Neutrophil-endothelial interaction in ANCA associated vasculitis

Hu, Nan

Chapter

5

Membrane-bound Proteinase 3 and its receptors

relevance for the pathogenesis of Wegener's Granulomatosis

Nan Hu
Johanna Westra
Cees GM Kallenberg

Autoimmun Rev. 2009 8:510-4

Abstract

Wegener's Granulomatosis (WG) is a life-threatening autoimmune disease. A pathogenic role for anti-neutrophil cytoplasmic autoantibodies (ANCAs) by inducing necrotizing damage to the vessel wall has been strongly suggested by in vitro and in vivo experimental data. Proteinase 3 (PR3), a serine protease mainly stored in the azurophilic granules of neutrophils, has been identified as a major ANCA-antigen in WG. Elevated expression levels of membrane-bound PR3 (mPR3) has been observed in WG and some other chronic inflammatory diseases, suggesting a pathogenic role of mPR3 by allowing interaction with PR3-ANCA. Recent studies revealed CD177 as a receptor for mPR3 on the neutrophil membrane. However, we recently showed that CD177 negative neutrophils also express mPR3 and are susceptible to PR3-ANCA induced neutrophil activation. Therefore, it is of interest to further investigate the functional consequences of binding of mPR3 to CD177, to explore other binding partners for mPR3 on the neutrophil membrane, and to study the relevance of colocalization of these molecules for disease pathogenesis. This review gives updated information on the mechanism of mPR3 expression and the relevance of colocalization of mPR3 with other molecules on the neutrophil membrane for the pathophysiological events occurring in WG.

Introduction

Proteinase 3 (PR3) is a neutrophil-derived serine protease, homologous to leukocyte elastase (HLE), cathepsin G (CG) and inactive azurocidin. PR3 is mainly stored in azurophilic (primary) granules, and, to a lesser extent, in specific (secondary) granules and secretory vesicles, and differentially expressed on the plasma membrane of neutrophils.[1,2] These serine proteases share similar structural elements and biological functions, but PR3 is unique in many aspects. PR3, together with myeloperoxidase (MPO), are major autoantigens of anti-neutrophil cytoplasmic autoantibodies (ANCAs), and ANCA directed to PR3 (PR3-ANCA) have been detected in more than 70% of patients with Wegener's Granulomatosis (WG).[3]

WG is a life-threatening disease characterized by granuloma formation in the upper/lower airways, glomerulonephritis and necrotizing small-vessel vasculitis.[3,4] Although an animal-model for PR3-ANCA associated vasculitis is not available, the pathogenic role of PR3-ANCA has been well established in vitro. The central mechanism of vessel-damage starts with ANCA-binding to their antigens expressed on the surface of cytokine-primed neutrophils resulting in neutrophil activation, in terms of neutrophil degranulation and oxidative burst. Released proteolytic enzymes and reactive oxygen species further cause necrotizing damage to the vessel wall. Therefore, presence of membrane-bound PR3 (mPR3) is a prerequisite for ANCA-binding and ANCA-mediated vessel damage.[3,5] Deficiency of the PiZ-allele of α1-antitrypsin, which is the endogenous inhibitor of PR3, is associated with WG, stressing the pathogenic role of PR3 in the development of WG.[6] This article reviews current knowledge on the mechanism of PR3 membrane-binding and the relevance of colocalization of PR3 with other molecules on the neutrophil membrane for the pathophysiological events occurring in WG.

PR3 expression in neutrophils

Even though large amounts of PR3 are stored in the granules and vesicles of neutrophils, low expression of PR3 can be detected as well on the membrane of isolated neutrophils. Priming of neutrophils with low-dose of TNF-α, which brings neutrophils to a preactivated state, may translocate PR3 to the plasma membrane and raise the expression level up to two- to three- folds of that on resting neutrophils.[7] IL-8, TGF-β and GM-CSF have also been reported to upregulate mPR3 expression on neutrophils.[8,9]

Interestingly, mPR3 is not uniformly expressed on the whole population of neutrophils from most individuals. Primed neutrophils can be divided into two

subsets according to the amount of mPR3 expressed on their membrane. One subset shows a rather low level of mPR3 expression and the other subset expresses a substantial amount of mPR3, indicating a bimodal-pattern of membrane staining for PR3 by flowcytometry.[1] This phenomenon is seldom seen for membrane proteins of neutrophils, and has not been found for other family members of neutrophil serine proteases. The percentage of mPR3high expressing neutrophils, namely the size of the mPR3high subset, ranges from 0% to 100% of the total number of neutrophils in a healthy population and remains strikingly constant within one individual over time.[1] The influence of genetic factors has been verified by Schreiber et al., by showing a strong correlation of the percentages of mPR3high neutrophils between monozygotic twins but not in dizygotic twins.[10] However, increased expression of mPR3 on neutrophils has been observed in several clinical conditions. Neutrophils from patients with PR3-ANCA associated vasculitis and some other chronic inflammatory diseases show higher levels of mPR3 expression than those from healthy controls,[11,12] and a high proportion of mPR3 expressing neutrophils is associated with more frequent relapse of WG.[13] These observations suggest that abnormally expressed mPR3 is involved in the development and severity of WG.

To explain the differential expression of mPR3, Gencik et al. identified 10 polymorphisms in the promoter region of the PR3 gene, but only one of them is possibly associated with a high percentage of mPR3high neutrophils.[14] However, Abdgawad et al. were not able to confirm this result in their cohort.[15] Later, Vietinghoff et al. found that HLA antigen matched siblings showed comparable percentages of mPR3high neutrophils, which in fact was similar to the correlation between monozygotic twins, suggesting that the HLA region is responsible for the genetic influence on the percentage of mPR3 presenting neutrophils.[16] In normal conditions, transcription of proteins stored in azurophilic granules is in silence once a granulocyte matures and is transferred into the circulation.[17] However, Yang et al. found that the gene encoding PR3 is reactivated in circulating neutrophils and monocytes from patients with ANCA-associated vasculitis.[18] Therefore, it seems that abnormal PR3 expression in WG is being regulated from various stages of neutrophil maturation and PR3 production. Taken together, the mechanism underlying the regulation of mPR3 expression still needs further elucidation.

Interaction of mPR3 with other molecules on the neutrophil membrane

The membrane-binding mechanism of PR3 is unknown. In early studies, Witko-Sarsat et al. demonstrated that mPR3 binding did not occur in a charge-

dependent manner, by showing that mPR3 could not be eluted off the membrane by drastic pH changes,[2] while Goldmann et al. showed that purified PR3 interacted with lipid bilayers by hydrophobic insertion.[19] Along with observations on the membrane-binding mechanism of HLE and CG, which are homologues to PR3, David et al. recognized CD11b/CD18 (Mac-1, β2-integrin) as a binding-partner of mPR3.[20] Later on, they found that FcγRIIIb also colocalizes with PR3 on the neutrophil membrane.[21] However, both CD11b/CD18 and FcγRIIIb are universally expressed on neutrophils; therefore, differential expression of mPR3 could not be fully explained until Vietinghoff et al. revealed that a substantial amount of PR3 was exclusively expressed on CD177 (NB1) expressing neutrophils.[22]

CD177 is the coding gene for the NB1 glycoprotein. Characteristics of CD177 and NB1gp have in depth been reviewed by Stroncek et al.,[23] and it is of interest that CD177 also shows differential expression on the neutrophil surface. The percentage of CD177 expressing neutrophils, similar to mPR3, ranges from 0% to 100% in a healthy population.[23] Vietinghoff et al. showed that CD177 is the receptor of mPR3 and mediates PR3 expression on the neutrophil membrane.[22] Our group confirmed this finding by showing an elevated percentage of CD 177 expressing neutrophils in patients with ANCA-associated vasculitis and SLE, which may account for the increased expression of mPR3 in these clinical conditions.[24] The mechanism involved in the physical binding between mPR3 and CD177 is largely unknown. Active PR3, but not proPR3 can bind to the surface of CD177-transfected HEK293 cells, suggesting that N-terminal processing is important for binding of PR3 to CD177.[25] Meanwhile, Korkmaz et al. predicted that the unique hydrophobic cluster of PR3, as compared to HLE and CG, probably mediates PR3-CD177 interaction.[26]

It should be mentioned that mPR3-expression has been observed on also the mPR3low subset in previous studies.[5] Data from our group also showed that neutrophils from CD177 negative individuals express low levels of PR3, but not CD177, on their membrane after priming with TNF-α, and these primed neutrophils were also susceptible for PR3-ANCA induced neutrophil activation.[24] These results indicate that CD177 is not an exclusive binding partner of mPR3, and other binding site(s) are present as well on neutrophils and mediate a low amount of mPR3 expression, such as CD11b/CD18 and FcγRIIIb mentioned before. In addition, HLE and CG have recently been shown to bind to chondroitin sulfate- and heparin sulfate-containing proteoglycans, which provide low affinity but high capacity binding sites for cationic proteins on the neutrophil membrane. PR3 competes with HLE and CG for these binding sites.[27] Moreover, Hajjar et al.

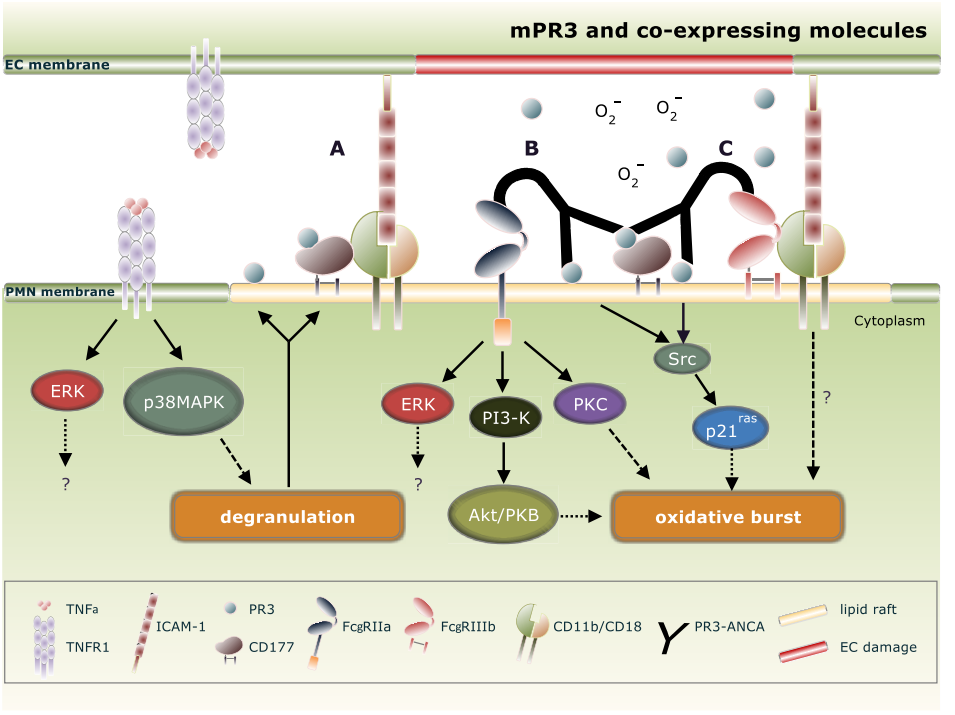

Figure 1. Relevance of mPR3-coexpressing molecules in the pathophysiology of PR3-ANCA-mediated neutrophil activation. (A) TNF-α primes neutrophils and translocates PR3 to the cell membrane. PR3-CD177 binding might activate β2-integrins and promote neutrophil firm adhesion. (B) PR3-ANCA cross-link mPR3 and FcγRIIa, which further induces the oxidative burst of neutrophils. Released proteolytic enzymes and reactive oxygen species cause vessel damage. (C) FcγRIIIb engagement activates β2-integrin, and the latter binds to ICAM-1 expressed on endothelial cells and, on the other hand, mediates PR3-ANCA induced neutrophil activation. EC: endothelial cell; PMN: polymorphonuclear neutrophil.

presumed that PR3 is a peripheral membrane protein that directly interacts with the neutrophil membrane through its hydrophobic region, based on studies on a membrane model using molecular dynamics simulation.[28] Researchers from the same group also demonstrated that PR3 is externalized during neutrophil apoptosis independent of degranulation. This process is mediated by phospholipid scramblase 1 (PLSCR1), a protein related to the bidirectional movement of plasma-membrane phospholipids.[29] On the whole, CD177, as a receptor of mPR3, accounts for substantial membrane-expression of PR3, while the expression of smaller amounts of mPR3 is probably mediated by other mechanism(s) mentioned before. These latter mechanisms allow the subset of mPR3low neutrophils to be involved in the pathophysiology of PR3-ANCA associated vasculitis as well.

Relevance of mPR3-coexpressing molecules in the pathophysiology of WG

Binding partners of mPR3 might have a role in the process of PR3-ANCA induced vessel damage. The signal transduction pathways mediating PR3-ANCA induced neutrophil activation have been reported,[30] and it is well accepted that cross-linking of PR3-ANCA/MPO-ANCA with FcγRIIa on primed neutrophils leads to the oxidative burst (Figure 1B). However, neutrophils, generally, are not activated by ANCA in the circulation, but TNF-α induced neutrophil adhesion to the endothelium has been shown to be a requirement for inducing the neutrophil oxidative burst. Reumaux et al. found that PR3- or MPO-ANCA induced neutrophil activation is strongly impaired when neutrophils are not allowed to adhere by persistent stirring or by blocking CD18.[7] On the other hand, FcγRIIIb is a glycosyl phosphatidylinositol (GPI)-linked protein which is not capable of mediating transmembrane signals, and signal transduction here probably occurs by molecules colocalizing with FcγRIIIb, such as β2-integrins[7]. It has been shown that engagement of FcγRIIIb by immune complexes in the circulation may activate β2-integrins and lead to a proadhesive phenotype likely to promote systemic vascular damage (Figure 1C).[31]

In contrast to FcγRIIIb and β2-integrins, little is known about the function of CD177. CD177 is also a GPI-anchored protein lacking the capability of mediating signal transduction.[23] We have shown that CD177-deficient neutrophils also could be activated by PR3-ANCA in vitro, indicating that CD177 is not necessary for this signaling.[24] Urokinase-type plasminogen activator receptor (uPAR) is a homologue of CD177,[32] and the uPA-uPAR system has a role in angiogenesis and tumor cell migration in cancer. The function of uPAR in cell-cell interaction has been well established. A series of studies have identified a specific binding site for uPAR in the CD11b/CD18 complex, and the binding of uPA to uPAR, similar to mPR3-CD177 interaction, may lead to conformational changes in β2-integrins (Figure 1A).[33,34] Moreover, in a mouse model of Pseudomonas aeruginosa infection, it was observed that recruitment of neutrophils to the lung occurred rapidly in uPAR+/+ mice, and was drastically reduced in uPAR-/- mice, suggesting a role of uPAR in neutrophil transendothelial migration which is probably dependent on β2-integrins.[35] In addition, Sachs and colleagues recently showed that CD177 is a counter-receptor for platelet endothelial cell adhesion molecule-1 (CD31) which is predominantly expressed on the membrane of endothelial cells.[36] These data support the hypothesis that mPR3 binds to CD177 on the neutrophil membrane upon priming and, consequently, activates β2-integrins co-expressed in the same complex, and that the complex further accelerates neutrophil firm adhesion and transmigration.

Conclusion

The percentage of mPR3 expressing neutrophils is genetically determined. The presence of an elevated proportion of neutrophils expressing a substantial amount of mPR3 in patients with ANCA-associated vasculitis could also be related to genetic factors. With respect to the mechanisms underlying membrane expression of PR3, a complex of molecules that colocalize with PR3 on the neutrophil membrane appears to be involved. This complex probably functions in neutrophil recruitment bringing these effecter cells close to the endothelium and further mediate PR3-ANCA induced neutrophil activation and vessel damage. Either blocking the membrane presentation of PR3 or neutralizing the functions of its binding partners might generate novel therapeutic strategies for ANCA-associated vasculitis.

References

1. Halbwachs-Mecarelli L, Bessou G, *et al.* Bimodal distribution of proteinase 3 (PR3) surface expression reflects a constitutive heterogeneity in the polymorphonuclear neutrophil pool. FEBS Lett 1995;374:29-33.
2. Witko-Sarsat V, Cramer EM, *et al.* Presence of proteinase 3 in secretory vesicles: evidence of a novel, highly mobilizable intracellular pool distinct from azurophil granules. Blood 1999;94:2487-96.
3. Kallenberg CG, Heeringa P, *et al.* Mechanisms of Disease: pathogenesis and treatment of ANCA-associated vasculitides. NatClinPractRheumatol 2006;2:661-70.
4. Jennette JC, Falk RJ. Small-vessel vasculitis. NEnglJMed 1997;337:1512-23.
5. Schreiber A, Luft FC, *et al.* Membrane proteinase 3 expression and ANCA-induced neutrophil activation. Kidney Int 2004;65:2172-83.
6. Segelmark M, Elzouki AN, *et al.* The PiZ gene of alpha 1-antitrypsin as a determinant of outcome in PR3-ANCA-positive vasculitis. Kidney Int 1995;48:844-50.
7. Reumaux D, Vossebeld PJ, *et al.* Effect of tumor necrosis factor-induced integrin activation on Fc gamma receptor II-mediated signal transduction: relevance for activation of neutrophils by anti-proteinase 3 or anti-myeloperoxidase antibodies. Blood 1995;86:3189-95.
8. Csernok E, Szymkowiak CH, *et al.* Transforming growth factor-beta (TGF-beta) expression and interaction with proteinase 3 (PR3) in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis. Clin Exp Immunol 1996;105:104-11.
9. Hellmich B, Csernok E, *et al.* Granulocyte-macrophage colony-stimulating factor (GM-CSF) but not granulocyte colony-stimulating factor (G-CSF) induces plasma membrane expression of proteinase 3 (PR3) on neutrophils in vitro. Clin Exp Immunol 2000;120:392-8.
10. Schreiber A, Busjahn A, *et al.* Membrane expression of proteinase 3 is genetically determined. JAmSocNephrol 2003;14:68-75.
11. Van Rossum AP, Huitema MG, *et al.* Standardised assessment of membrane proteinase 3 expression. Analysis in ANCA-associated vasculitis and controls. AnnRheumDis 2007;66:1350-5.
12. Witko-Sarsat V, Lesavre P, *et al.* A large subset of neutrophils expressing membrane proteinase 3 is a risk factor for vasculitis and rheumatoid arthritis. JAmSocNephrol 1999;10:1224-33.
13. Rarok AA, Stegeman CA, *et al.* Neutrophil membrane expression of proteinase 3 (PR3) is related to relapse in PR3-ANCA-associated vasculitis. JAmSocNephrol 2002;13:2232-8.
14. Gencik M, Meller S, *et al.* Proteinase 3 gene polymorphisms and Wegener's granulomatosis. Kidney Int 2000;58:2473-7.
15. Abdgawad M, Hellmark T, *et al.* Increased neutrophil membrane expression and plasma level of proteinase 3 in systemic vasculitis are not a consequence of the - 564 A/G promotor polymorphism. Clin Exp Immunol 2006;145:63-70.

16. Vietinghoff S, Busjahn A, *et al.* Major histocompatibility complex HLA region largely explains the genetic variance exercised on neutrophil membrane proteinase 3 expression. J Am SocNephrol 2006;17:3185-91.
17. Cowland JB, Borregaard N. The individual regulation of granule protein mRNA levels during neutrophil maturation explains the heterogeneity of neutrophil granules. JLeukocBiol 1999;66:989-95.
18. Yang JJ, Pendergraft WF, *et al.* Circumvention of normal constraints on granule protein gene expression in peripheral blood neutrophils and monocytes of patients with antineutrophil cytoplasmic autoantibody-associated glomerulonephritis. JAmSocNephrol 2004;15:2103-14.
19. Goldmann WH, Niles JL, *et al.* Interaction of purified human proteinase 3 (PR3) with reconstituted lipid bilayers. EurJ Biochem 1999;261:155-62.
20. David A, Kacher Y, *et al.* Interaction of proteinase 3 with CD11b/CD18 (beta2 integrin) on the cell membrane of human neutrophils. J LeukocBiol 2003;74:551-7.
21. David A, Fridlich R, *et al.* The presence of membrane Proteinase 3 in neutrophil lipid rafts and its colocalization with FcgammaRIIIb and cytochrome b558. Exp Cell Res 2005;308:156-65.
22. Von Vietinghoff S, Tunnemann G, *et al.* NB1 mediates surface expression of the ANCA antigen proteinase 3 on human neutrophils. Blood 2007;109:4487-93.
23. Stroncek DF, Caruccio L, *et al.* CD177: A member of the Ly-6 gene superfamily involved with neutrophil proliferation and polycythemia vera. J TranslMed 2004;2:8.
24. Hu N, Westra J, *et al.* Coexpression of CD177 and membrane proteinase 3 on neutrophils in antineutrophil cytoplasmic autoantibody-associated systemic vasculitis: anti-proteinase 3-mediated neutrophil activation is independent of the role of CD177-expressing neutrophils. Arthritis Rheum 2009;60:1548-57.
25. Von Vietinghoff S, Eulenberg C, *et al.* Neutrophil surface presentation of the anti-neutrophil cytoplasmic antibody-antigen proteinase 3 depends on N-terminal processing. ClinExpImmunol 2008;152:508-16.
26. Korkmaz B, Kuhl A, *et al.* A hydrophobic patch on proteinase 3, the target of autoantibodies in Wegener granulomatosis, mediates membrane binding via NB1 receptors. JBiolChem 2008;283:35976-82.
27. Campbell EJ, Owen CA. The sulfate groups of chondroitin sulfate- and heparan sulfate-containing proteoglycans in neutrophil plasma membranes are novel binding sites for human leukocyte elastase and cathepsin G. J BiolChem 2007;282:14645-54.
28. Hajjar E, Mihajlovic M, *et al.* Computational prediction of the binding site of proteinase 3 to the plasma membrane. Proteins 2008;71:1655-69.
29. Kantari C, Pederzoli-Ribeil M, *et al.* Proteinase 3, the Wegener autoantigen, is externalized during neutrophil apoptosis: evidence for a functional association with phospholipid scramblase 1 and interference with macrophage phagocytosis. Blood 2007;110:4086-95.
30. Rarok AA, Limburg PC, *et al.* Neutrophil-activating potential of antineutrophil cytoplasm autoantibodies. J LeukocBiol 2003;74:3-15.
31. Kocher M, Siegel ME, *et al.* Cross-linking of Fc gamma receptor IIa and Fc gamma receptor IIIb induces different proadhesive phenotypes on human neutrophils. J Immunol 1997;159:3940-8.
32. Kissel K, Santoso S, *et al.* Molecular basis of the neutrophil glycoprotein NB1 (CD177) involved in the pathogenesis of immune neutropenias and transfusion reactions. EurJ Immunol 2001;31:1301-9.
33. Simon DI, Wei Y, *et al.* Identification of a urokinase receptor-integrin interaction site. Promiscuous regulator of integrin function. J BiolChem 2000;275:10228-34.
34. Tang ML, Vararattanavech A, *et al.* Urokinase-type plasminogen activator receptor induces conformational changes in the integrin alphaMbeta2 headpiece and reorientation of its transmembrane domains. J BiolChem 2008;283:25392-403.
35. May AE, Neumann FJ, *et al.* VLA-4 (alpha(4)beta(1)) engagement defines a novel activation pathway for beta(2) integrin-dependent leukocyte adhesion involving the urokinase receptor. Blood 2000;96:506-13.

36. Sachs UJ, ndrei-Selmer CL, *et al.* The neutrophil-specific antigen CD177 is a counter-receptor for platelet endothelial cell adhesion molecule-1 (CD31). J BiolChem 2007;282:23603-12.