Received: 31 October 2021 | Accepted: 22 December 2021

DOI: 10.1002/cbin.11757

MINI REVIEW

Cell Biology International WILEY

Proteinase 3 associated with Wegener's granulomatosis

Wolfgang H. Goldmann

Department of Biophysics, Friedrich-Alexander-University of Erlangen-Nuremberg, Erlangen, Germany

Correspondence
Wolfgang H. Goldmann, Department of Biophysics, Friedrich-Alexander-University of Erlangen-Nuremberg, Henkstr. 91, D-91052 Erlangen, Germany.
Email: wolfgang.goldmann@fau.de

Funding information
European Union Horizon 2020 Program (Phys2BioMed), Grant/Award Number: Grant 812772; German Science Foundation, DFG, Grant/Award Number: FA 336/13-1

Abstract

Wegener's granulomatosis (WG) is a form of systemic vasculitis characterized by granulomatous inflammation of the upper and lower airways, vasculitis, and necrotizing glomerulonephritis. It is strongly associated with anti-neutrophil cytoplasmic antibodies against proteinase 3 (PR3-ANCAs). Various in vitro observations provided strong evidence that autoimmune PR3-ANCAs are directly involved in glomerular and vascular inflammation. However, little is known about the pathogenic significance of PR3-ANCAs in vivo. Therefore, the generation of animal models helped to validate the suggested autoimmune origin and pathophysiology in WG. To characterize and improve the models, numerous studies were carried out to elucidate the effect of mouse/rat PR3-ANCAs on neutrophil function as well as the role of CD4/CD8 in T and B cells and antibodies in the pathogenesis of the disease. Understanding the pathogenesis is therefore critical to relate these models to human studies hoping that they will be useful for better insight of WG and the development of specific therapies for the disease.

KEYWORDS

animal models mice/rats, antineutrophil cytoplasmic antibodies, antineutrophil/anti-PR3 myeloperoxidase, kinetics, lipid membrane binding, proteinase 3, Wegener's granulomatosis

1 | INTRODUCTION

Wegener's granulomatosis (WG) is a debilitating and life-threatening autoimmune disease of unknown etiology and a major cause of pauci-immune necrotizing and crescentic glomerulonephritis. The classical triad of WG consists of (i) necrotizing granuloma of the upper and lower respiratory tract typically with mucosal inflammation and ulceration, (ii) necrotizing vasculitis involving both arteries and veins, and (iii) nephritis, which is a focal necrotizing glomerulitis with thrombosis of capillary loops. It has a strong and specific association with autoantibodies directed against proteinase 3 (PR3) (Van der Geld et al., 2001). These circulating antineutrophil cytoplasmatic autoantibodies (ANCAs) are directed against conformational epitopes of PR3 and are highly sensitive and specific markers for the disease. The involvement of ANCA in the inflammatory tissue injury is supported by several observations and ANCA titers are well correlated with disease activity in several clinical studies (Franssen et al., 2000; Specks, 2000). In vitro, the interaction of ANCA with neutrophils resulted in the activation of polymorphonuclear neutrophil degranulation, superoxide secretion, release of lipid mediators, stimulation of neutrophil-related endothelial cytotoxicity, and secretion of cytokines (interleukin-1β) (Daouk et al., 1995; Han et al., 2003; Harper & Savage, 2000; Müller-Kobold et al., 1998).

PR3 is a neutral serine protease found in neutrophils and monocytes (Pankhurst & Savage, 2006). It is a highly folded protein with four disulfide bridges keeping its three-dimensional (3D) structure intact (Goldmann et al., 1999). One of its biological roles is the microbicidal activity independent of the proteolytic activity (Brooks

et al., 1996). PR3 has an elastase-like enzymatic activity and can degrade extracellular matrix and basement membrane proteins leading to the migration of neutrophils through the basement membranes (Fujinaga et al., 1996; Pankhurst & Savage, 2006). In azurophilic granules as well as secretory vesicles, PR3 expression is also observed on the plasma membrane of resting neutrophils (Wiesner et al., 2005). The PR3 expression on neutrophils is increased in patients with active WG, and the expression level is correlated with disease activity (Niles et al., 1991).

2 | PR3-BINDING TO LIPID MEMBRANES, SIMULATIONS, AND MODELING

Goldmann et al. (1999) showed that PR3 inserts into the hydrophobic region of liposomes and that the viability of this enzyme in the lipid environment is accessible to the natural and pathologic inhibitors such as α1-PI and ANCA. Although the attachment of PR3 to liposomes decreased its esterolytic activity by approximately 50% compared to control, that is, in the absence of lipids, the enzyme/substrate complex did not induce a conformational change in PR3 when associating with liposomes. The insertion of PR3 into liposomes impaired either the "proper positioning" of PR3 for the substrate or the active site. However, the binding of the natural inhibitor α1-PI to PR3 showed a higher reduction of the enzyme activity in the presence compared to the absence of liposomes, whilst the autoantibody (ANCA) bound to PR3 in the presence of lipids was slightly more effective. These researchers also showed that the 3D structure of PR3 is like other proteases of the chymotrypsin family proteases and that its quaternary arrangement allows the formation of a hydrophobic "pore-like" structure, with Phe166, Ile217, Trp218, Leu223, and Phe224 from each monomer contributing to this hydrophobic patch and to the formation of the central cavity of the crystallographic tetramer (Goldmann et al., 1999). As the catalytic triad (Ser195, His57, and Asp102) and the putative substrate-binding site lies within a shallow depression on the "front" surface of PR3 and the antigenic loop for ANCA on the "back," that is, relatively remote from the surface of PR3 and from the catalytic and substrate-binding pocket, Goldmann et al. (1999) speculated that the hydrophobic patch may be involved in the insertion of PR3 into the lipid membrane. Whether additional surface receptor(s) can contribute to this interaction was also addressed in a study by Witko-Sarat et al. (1999).

Broemstrup and Reuter (2010) carried out molecular dynamics simulations of PR bound to pure dimyristoylphosphatidylcholine (DMPC) and dimyristoyl-phosphatidylglycerol lipid bilayer and mixed bilayer of equimolar ratio. In this comprehensive study, these researchers described the interaction of the enzyme PR3 with the lipid membranes and proposed three types of interaction: (i) that five basic amino acids of the enzyme associate with the phosphate head groups via hydrogen bounds; (ii) that six hydrophobic amino acids V163, F165, F166, I217, L223, and F224 insert into the hydrophobic core below the carbonyl groups of the bilayers which confirmed experimental data from Goldmann et al. (1999); and (iii) that those six amino acids of the aromatic side-chain interact via cation–pi interaction with the choline groups of DMPC. They further found that amino acids of PR3 (believed to be important for membrane binding) are not conserved in the homolog of human neutrophil elastase (HNE) and therefore proposed that the amino acids R186, K187, R222, and W218 make major contributions to the stabilization and orientation of PR3 in the interfacial region which are not conserved in HNE. These observations are in accordance with reports from (Goldmann et al., 1999; Witko-Sarat et al., 1999) that PR3 has only one specific membrane binding site compared to HNE. The esterolytic activity of PR3 is not impaired according to Campbell et al. (2000); however, Goldmann et al. (1999) reported an approximately 50% reduction of its catalytic activity when inserted in membrane liposomes.

Further, Hajjar et al. (2010) described a specific association of PR3 with the plasma membrane which is stronger than simply an ionic interaction. They identified several proteins as potential partners of PR3 at the membrane which might be of critical importance for the understanding of its involvement in WG. For instance, David et al. (2003a2003b, 2005) provided evidence for the colocalization of PR3 with integrin CD11b⁄CD18 (β2-integrin) and Fcγ-receptors RIIA and RIIIB with the lipid membrane (Figure 1). Bauer et al. (2007) also proposed that PR3 membrane expression could be mediated through CD177 binding. In addition, molecular studies comparing between human and mouse PR3 clearly point to major differences in their catalytic properties and ability to interact with membranes which is a key determinant of the accessibility for specific substrates and their use.

In their review, Hajjar et al. (2010) also emphasized the importance of the structural characteristics of human PR3 and HNE in studies and their pathophysiological role in general and in the design of specific ligands and/or drugs. Much progress has been made in recent years with respect to our understanding of the structure–function relationship of human PR3; however, the field would benefit immensely from new structures of enzymes complexed with their inhibitors as well as from new biophysical studies investigating their interaction with lipid bilayers and associated proteins.

Granel et al. (2021) identified different epitopes and regions with polyclonal immunoglobulins from patients and murine/chimeric anti-PR3 monoclonal antibody (mAb), but not with human anti-PR3 mAb. They described epitopes recognized during the active phase of the disease and showed that pathogenic PR3-ANCAs have common characteristics, that is, binding of PR3 close to the active site and modulation of the enzymatic activity, which suggest that PR3-ANCAs might have a direct pathogenic role through the Fab fragment in vitro.

3 | IN VIVO ANIMAL STUDIES

Previously, numerous in vitro observations provided strong evidence that autoimmune PR3-ANCA is directly involved in glomerular and vascular inflammation in WG (McKinney et al., 2014; Niles et al.,

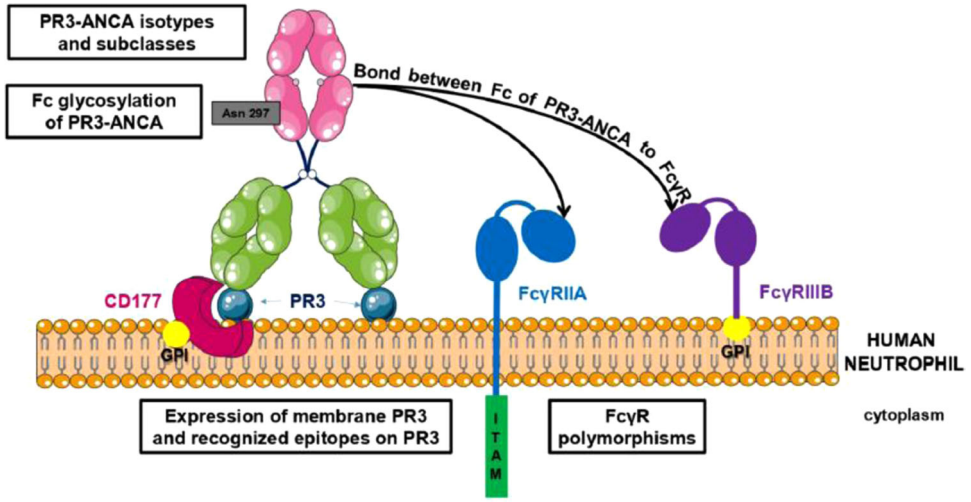

FIGURE 1 PR3-ANCA pathogenicity. Two types of interaction between PR3-ANCA and neutrophils are presented by Granel et al. (2021): "one includes a link between the PR3-ANCA Fab and mbPR3 exposed at the surface of neutrophils and the other involves a bond between Fc of PR3-ANCA and FcgR. Pathogenicity of PR3-ANCA depends on many factors such as the expression of membrane PR3 on neutrophils, recognized epitopes, the presence/absence of Fcg-receptor polymorphisms, subclasses, and isotypes of PR3-ANCA, and finally the Fc glycosylation of PR3-ANCA" (taken from Granel et al. (2021) in Frontiers in Immunology with permission). ANCA, antineutrophil cytoplasmic antibody; PR3, proteinase 3

1989). However, little is known about the pathogenic significance of PR3-ANCA in vivo. Therefore, Primo et al. (2010) generated a mouse model of WG that could help to validate the suggested autoimmune origin and its pathophysiology. The purpose of this study was to break the tolerance towards self-PR3 and to evaluate the role of ANCA in vivo. These researchers successfully expressed recombinant mouse PR3 (rmPR3) in insect cells and demonstrated its functional status by proteolytic activity towards a specific substrate. Non-obese diabetic (NOD) mice were immunized with rmPR3 protein, which developed high levels of PR3-c-ANCA autoantibodies. The transfer of splenocytes from NOD mice immunized with rmPR3 in NOD-severe combined immuno-deficiency (SCID) mice resulted in the reconstitution of the immune system and appearance of PR3-ANCA antibodies. The immunization of mice with rmPR3 led to breaking the tolerance towards self-PR3 and to high levels of circulating and bound PR3-ANCA. Further validation of animal models of human WG in mice and rats is still ongoing (Shochet et al., 2020).

4 | ANCA-ASSOCIATED VASCULITIS IN ANIMAL STUDIES

Several in vivo studies suggested that ANCA is associated with the pathogenesis of the disease in both the spontaneous necrotizing vasculitis mouse models (BXSB, MRL/*lpr*, and SCG/Kj) and in induced models (mercuric chloride-treated rats, autoantigen- and autoantibody-immunized mice; Huugen et al., 2004; Mathieson et al., 1993). However, direct evidence of the pathogenic role of ANCA remained elusive. To provide direct proof for an antibody-dependent mechanism of tissue damage and neutrophil activation, appropriate animal models were developed. These models were needed to better understand the pathogenesis of the disease which allowed the development of novel therapeutic modalities.

Primo et al. (2010) successfully expressed and purified murine PR3 and confirmed the rPR3 specificity using two specific antibodies. They demonstrated the functional status of rPR3 in proteolytic assays of a specific substrate that indicated a mature and functional protein. These researchers showed that NOD mice immunized with mouse rPR3 protein developed high levels of PR3-c-ANCA autoantibodies, while these were not detected in control mice immunized with human purified PR3. The transfer of splenocytes from NOD mice immunized with mPR3 into NOD-SCID mice resulted in the reconstitution of an immune system and the appearance of PR3-ANCA antibodies. Several other animal models have also been tested which showed antibodies to human PR3 in rats (Van der Geld et al., 2007; Shochet et al., 2020). However, rat antibodies against human PR3 did not react with rat PR3 and rat myeloid cells, that is, there is probably no strong homology between rodents and human PR3.

Jenne et al. (1997) found that human ANCA antibodies from 40 different patients with WG did not bind to the murine homolog of PR3. Other studies also showed that the epitopes of human PR3 recognized by the human autoantibody are not preserved on mouse PR3 (Shochet et al., 2020). In fact, the cloning of mouse PR3 demonstrated that the nucleotide sequence of murine PR3 is only 73% and the protein sequence only 69% identical to human PR3 sequences within the coding region of the mature PR3 enzyme (Jenne et al., 1997). Additionally, significant species' differences in physiochemical properties, that is, substrate specificities, enzyme kinetics towards synthetic peptide substrates, oxidized insulin B chains, and

fibrinogen were detected between mouse and human PR3 (Wiesner et al., 2005).

Another way to induce PR3-ANCA in mice has been described by Rauova et al. (2002). Two weeks after the injection of human PR3-ANCA, mice developed PR3-specific anti-idiotypic antibodies and months later were positive for anti-idiotypic antibodies that reacted with PR3. Surprisingly, serum from these mice also reacted to human myeloperoxidase (MPO) and endothelial surface proteins. Some mice developed inflammatory lung lesions after 8 months, but neither vasculitis nor glomerulonephritis was observed. Furthermore, no autoreactivity to mouse PR3 was demonstrated. In another study, mouse immunization with the middle region of cPR3 (antisense peptide) resulted in the development of antibodies against cPR3 and PR3 (Pendergraft et al., 2004). Notwithstanding, neither pathological injury nor disease have been observed. Pfister et al. (2004) showed that PR3/neutrophil elastase in doubly deficient mice immunized with recombinant murine PR3 developed anti-PR3 antibodies. The passive transfer of anti-PR3 serum to wild-type mice did not induce disease-specific symptoms, but only aggravated the local inflammatory response elicited by local tumor necrosis factor-α administration. Thus, no satisfying models for PR3-ANCA-associated diseases are yet available. In the absence of the animal models, which could explain the mechanisms that trigger and perpetuate WG and other forms of systemic vasculitis, it is highly doubtful that further improvements during these chronic and life-threatening diseases can be achieved.

Primo et al. (2010), however, used a different approach to model the human disease more closely. In the absence of autoimmunity, they expected that T and B cells are tolerated (through deletion or any other tolerating mechanisms) towards the majority of expressed self-proteins which are hard to break. To increase their chances of success in breaking tolerance, Primo et al. (2010) started the experiment in the autoimmunity-prone NOD mouse strain. This strain, prone to spontaneously developing autoimmune type I diabetes and several other autoimmune disorders, is homozygous for a unique H-2 haplotype (H-2^{g7}) (Tisch & McDevitt, 1996). The mechanism of the association of major histocompatibility complex (MHC) class II molecules with autoimmune disease is not well understood, but a model by Ridgeway et al. (1999) suggests that NOD MHC class II molecules (binding self-peptides with low efficiency during thymic selection) permit self-reactive T cells to enter the periphery after escaping negative selection processes. In support of this hypothesis, reports by Latek et al. (2000) indicated that I-A^{g7} is unstable and a "poor peptide binder," and the immunization with multiple self-peptides induces an autoimmune response in NOD mice due to their lower threshold for breaking self-tolerance (Ridgeway et al., 1996). Therefore, Primo et al. (2010) hypothesized that it would be easier to induce a response to self-PR3 in NOD mice than in other mouse strains.

Wiesner et al. (2005) reported a higher kinetic value (K_m) and a lower value for (k_{cat}) for murine PR3 compared to Goldmann et al. (1999). One reason for the difference between the kinetic measurements could be the use of different expression systems. The enzymatic behavior of the rmPR3 and the high level of antibodies induced in NOD mice are indicative of its effectiveness. However, Primo et al. (2010) indicated that NOD mice immunized with mouse PR3 protein developed high levels of PR3-c-ANCA autoantibodies on Days 21, 42, and 63 postimmunization and their sera produced an intense granular cytoplasmatic PR3-ANCA like pattern such as PR3-ANCAs on human neutrophils. Fluorescence activated cell sorting analyses of peripheral blood showed that approximately 20% of circulating neutrophils/monocytes cells were coated with anti-PR3-c-ANCA which were consistent with observations in clinical settings. There is a strong association between the disease and the detection of high titers of c-ANCA and the presence of a granular cytoplasmatic staining pattern using an indirect immunofluorescence to ethanol-fixed human neutrophils (Ewert et al., 1991; Franssen et al., 2000). In addition, activated granulocytes are present in the circulation of WG patients and the amount of surface-expressed PR3 correlates with disease activity (Müller-Kobold et al., 1998). Despite some similarities to the human disease, the presence of mPR3-ANCAs was not sufficient to produce a clinical manifestation of WG in these mice.

Xiao et al. (2002) reported clinical abnormalities and pathological manifestations of glomerulonephritis and vasculitis after the adoptive transfer of different numbers of splenocytes from myeloperoxidase $MPO^{-/-}$ mice immunized with murine MPO to $Rag^{-/-}$ mice. The transfer of splenocytes introduced mMPO-specific T lymphocytes and antibodies produced by mMPO-specific B lymphocytes. On the other hand, several studies have used passive transfer experiments to induce the disease. Both cellular types could be playing an important role in this model, but much more severe disease manifestations were observed after adoptive transfer. Therefore, to produce the maximum possible effect, Primo et al. (2010) pursued the latter approach, transferring splenocytes from NOD mice immunized with mouse PR3 into NOD-SCID mice. They observed a sudden rise in PR3-c-ANCAs between Days 4 and 14 after adoptive transfer in the sera of all secondary recipients. Twenty days after the adoptive transfer, one recipient developed generalized sickness and acute kidney failure followed by death. High values of blood urea nitrogen and creatinine in the serum of this mouse were consistent with rapidly progressive glomerulonephritis. They could also show that immunization with mouse PR3 protein in NOD mice resulted in breaking the tolerance towards self-PR3 and the development of high titers of PR3-c-ANCA autoantibodies. These antibodies were detected bound to neutrophils/monocytes. The NOD mice did not develop any apparent signs of autoimmunity up to 3 months postimmunization despite the high titer of autoantibodies. The adoptive transfer of splenocytes from NOD mice immunized with PR3 into immunodeficient NOD-SCID mice resulted in the reconstitution of an immune system and the appearance of PR3-ANCA antibodies. In striking contrast to NOD mice that had a high titer of PR3-ANCA autoantibodies, the appearance of PR3-ANCAs in NOD-SCID mice resulted in kidney failure and death in the secondary recipients of splenocytes from rPR3 immunized hosts.

In summary, to characterize and improve the various models of WG, further studies are needed to elucidate the effect of murine PR3-ANCA on neutrophil function. An understanding based on its

pathogenesis is therefore critical to relate these models to human studies. More recently, Granel et al. (2020) developed a non-activating human monoclonal antibody after testing various membrane proteins (CD 11b, CD 18, and CD 63) as well as the metabolic product ROS from neutrophils, which offers promising results in understanding the PR3-ANCA pathogenicity and possible future therapeutic application.

ACKNOWLEDGMENTS

The author thanks Dr. B. Nikolic for his encouraging feed-back and Dr. V. C. Primo for her support as well as Celia Marshall (MA) for proofreading and editing the manuscript. This study was supported by the German Science Foundation (DFG; FA 336/13-1) and European Union Horizon 2020 Program (Phys2BioMed; Grant 812772). Open access funding enabled and organized by Projekt DEAL.

DATA AVAILABILITY STATEMENT

Data availability statement will be published.

ORCID

Wolfgang H. Goldmann http://orcid.org/0000-0003-0738-2665

REFERENCES

Bauer, S., Abdgawad, M., Gunnarsson, L., Segelmark, M., Tapper, H., & Hellmark, T. (2007). Proteinase 3 and CD177 are expressed on the plasma membrane of the same subset of neutrophils. *Journal of Leukocyte Biology*, *81*, 458–464.

Broemstrup, T., & Reuter, N. (2010). How does proteinase 3 interact with lipid layers? *Physical Chemistry Chemical Physics*, *12*, 7487–7496.

Brooks, C. J., King, W. J., Radford, D. J., Adu, D., McGrath, M., & Savage, C. O. (1996). IL-1 beta production by human polymorphonuclear leukocytes stimulated by anti-neutrophil cytoplasmatic autoantibodies: Relevance to systemic vasculitis. *Clinical Experimental Immunology*, *106*, 273–279.

Campbell, E. J., Campbell, M. A., & Owen, C. A. (2000). Bioactive proteinase 3 on the cell surface of human neutrophils: Quantification, catalytic activity, and susceptibility to inhibition. *Journal of Immunology*, *165*, 3366–3374.

Daouk, G. H., Palsson, R., & Arnaout, M. A. (1995). Inhibition of proteinase 3 by ANCA and its correlation with disease activity in Wegener's granulomatosis. *Kidney International*, *47*, 1528–1536.

David, A., Fridlich, R., & Aviram, I. (2005). The presence of membrane proteinase 3 in neutrophil lipid rafts and its colocalization with FcγRIIIb and cytochrome b558. *Experimental Cell Research*, *308*, 156–165.

David, A., Kacher, Y., Specks, U., & Aviram, I. (2003a). Interaction of proteinase 3 with CD11b/CD18 (beta2 integrin) on the cell membrane of human neutrophils. *Journal of Leukocyte Biology*, *74*, 551–557.

David, J., Rasmussen, N., Andrassy, K., Bacon, P., Tervaert, J. W. C., Dadoniené, J., Ekstrand, A., Gaskin, G., Gregorini, G., de Groot, K., Gross, W., Hagen, E. C., Mirapeix, E., Pettersson, E., Siegert, C., Sinico, C., Tesar, V., Westman, V., & Pusey, C. (2003b). A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. *New England Journal of Medicine*, *349*, 36–44.

Ewert, B. H., Jennette, J. C., & Falk, R. J. (1991). The pathogenic role of antineutrophil cytoplasmic autoantibodies. *American Journal of Kidney Diseases*, *18*, 188–195.

Franssen, C. F., Stegeman, C. A., Kallenberg, C. G. M., Gans, R. O., De Jong, P. E., Hoorntje, S. J., & Tervaert, J. W. (2000). Antiproteinase 3- and antimyeloperoxidase-associated vasculitis. *Kidney International*, *57*, 2195–2206.

Fujinaga, M., Chernaia, M. M., Halenbeck, R., Koths, K., & James, N. M. (1996). The crystal structure of PR3, a neutrophil serine proteinase antigen of Wegener's granulomatosis antibodies. *Journal of Molecular Biology*, *261*, 267–278.

Van der Geld, Y. M., Hellmark, T., Selga, D., Heeringa, P., Huitema, M. G., Limburg, P. C., & Kallenberg, C. G. M. (2007). Rats and mice immunised with chimeric human/mouse proteinase 3 produce autoantibodies to mouse PR3 and rat granulocytes. *Annals of the Rheumatic Disease*, *66*, 1679–1682.

Van der Geld, Y. M., Limburg, P. C., & Kallenberg, C. G. M. (2001). Proteinase 3 Wegener's autoantigen: From gene to antigen. *Journal of Leukocyte Biology*, *69*, 177–190.

Goldmann, W. H., Niles, J. L., & Arnaout, M. A. (1999). Interaction of purified human proteinase 3 (PR3) with reconstituted bilayers. *European Journal of Biochemistry*, *261*, 155–162.

Granel, J., Korkmaz, B., Nouar, D., Weiss, S. A. I., Jenne, D. E., Lemoine, R., & Hoarau, C. (2021). Pathogenicity of proteinase 3-antineutrophil cytoplasmic antibody in granulomatosis with polyangiitis: Implications as biomarker and future therapies. *Frontiers in immunology*, *12*, 571933.

Granel, J., Lemoine, R., Morello, E., Gallais, Y., Mariot, J., Drapeau, M., Musnier, A., Poupon, A., Pugniere, M., Seren, S., Nouar, D., Gouillex-Gruart, V., Watier, H., Korkmaz, B., & Hoarau. (2020). C3 human monoclonal antibody: A proof of concept for non-pathogenic proteinase 3 anti-neutrophil cytoplasmic antibodies in granulomatosis with polyangiitis. *Frontiers in Immunology*, *11*. https://doi.org/10.3389/fimmu.2020.573040

Hajjar, E., Broemstrup, T., Kantari, C., Witko-Sarsat, V., & Reuter, N. (2010). Structure of human proteinase 3 and neutrophil elastase—so similar yet so different. *FEBS Letters*, *277*, 2238–2254.

Han, W. K., Choi, H. K., Roth, R. M., McCluskey, R. T., & Niles, J. L. (2003). Serial ANCA titers: Useful tool for prevention of relapses in ANCA-associated vasculitis. *Kidney International*, *63*, 1079–1085.

Harper, L., & Savage, C. O. (2000). Pathogenesis of ANCA-associated systemic vasculitis. *Journal of Pathology*, *190*, 349–359.

Hattar, K., Sibelius, U., Bickenbach, A., Csernok, E., Seeger, W., & Grimminger, F. (2001). Subthreshold concentrations of anti-proteinase 3 antibodies (c-ANCA) specifically prime human neutrophils for fMLP-induced leukotriene synthesis and chemotaxis. *Journal of Leukocyte Biology*, *69*, 89–97.

Huugen, D., Terwaert, J. W. C., & Heeringa, P. (2004). Antineutrophil cytoplasmic antoantibodies and pathophysiology: New insights from animal models. *Current Opinion in Rheumatology*, *16*, 4–8.

Jenne, D. E., Fröhlich, L., Hummel, A. M., & Specks, U. (1997). Cloning and functional expression of the murine homologue of proteinase 3: Implication for the design of murine models of vasculitis. *FEBS Letters*, *408*, 187–190.

Kitching, A. R., Anders, H. J., Basu, N., Brouwer, E., Gordon, J., Jayne, D. R., Kullman, J., Lyons, P. A., Merkel, P. A., Savage, C. O. S., Specks, U., & Kain, R. (2020). ANCA-associated vasculitis. *Nature Reviews Disease Primers*, *6*, 71.

Latek, R. R., Suri, A., Petzold, S. J., Nelson, C. A., Kanagawa, O., Unanue, E. R., & Fremont, D. H. (2000). Structural basis of peptide binding and presentation by the type I diabetes-associated MHC class II molecule of NOD mice. *Immunity*, *12*, 699–710.

Mathieson, P. W., Qasim, F. J., Esnault, V. L., & Oliveira, D. B. (1993). Animal models of systemic vasculitis. *Journal of Autoimmunology*, *6*, 251–264.

McKinney, E. F., Wilcocks, L. C., Broecker, V., & Smith, K. G. C. (2014). The immunopathology of ANCA-associated vasculitis. *Seminars in Immunopathology*, *36*, 461–478.

Müller, A., Krause, B., Kerstein-Stähle, A., Condühr, S., Klapa, S., Ullrich, S., Holl-Ulrich, K., & Lamprecht, P. (2021). Granulomatous inflammation in ANCA-associated vasculitis. *International Journal of Molecular Science*, *22*, 6474.

Müller-Kobold, A. C., Kallenberg, C. G. M., & Tervaert, J. W. (1998). Leucocyte membrane expression of proteinase 3 correlates with disease activity in patients with Wegener's granulomatosis. *British Journal Rheumatology*, *37*, 901–907.

Niles, J. L., McCluskey, R. T., Ahmad, M. F., & Arnaout, M. A. (1989). Wegener's granulomatosis autoantigen is a novel neutrophil serine proteinase. *Blood*, *74*, 1888–1893.

Niles, J. L., Pan, G. L., Collins, A. B., Shannon, T., Skates, S., Fiensberg, R., Arnaout, M. A., & McCluskey, R. T. (1991). Antigen-specific radio-immunoassays for anti-neutrophil cytoplasmic antibodies in the diagnosis of rapidly progressive glomerulonephritis. *Journal of American Society of Nephrology*, *2*, 27–36.

Pankhurst, T., & Savage, C. O. (2006). Pathogenic role of anti-neutrophil cytoplasmic antibodies in vasculitis. *Current Opinion in Pharmacology*, *6*, 190–196.

Pendergraft, W. F., Preston, G. A., Shah, R. R., Tropsha, A., Carter, C. W., Jennette, J. C., & Falk, R. J. (2004). Autoimmunity is triggered by cPR-3 (105-201), a protein complementary to human autoantigen proteinase-3. *Nature Medicine*, *10*, 72–79.

Pfister, H., Ollert, M., Fröhlich, L. F., Quintanilla-Martinez, L., Colby, T. V., Specks, U., & Jenne, D. E. (2004). Antineutrophil cytoplasmic autoantibodies against the murine homolog of proteinase 3 (Wegener autoantigen) are pathogenic in vivo. *Blood*, *104*, 1411–1418.

Primo, V. C., Marusic, S., Franklin, C. C., Goldmann, W. H., Achaval, C. G., Smith, R. N., Arnaout, M. A., & Nikolic, B. (2010). Anti-PR3 immune responses induce segmental and necrotizing glomerulonephritis. *Clinical and Experimental Immunology*, *159*, 327–337.

Rauova, L., Gilburd, B., Zurgil, N., Blank, M., Guegas, L. L., Brickman, C. M., Cebecauer, L., Deutsch, M., Wiik, A., & Shoenfeld, Y. (2002). Induction of biologically active antineutrophil cytoplasmic antibodies by immunization with human apoptotic polymorphonuclear leukocytes. *Clinical Immunology*, *103*, 69–78.

Ridgeway, W. M., Fasso, M., & Fathman, C. G. (1999). A new look at MHC and autoimmune disease. *Science*, *284*, 749–751.

Ridgeway, W. M., Fasso, M., Lanctot, A., Garvey, C., & Fathman, C. G. (1996). Breaking self-tolerance in non-obese diabetic mice. *Journal of Experimental Medicine*, *183*, 1657–1662.

Shochet, L., Holdsworth, S., & Kitching, A. R. (2020). Animal models of ANCA-associated vasculitis. *Frontiers in immunology*, *11*, 525–528.

Specks, U. (2000). What you should know about PR3-ANCA. Conformational requirements of proteinase 3 (PR3) for enzymatic activity and recognition by PR3-ANCA. *Arthritis Research*, *2*, 263–267.

Tisch, R., & McDevitt, H. (1996). Insulin-dependent diabetes mellitus. *Cell*, *85*, 291–297.

Von Vietinghoff, S., Tunnemann, G., Eulenberg, C., Wellner, M., Cardoso, M. C., Luft, F. C., & Kettritz, R. (2007). NB1 mediates surface expression of the ANCA antigen proteinase 3 on human neutrophils. *Blood*, *109*, 4487–4493.

Wiesner, O., Litwiller, R. D., Hummel, A. M., Viss, M. A., McDonald, C. J., Jenne, D. E., Fass, D. N., & Specks, U. (2005). Differences between human proteinase 3 and neutrophil elastase and their murine homologues are relevant for murine model experiments. *FEBS Letters*, *579*, 5305–5312.

Witko-Sarat, V., Cramer, E. M., Hieblot, C., Guichard, J., Nusbaum, P., Lopez, S., Lesavre, P., & Halbwachs-Mecarelli, L. (1999). Presence of proteinase 3 in secretory vesicles: Evidence of a novel, highly mobilizable intracellular pool distinct from azurophil granules. *Blood*, *94*, 2487–2496.

Xiao, H., Heeringa, P., Hu, P., Liu, Z., Zhao, M., Aratani, Y., Maeda, N., Falk, J. R., & Charles, J. (2002). Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *Journal of Clinical Investigation*, *110*, 955–963.

How to cite this article: Goldmann, W. H. (2022). Proteinase 3 associated with Wegener's granulomatosis. *Cell Biology International*, 46, 548–553. https://doi.org/10.1002/cbin.11757