β, IL-6 and TNF-α), vasodilatory peptides (VIP and substance P) and neuroendocrine hormones such as endorphins, enkephalins, somatostatin and angiotensin, all implicated in many functions of the immune system and the inflammatory responses (Lotz *et al.*, 1988; Metcalf, 1989; Gijbels and Billiau, 1992; Van Den Bergh *et al.*, 1992). Likewise, immunoregulatory properties of substance P (SP) after interaction with specific SP receptors include *in vitro* stimulation of T cell proliferation, the augmentation of Ig synthesis, macrophage and neutrophil activation and the modulation of lymphocyte traffic (reviewed in Calvo *et al.*, 1992). As seen in Table 1, there is therefore much interest in the role of cell-surface proteases such as NEP, ACE and DPP IV in inactivating SP.

Many integral membrane proteins lead to a dual existence as both membrane-bound and soluble isoforms (Ehlers and Riordan, 1991). Such a proteolytic mechanism leads to the release of a large variety of proteins, including growth factors and cytokines (TGF-α, TNF-α, CSF-1 (*colony stimulating factor*), EGF (*epidermal growth factor*) and S-CGF (*stem cell growth factor*), growth factor and cytokine receptors (TNF-α, NGF (*nerve growth factor*), CSF-1, IL-1, IL-2 and IFN-γ), leukocyte antigens (FcRIII/CD16, FcRII/CD23, CD8, Mel14) and ectoenzymes themselves (cholinesterase, sialyltransferase/CD75, NEP, DPP IV, AP-N and ACE). An endogenous EDTA-sensitive membrane-associated protease from pig kidney has been shown to release ACE in a soluble form (Hooper *et al.*, 1987). This example lends support to the concept that the proteolytic release of membrane proteins can be a specific event regulated by cell-surface proteases themselves.

Role of haemopoietic cell-surface proteases in signal transduction

Besides the CD3/TCR complex which activates T cells and leads to the biological expression of the T cell functional programme, other non-TCR structures including CD2, CD4, CD8, CD28, Thy-1, LFA-

1, CD49d/CD29 (VLA-4), CD49e/CD29 (VLA-5) can induce or modulate cell activation. Recently, monoclonal antibodies (mAbs) against the human CD26/DPP IV have been shown to co-stimulate T cell proliferation, IL-2 secretion and cytoxicity (Fleisher, 1987; Dang *et al.*, 1990, 1991; Hegen *et al.*, 1990). In the mouse and in the rat, mAbs anti-CD26 have similar effects on immature and mature T cell proliferation (Naquet *et al.*, 1989; Vivier *et al.*, 1991; Bristol *et al.*, 1992). Moreover, mAb against rat CD26 can also mediate a co-stimulatory signal for granulocyte and macrophage colony formation (Bristol *et al.*, 1992).

There is current evidence that leukocyte integrins are signal transducers. Indeed, collagen-dependent CD4 T cell activation is found to involve the integrin VLA-3 (CD49c/CD29) but also a second collagen receptor represented by CD26/DPP IV, indicating that cell adhesion *via* two different families of antigens **i.e.** integrins and proteases can contribute to cell activation (Dang *et al.*, 1990).

How does CD26/DPP IV signal? The biochemical signals delivered by CD26 are distinct from signals derived from an increased intracellular calcium level (Bristol *et al.*, 1992) but seem to require protein kinase C activation (Dang *et al.*, 1990; Vivier *et al.*, 1991; Bristol *et al.*, 1992). CD26 on human T cells is physically associated to CD45RO, one form of the tyrosine phosphatase leukocyte common antigen (Torimoto *et al.*, 1991). Anti-CD26 mAb treatment down-modulates both CD26 and CD45RO expression and results in increased tyrosine phosphorylation of two proteins critical in T cell activation i.e. the TCRζ chain and $p56^{lck}$ kinase (Torimoto *et al.*, 1991). Likewise, similar activation signal transducing properties are now reported with two other cell-surface proteases CD13/AP-N and CD10/NEP. Anti-CD13 mAb is shown to inhibit rosette formation by neutrophils and erythrocyte-antibody complement (O'Connell *et al.*, 1989), whereas anti-CD10 mAb significantly decreased IL-2 production in activated Jurkat T cells (Mari *et al.*, 1992). Outside of the haemopoietic system, specific binding of enzymatically active uPA with its receptor is mitogenic in malignant cells (Kirchheimer *et al.*, 1989). Altogether, these results emphasize the emerging theme of signal transduction by haemopoietic cell-surface proteases.

Other roles?

Cell-surface proteases as adhesion molecules

Outside of the haemopoietic system, CD26/DPP IV has been shown to have a binding affinity for collagen and fibronectin (Hanski *et al.*, 1988; Bauvois, 1988; Piazza *et al.*, 1989) and was strongly suggested to participate in adhesion of rat hepatocytes and murine fibroblasts to collagen (Hanski *et al.*, 1988; Bauvois, 1988). Since then, DPP IV on human T cells has been demonstrated to be a functional collagen receptor leading to cell activation (Dang *et al.*, 1990). Thus, ectoproteases can directly serve as cell adhesion molecules that further modulate intracellular activation signals. Figure 1 depicts a highly speculative hypothesis regarding the potential role of ectoproteases such as DPP IV in regulation of haemopoietic cell adhesion: in a first step, cells may contact the ECM protein (fibronectin/collagen) via specific cell-surface proteases and resulting adhesion could result in signal transduction and cell activation. In a second step, activated cells no longer could bind to ECM, consistent with the detachment of cells and concomitant degradation or not of ECM. Such flexibility provided by cell-surface proteases could be important in attachment and migration of haemopoietic cells occurring in various physiological (development) and pathological processes (inflammation, cancer).

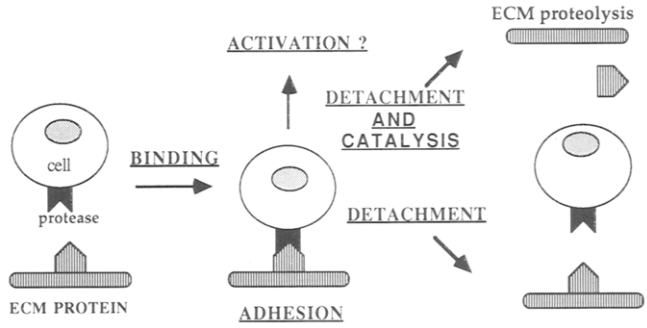

Fig. 1.

Cell-surface proteases as virus receptors

It is known that viruses make use of cell-surface proteins as cellular receptors to enter and infect cells. Two recent reports provide strong evidence that epithelial cell CD13/AP-N serves as a receptor for at least two viruses i.e. TGEV (transmissible gastroenteritis virus) and HCV-229E human coronavirus (Delmas *et al.*, 1992; Yeager *et al.*, 1992). Interestingly, very recent data have reported that human immunodeficiency virus (HIV) can bind an integral type II protein which shares close homology with CD23/FcRII (Curtis *et al.*, 1992) suggesting that non-CD4 HIV receptors with structural features of some integral proteases can be important in HIV infection.

Cell-surface proteases and transpeptidation

Proteases are found to be strong catalysts for transpeptidation reactions (reviewed in Kasche, 1989). Indeed, CD26/DPP IV *in vitro* expresses both peptidase and acyl transferase activity (Yoshimoto *et al.*, 1978). In our laboratory, we have recently shown that cells of the granulocyte-macrophage lineage degrade IL-6 into active 8 kDa peptides. Further inactivation involves formation of a 16 kDa complex due to dimerization of the 8 kDa-derived IL-6 peptides resulting from a peptidyl transferase-catalysed reaction occurring at the cell surface (Laouar *et al.*, 1993). Our data may explain the origin of high molecular weight IL-6 species (45–85 kDa) detected in human serum (May *et al.*, 1991).

Conclusions and prospects

As summarized here, the structural features of cell-surface proteases in the haemopoietic system are becoming well understood. What has become clear in the last few years is that haemopoietic cell-surface proteases are not simply proteolytic enzymes but they can be considered as receptors in the sense of transmitting signals both into and out of the cells.

Primary function of such enzymes is to activate/inactivate *via* degradation a variety of molecules including hormones, neuropeptides, cytokines and ECM proteins. Apart from their degradation role, the second major property of cell-surface proteases is their role as signal transducing molecules: (perhaps all) cell-surface proteases mediate information transfer into cells by acting as coreceptors with more traditional receptors. It is reasonable to propose that interaction of these enzymes with their biological substrate(s) can trigger signalling events. One of the unanswered questions is how proteases

signal and a rapidly advancing area is presently the dissection of the molecular basis of signal transduction through proteases.

Studies on the role of cell-surface proteases outside the haemopoietic system led to the surprising finding that enzymes such as DPP IV/CD26 may be receptors by which cells attach to ECM. This is an attractive model in which leukocytes *via* cell-surface proteases could exhibit combined adhesion and migration activities through lymphoid areas and peripheral tissues. This hypothesis will surely benefit from progress in studies of cell adhesion.

A current speculation concerns cell-surface proteases and their contribution in the conversion of peptides and proteins. Further studies are needed to improve the 'planning' of efficient and selective cell-surface protease-catalyzed reactions in biological processes.

Through these activities above, there is emerging evidence that haemopoietic cell-surface proteases may play a dynamic role in development and in adult organisms by regulating cell proliferation and differentiation, secretion, cell-mediated cytotoxicity and cell migration. Cell-surface proteases are subject to fine control such that their expression at any point in time is being regulated by a variety of factors including the immediate microenvironment (cytokines, ECM, inhibitors), the state of cell activation and maturation and in turn they can modulate cell functions.

Definitive evidence for the physiological relevance of a given action of a haemopoietic cell-surface protease requires tests in the intact organism. Obviously, such tests are difficult to carry out and an alternative strategy is the association of modified states of cell-surface proteases with diseases as diverse as thrombosis, inflammation and leukaemias. A number of pathologic conditions are already shown to be associated with up and/or down regulation of cell-surface proteases and they therefore may serve as independent diagnostic predictors. For example, the clinical course of pre-B and T acute lymphoid leukaemias are associated with high content of CD10/NEP (Foon and Todd, 1986). Both an increase or decrease in CD6/DPP IV activity has been used as a marker of lymphoid leukaemias (Scott *et al.*, 1988) and HIV infections (De Pasquale *et al.*, 1989). Thus, the subsequent elucidation of the role(s) of these cell-surface enzymes on leukocytes that possess modified properties should contribute to our understanding of the multifunctional roles of cell-surface proteases.

Finally, one intriguing question is how cell-surface protease molecules can contribute to the infectious process. As promising candidates for receptors of a large variety of mammalian viruses, they may represent a novel area for antiviral research and therapy.

In all of these aspects, we expect considerable progress in the next few years; however, haemopoietic cell-surface proteases already appear as flexible molecules with varied mechanisms directing a multiplicity of cell functions.

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