Signaling pathways involved in matrix metalloproteinase–9 (MMP–9) release from human neutrophils.

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Inflammatory disease, protease–assisted invasion, or the killing of microorganisms is commonly associated with proteases such as the matrix metalloproteinases (MMPs) and cathepsins. MMPs are collectively capable of degrading the entire extracellular matrix and have been implicated in tumour progression, invasion and metastasis, while MMP-8 and -9 may also be involved in the invasion of professional blood phagocytes [e.g. neutrophils (PMNs)] and inflammation. MMP–induced inflammation, tissue damage and invasion is normally limited by the natural tissue inhibitors of metalloproteinases 1–4 (TIMP-1, -2, -3 and -4). However, TIMP-1 has inconsistent and unanticipated roles in these processes. In certain cancers, TIMP–1 causes increased invasiveness e.g. when present at high levels in Burkitts lymphoma, colorectal carcinomas and breast cancers. In other types of cancers (such as amnion sarcomas and various cancer cell lines) the presence of lower levels of TIMP may suppresses tumour growth, invasion and metastasis. Additionally, increased resistance to bacteria in the absence of TIMP-1 has been demonstrated in corneal infections in mice. Here the ratio and order of protease/TIMP-1 secretion may influence the complement-dependent immune response by affecting the character of opsonins which coat bacteria for phagocytosis. This, in turn, may affect receptor recognition, subsequent signal transduction during phagocytosis, and the final killing–potential of the phagosome, by influencing the fusion of various protease granules and antibacterial substances with the phagosome. Since TIMP-1 has been localised to a distinct granule population in PMNs, separate from its target MMPs, it is important to establish the mode of differential release of the MMP and TIMP–1 granules in order to establish how MMP and/or TIMP-1 release may be triggered both by microorganisms, providing an opportunity for therapeutic intervention in inflammation, and during invasion, providing a similar opportunity for intervention in the prevention of metastatic tumour invasion. Preliminary results indicate that MMP-9 may be controlled by a protein tyrosine kinase (PTK) pathway, with the possible involvement of both Src and Syk family PTKs, as well as a secondary calcium-dependent phosphatidylinositol 3-kinase (PI-3K) pathway. Exposure of human PMNs to increasing extracellular calcium levels shows that MMP-9 is constitutively released from PMNs, but is also triggered by extracellular calcium. Genistein (100 M, broadspectrum PTK inhibitor), however, abolishes the release of PMN MMP-9, in the presence and absence of extracellular calcium. Both PP2 (4-amino-5-(4-chlorophenyl)-7-(t-butyl) pyrazolo[3,4-d]pyrimidine), a Src family PTK inhibitor, and piceatannol, a Syk family PTK inhibitor, reduce MMP-9 release substantially, indicating that multiple PTK families might be involved. Low levels of wortmannin (100 nM, inhibition of PI–3K) abolish the release of MMP-9, in the absence of calcium, and reduce MMP-9 release in the presence of calcium. Investigations into the signalling pathways involved in TIMP-1 release are continuing.