Novel roles of protease inhibitors in infection and inflammation

P. S. Hiemstra

Department of Pulmonology, Building 1, C3-P, Leiden University Medical Center (LUMC), P.O. Box 9600, 2300 RC Leiden, The Netherlands

Abstract

The local balance between proteinase inhibitors and proteinases determines local proteolytic activity. Various studies have demonstrated the importance of serine proteinase inhibitors in regulating the activity of serine proteinases that are released by leucocytes during inflammation. Recently it has been shown that these inhibitors may also display functions that are distinct from those associated with the inhibition of leucocyte-derived proteinases. In this review the results of selected studies focusing on three inhibitors of neutrophil elastase, i.e. α1-proteinase inhibitor, secretory leucocyte proteinase inhibitor and elafin, are presented, with the aim of illustrating their possible involvement in the regulation of inflammation, host defence against infection, tissue repair and extracellular matrix synthesis.

Introduction

Proteinases are involved in a wide variety of biological processes, including inflammation and tissue injury. Various studies have focused on the role of proteinases in tissue injury, and it was thought that the balance between proteinases and proteinase inhibitors is a major determinant in maintaining tissue integrity. Serine proteinases from inflammatory cells, including neutrophils, are implicated in various inflammatory disorders, such as pulmonary emphysema. Pulmonary emphysema is characterized by alveolar destruction leading to a major impairment in lung function. Deficiency of α1-proteinase inhibitor (α1-PI) is a well established risk factor for the development of pulmonary emphysema [1,2]. α1-PI (also known as α1-antitrypsin) is the major serine proteinase inhibitor present in the circulation, and it is produced predominantly by hepatocytes, but also locally by macrophages and epithelial cells. It is a member of the family of serine proteinase inhibitors termed serpins, and is an acute-phase reactant.

Early observations on the role of neutrophil elastase and α1-PI in inflammation prompted researchers to explore further the role of proteinase inhibitors and to search for previously unidentified inhibitors that are produced locally at the mucosa. This led to the discovery of the locally produced elastase inhibitors secretory leucocyte protease inhibitor (SLPI) and elafin. SLPI is an 11.7 kDa cationic, non-glycosylated serine proteinase inhibitor that is present in fluids lining mucosal surfaces [3,4]. It inhibits a variety of proteinases, including proteinases from neutrophils (neutrophil elastase, cathepsin G), pancreatic acinar cells (trypsin, chymotrypsin) and mast cells (chymase and tryptase). The molecule is composed of two highly homologous cysteine-rich domains, and the C-terminal domain contains the elastase-inhibitory activity. SLPI is produced by various epithelia, and also possibly by neutrophils, mast cells and macrophages. Major sites of SLPI synthesis in the lung are Clara and goblet cells of the surface epithelium, and the serous cells of the submucosal glands. Elafin, also known as skin-derived antileucoproteinase, is a proteinase inhibitor that is structurally similar to SLPI, with similar patterns of expression [5]. There are differences regarding the target specificity of SLPI and elafin for neutrophil-derived serine proteinases: whereas SLPI is a potent inhibitor of elastase and cathepsin G, elafin inhibits elastase and proteinase 3.

Role in inflammation

Various observations indicate that serine proteinase inhibitors such as α1-PI, SLPI and elafin exert their anti-inflammatory effects not only by inhibiting neutrophil elastase and other leucocyte-derived serine proteinases. This is shown, for example, by results from studies in which it was demonstrated that α1-PI and SLPI suppress bleomycin-induced lung injury in vivo in an animal model [6,7]. In these studies both SLPI and α1-PI were found to reduce lung injury and fibrosis.
without affecting the elevated elastase levels in the lung. Various examples of modulatory activities of, for example, SLPI and $\alpha_1$-PI on inflammation that are distinct from inhibition of leucocyte-derived serine proteinases have been reported. Elastase-$\alpha_1$-PI complexes and proteolytically modified $\alpha_1$-PI have neutrophil chemotaxant activity [8], and elastase-$\alpha_1$-PI complexes stimulate $\alpha_1$-PI production by macrophages [9], effects that are thought to be mediated by a cell surface receptor termed serpin–enzyme complex receptor [10].

SLPI inhibits the pro-inflammatory activity of bacterial products such as lipopolysaccharide [11,12], and regulates the activity of inflammatory cells. This has been suggested to be due to inhibition of activation of the transcription factor nuclear factor-$\kappa$B (NF-$\kappa$B) by SLPI, as a result of inhibition of the proteolytic degradation of I$\kappa$B$\alpha$, the inhibitor of NF-$\kappa$B [13,14]. In unstimulated cells, NF-$\kappa$B is retained in the cytoplasm in complex with I$\kappa$B proteins. Upon cellular activation, I$\kappa$B is degraded and NF-$\kappa$B is released, allowing it to move to the nucleus and influence gene expression. Therefore SLPI-mediated protection of I$\kappa$B from proteolytic degradation may inhibit NF-$\kappa$B activity and its ability to increase the expression of pro-inflammatory genes.

**Host defence against infections**

Neutrophil elastase has been shown to impair host defence against infection by degrading phagocyte surface receptors and opsonins [15,16]. Therefore inhibitors of neutrophil elastase such as $\alpha_1$-PI, SLPI and elafin may protect the body from this elastase-mediated impairment of host defence. Several studies have revealed that inhibitors such as SLPI and elafin may also directly display broad-spectrum antimicrobial activity [17,18]. Since the epithelium is a major source of these proteinase inhibitors, SLPI and elafin may form part of the array of antimicrobial polypeptides that is secreted by epithelial cells to defend the mucosa against infections with pathogens. Other peptides produced by the epithelium include lysozyme, lactoferrin, $\beta$-defensins and cathelicidins [19]. The antimicrobial activity of SLPI appears to be independent of its ability to act as an elastase inhibitor, and SLPI is active against Gram-positive and Gram-negative bacteria [17], fungi [20] and selected viruses, including HIV-1, Sendai and influenza A [21].

Proteinase inhibitors may also regulate host defence via their ability to control the proteolytic processing of antimicrobial peptides. Most antimicrobial peptides are produced as proproteins that require processing before the active peptide is released. Cathelicidins are a class of antimicrobial peptides that are stored in an inactive pro-form that requires proteolytic processing by serine proteinases to release the active cathelicidin. In a pig model, inhibition of elastase activity in wounds following the addition of proteinase inhibitors blocked the proteolytic generation of active cathelicidins and thus inhibited the antimicrobial activity of the wound fluid [22]. This observation demonstrates the ability of serine proteinase inhibitors to regulate the processing of antimicrobial peptides.

**Repair processes and cell growth**

As discussed above, proteinase inhibitors restrict extracellular proteolytic activity and thus provide protection against proteolytic destruction. However, these inhibitors may also provide anti-inflammatory activities by other mechanisms. This is shown by a selection of studies suggesting that proteinase inhibitors such as SLPI and $\alpha_1$-PI may be involved in tissue repair reactions that follow injury. A role for SLPI in tissue repair was suggested by the observation that the epithelial expression of SLPI is increased upon cutaneous injury in humans [23]. Whereas these observations suggest an association between tissue repair and SLPI expression, studies in mice rendered SLPI-deficient by targeted recombination demonstrated a role for SLPI in cutaneous wound healing in mice in vivo [24]. In that study it was shown that the absence of SLPI resulted in delayed cutaneous wound healing, which was attributed to an increased and prolonged inflammatory response during the repair process, and delayed matrix accumulation. Three activities of SLPI were considered as being elements in the repair-promoting activity of this proteinase inhibitor: inhibition of locally released elastase; control of the activity of leucocytes; and a decrease in the activation of transforming growth factor-$\beta$ (TGF-$\beta$). TGF-$\beta$ is a growth factor displaying a variety of activities that has been suggested to be involved in tumour growth, control of inflammatory processes and immune responses, repair processes and matrix accumulation [25]. The authors suggested that there is an increased local activation of TGF-$\beta$ in SLPI-deficient mice. This may cause an influx in inflammatory cells as a result of the
chemotactic activity of TGF-β [24,26], and thus lead to a sustained inflammatory response in the wound area.

In vitro studies provide further evidence for a role for proteinase inhibitors in cell growth and tissue repair. Turnover of the extracellular matrix is the net result of synthesis and degradation, and matrix metalloproteinases and their inhibitors are well known regulators of extracellular matrix degradation. Various studies have shown that proteinase inhibitors may also affect the proliferation of fibroblasts and their ability to produce matrix components. Z1-PI was found to stimulate fibroblast proliferation and procollagen synthesis in culture, which was mediated by activation of mitogen-activated protein kinases [27]. Interestingly, in tumour cell lines Z1-PI may act as a growth inhibitor, an activity that may be mediated by its ability to block transferrin-receptor binding and iron uptake by these cells [28] or to inhibit the pericellular release of TGF-β [29]. Therefore the effects of Z1-PI on cell proliferation may differ in various cell types. Both Z1-PI and SLPI stimulate the production of hepatocyte growth factor (HGF; also known as scatter factor (SF)) in human lung fibroblasts [30]. HGF is a major cytokine product of mesenchymal cells and has been implicated in the regulation of mitogenesis, motogenesis and morphogenesis [31]. Interestingly, SLPI did not affect HGF production by skin fibroblasts, cells that, like lung fibroblasts, are able to increase HGF expression upon stimulation by, for example, interleukin-1β. Finally, the involvement of the proteinase-inhibitory activity of SLPI in this stimulatory activity was suggested from observations on the effect of oxidative inactivation of the antiprotease activity of SLPI, and studies comparing the activities of the C- and N-terminal domains of the molecule.

In addition to regulating HGF production by fibroblasts, SLPI also affects other functions of these cells, such as their ability to contract collagen gels in vitro [32]. Collagen gel contraction is thought to result from the ability of fibroblasts to reorganize and compact collagen fibres, and the model is considered as an in vitro model of wound healing and scar formation. Analysis of the ability of conditioned medium from cultured human oral epithelial cells to contract collagen gels in vitro led to the identification of SLPI as the factor in this medium that inhibits fibroblast-mediated scar formation. It was suggested that SLPI thus might play an important role in the prevention of scar formation, an observation that is in line with the finding of increased SLPI expression in cutaneous injury [23].

Serine proteinase inhibitors also affect the growth of cells other than fibroblasts or tumour cells. This is demonstrated by the observation that SLPI displays marked growth-promoting activity towards glandular epithelial cells isolated from pig endometrium, as determined by assessment of stimulation of DNA synthesis [33]. A similar activity was noted for the serine proteinase inhibitor uterine plasmin/trypsin inhibitor (UPTI). Interestingly, in the mammalian uterus various classes of proteinase inhibitors are produced during early pregnancy, including SLPI, UPTI and tissue inhibitors of metalloproteinases, as has been demonstrated in the pig (reviewed in [33]). Although proteolytic mechanisms are considered essential for uterine tissue remodelling and implantation events, a tight control may be provided by this array of locally produced proteinase inhibitors. In addition, these studies suggest that SLPI and UPTI may serve as autocrine growth factors for the uterine epithelium.

Concluding remarks
There is increasing evidence that serine proteinase inhibitors such as Z1-PI, SLPI and elafin not only regulate inflammation by inhibiting the proteolytic activity of serine proteinases released by leucocytes (summarized in Figure 1). These inhibitors may also directly affect functions of leucocytes, such as chemotaxis and mediator release. In addition, they may contribute directly or indirectly to defence against invading microorganisms. Finally, these proteinase inhibitors may affect cell growth and extracellular matrix production, and thus may be involved in tissue repair reactions. These results suggest the existence of cellular receptors for proteinase inhibitors and/or for complexes of proteinases and proteinase inhibitors. The serpin–enzyme complex receptor and its role in cell stimulation by complexes of elastase and Z1-PI are discussed elsewhere in this review. There is also evidence for an SLPI-binding protein on the cell surface of monocytes. Monocytes have been shown to bind and internalize SLPI, and a 55 kDa SLPI-binding protein was identified on the cell surface [34]. Subsequent studies employing yeast two-hybrid screening using peripheral blood leucocyte cDNA demonstrated that the SLPI-binding protein might be the human scramblase protein [35].
Interestingly, scramblase is involved in the movement of membrane phospholipids, and it was suggested that the interaction between SLPI and scramblase might stabilize the membrane, thus preventing fusion of HIV-1 with the host membrane and providing an explanation for the anti-HIV-1 activity of SLPI.

In summary, whereas there is evidence for a variety of activities of proteinase inhibitors in inflammation, repair processes and host defence, many unanswered questions remain. These questions relate to the relative contributions of the various proteinase inhibitors to these processes in vivo, and to the mechanisms involved.Clarification of these unresolved issues will contribute to our insight into the role of serine proteinase inhibitors in a variety of inflammatory and infectious diseases.

References
Statins for the prevention of vein graft stenosis: a role for inhibition of matrix metalloproteinase-9

K. E. Porter1 and N. A. Turner
Integrated Molecular Cardiology Group, Institute for Cardiovascular Research, University of Leeds, Leeds LS2 9JT, U.K.

Abstract
Saphenous vein (SV) grafts are commonly used to bypass coronary arteries that are diseased due to atherosclerosis. However, the development of intimal hyperplasia in such grafts can lead to patency-threatening stenosis and re-occlusion of the vessel. The proliferation and migration of smooth muscle cells (SMC) play key roles in the development of intimal hyperplasia, and an agent that inhibits both of these processes therefore has therapeutic potential. A prerequisite for SMC proliferation and migration in vivo is degradation of the basement membrane, achieved by secretion of the matrix-degrading gelatinases matrix metalloproteinase-2 (MMP-2) and MMP-9. Statins are cholesterol-lowering drugs that also have direct effects on SMC function. Here we report that neointima formation in organ-cultured human SV segments is inhibited by simvastatin, an effect that is associated with reduced MMP-9 activity. Additionally, our work shows that simvastatin not only inhibits proliferation, but importantly also inhibits invasion (migration through a matrix barrier), of cultured human SV SMC. Thus simvastatin treatment appears to inhibit neointima formation as a result of combined inhibition of SMC proliferation and invasion. The potential intracellular mechanisms by which statins affect SMC proliferation and migration, and thus attenuate intimal hyperplasia, are discussed, with particular emphasis on the role of MMP-9.

Vein graft stenosis and intimal hyperplasia (IH)
Coronary artery bypass grafting using the autologous saphenous vein (SV) is used routinely to revascularize patients with atherosclerotic coronary artery disease. However, occlusions in such grafts are common, resulting in long-term patency rates of approx. 50% after 10 years [1]. The prevention of graft stenosis, rather than treatment of an established lesion, would make a significant impact on long-term patency, and in view of the large numbers of patients receiving venous bypass grafts, the development of preventative therapeutic approaches is an important aim. A large number of pharmacological agents have been shown to reduce stenosis in animal models, although no systemic agent has yet proven effective in humans.

The underlying pathological lesion of stenosis is IH, the characteristic histological features of which are a thickened intima (neointima) containing smooth muscle cells (SMC) in a stroma of mucopolysaccharide, collagen and elastin [2]. IH is a complex process that is initiated in the vessel wall following endothelial injury/denudation as a consequence of bypass grafting. Venous bypass graft failure is related principally to the biological properties of SMC and endothelial cells, and, in areas of endothelial loss, platelet aggregation favours thrombus formation and vascular occlusion [3]. Furthermore, SV SMC proliferate in response to a host of released growth factors and cytokines, including platelet-derived growth factor (PDGF), thrombin and endothelin-1 [3–5].

Key words: HMG-CoA reductase inhibitor, MMP-9, smooth muscle cell migration, smooth muscle cell proliferation.

Abbreviations used: FCS, foetal calf serum; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; IH, intimal hyperplasia; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; PDGF, platelet-derived growth factor; SMC, smooth muscle cell(s); SV, saphenous vein.

1To whom correspondence should be addressed (e-mail medkep@leeds.ac.uk).