Received: 5 June 2020 | Revised: 30 July 2020 | Accepted: 10 August 2020

DOI: 10.1111/sji.12958

SSI 50 YEARS ANNIVERSARY ARTICLE

SPECIAL MINI REVIEW

Scandinavian Journal of Immunology WILEY

Proteinase 3 Autoreactivity in Anti-Neutrophil Cytoplasmic Antibody–associated vasculitis—Immunological versus clinical features

Ravi K. Sharma[1] | **Björn Lövström**[1,2] | **Iva Gunnarsson**[1,2] | **Vivianne Malmström**

[1]Division of Rheumatology, Department of Medicine Solna, Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden

[2]Division of Rheumatology, Department of Medicine Solna, Karolinska Institutet and Unit of Rheumatology, Karolinska University Hospital, Stockholm, Sweden

Correspondence
Vivianne Malmström, Division of Rheumatology, Department of Medicine Solna, Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden.
Email: Vivianne.malmstrom@ki.se

Funding information
The authors would like to thank the Swedish research council (2019-01664), Karolinska Institutet's foundation grant (2019-01961) and ALF funding from Stockholm County council (2019-01664) for funding support.

Abstract

ANCA-associated vasculitis (AAV) is a group of chronic inflammatory diseases of small- and medium-sized vessels, which are broadly subdivided based on organ manifestations and disease-specific autoantibodies. The so called anti-neutrophil cytoplasmic antibodies (ANCA) mostly target one of the enzymes, proteinase 3 (PR3) or myeloperoxidase (MPO). Accumulating genetic data demonstrates that these two autoantibodies discriminate two distinct disease entities, more so than the clinical subdivision which is mainly criteria-based. Treatment of AAV includes heavy immunosuppression and is guided by organs that are involved. Generally, patients with PR3-ANCA display higher risk for disease relapse than patients with MPO-ANCA. In this review, we will focus on the autoimmune features of PR3+ AAV and our current understanding of its triggers and the potential translation into clinical practice.

1 | INTRODUCTION

The term 'vasculitis' literally means inflammation of blood vessels. These diseases can be acute or chronic, and triggers include trauma, infection and reactivity against self-components. A prominent feature of chronic vasculitis is immune-mediated triggering that causes persistent or recurrent inflammation, which often co-occurs with an increase of anti-neutrophil cytoplasmic antibodies (ANCA). These features identify ANCA-associated vasculitis (AAV), a chronic inflammatory rheumatic syndrome affecting small- and medium-sized vessels and which can be subdivided into three different subgroups (see Figure 1A). The primary autoantigens identified in AAV are two enzymes normally found in neutrophilic granules: proteinase 3 (PR3) and myeloperoxidase (MPO), respectively. Autoantibodies to these enzymes, PR3- and MPO-ANCA, are hallmarks of AAV, with PR3-ANCA being most common in granulomatosis with polyangiitis (GPA) while MPO-ANCA is most common in microscopic polyangiitis (MPA) and in approximately half of patients with eosinophilic GPA (EGPA). However, ANCA-negative cases with similar clinical presentation but without the presence of PR3 or MPO also occur and are today included in the AAV disease groups. Patients with

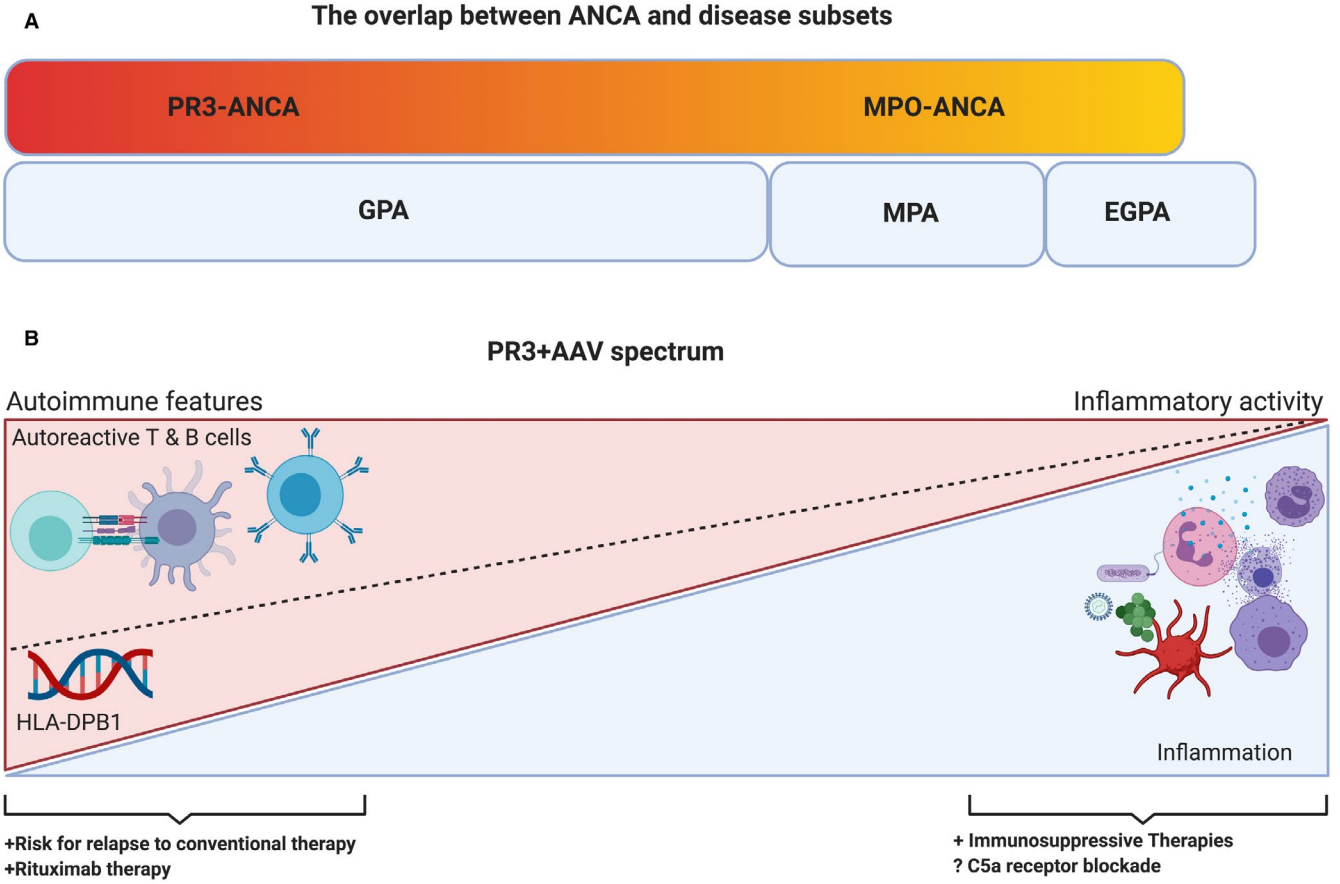

FIGURE 1 AAV disease subsets and immune features:
(A) Most AAV patients present with one of the ANCA autoantibodies, typically PR3- or MPO-ANCA. These roughly correlate with disease subgroup, so that EGPA and MPA patients have MPO-ANCA while GPA patients have PR3-ANCA. This subdivision is, however, not absolute, and therefore, PR3+ AAV is a more distinct subgroup than GPA. (B) PR3+ AAV patients may have more or less prominent autoimmunity versus general inflammation as schematically illustrated. The genetic risk alleles and autoreactive immune cell responses drive the autoimmune side of the spectrum whereas the inflammatory side is more related to innate immune triggers (eg infections). Patients with prominent autoimmunity may hence have better long-term outcome from therapies targeting T and/or B cells, while general immunosuppression may be more beneficial in patients having the inflammatory phenotype

MPO-ANCA-positive GPA have been shown to have a significantly different clinical courses compared to patients with PR3-ANCA-positive GPA,[1] and how to best subclassify AAV patients remains an issue of debate.[2] EGPA will not be further discussed in this review. Several aspects of the pathogenesis of AAV have been thoroughly reviewed recently, for example MPO-ANCA, the role of neutrophils and NETosis and animal models,[3-5] but not the role of autoreactive CD4+ T cells and autoantibodies in pathogenesis of PR3-AAV.

In this minireview, we will focus on PR3-ANCA and PR3-AAV in context of pathogenesis and antigen-specific T-cell responses. Although the MPA and GPA disease subsets partly overlap with regard to organ manifestation, autoantibody features and treatment options, it has been unequivocally demonstrated that patients with MPO- versus PR3-ANCA have distinctly different genetic associations,[6] suggesting that the underlying autoimmunity in different forms of AAV is caused by distinct pathways. Moreover, patients with PR3-ANCA have a higher risk of disease relapse,[7] implicating that these patients are more reflective of autoimmune pathology. Here, our aim is to summarize the current understanding of PR3+ AAV from an autoimmunity perspective and provide implications for clinical translational for this disease.

2 | CLINICAL FEATURES OF PR3+ AAV

AAV patients are mainly seen at rheumatology or nephrology clinics, depending on where the major disease manifestations occur. For the GPA/PR3 group, ear, nose and throat (ENT), kidney and lungs are commonly involved, while in the MPA/MPO group kidney manifestations are more common. At time of diagnosis, AAV usually presents as an inflammatory

disease and is treated with high dose of glucocorticoids and immunosuppressants including biological treatment with rituximab, a B-cell–depleting (BCD) therapy. The goal for treatment is remission, or at least minimizing organ damage and disease flares and thereby improving quality of life and life expectancy. In some cases, AAV presents as a very aggressive disease requiring dramatic intervention, for example plasmapheresis, which will clear the circulation from antibodies and inflammatory mediators. Despite treatment, the risk of relapse is relatively high, and currently, there are no good prediction tools for future disease recurrence.

Granuloma formation has historically been associated with disease phenotype in AAV.[8] Granuloma is better known and characterized as a containment response from invading pathogens, particularly intracellular microbes such as *Mycobacterium tuberculosis*. It is an organized structure formed by entrapment of pathogen by epithelial and phagocytic cells, primarily macrophages, which are surrounded by T cells and B cells. Indeed, macrophages and giant cells along with phagocytosed apoptotic bodies and apoptotic neutrophils have been shown in granulomatous regions in PR3+ AAV [9] implicating that all the components of an autoimmune response are present locally in the tissue. Today however, granulomas are rarely seen in affected ENT or kidney tissues in PR3+ AAV, but sometimes in affected lung.

3 | PREDISPOSING FACTORS

3.1 | Genetic factors contributing to PR3+ AAV

Ethnicity has been implicated to contribute as a predisposing factor for AAV,[10] where GPA (PR3+ AAV) was less commonly observed in patients of non-European origin than MPA.[10] This has been confirmed in studies across the globe (as reviewed in Watts et al).[11] Variation in HLA-alleles was shown to be responsible for this geographical bias in incidence, and HLA-DPB1*0401 was found to be a risk allele for PR3+ AAV.[6,12] The HLA-DP family of MHC class II molecules serve (just as HLA-DR and HLA-DQ) for presentation of peptides to CD4+ T cells. This indicates that autoreactive immune responses in PR3+ AAV are HLA-DP restricted. In addition, genetic association in close vicinity to the PR3 gene itself has also been demonstrated in PR3+ GPA, indicating altered expression levels or gene regulation.[13]

Additionally, SNPs in the vicinity of PTPN22, CTLA4 and SERPINA1 (α-1-antitrypsin gene) have also been shown to be risk alleles in PR3+ AAV.[6,12,14,15] Collectively, these genome-wide association studies (GWAS) and small cohort studies all point towards an autoimmune nature of the disease, a genetic predisposition to anti-PR3 response (ie ANCA) and a significant association of HLA-DP (Figure 2).

3.2 | Environmental factors contributing to PR3+ AAV

In addition to genetic predisposition, environmental factors might also contribute to disease pathogenesis (Figure 2). A relatively high prevalence of disease in middle-aged and elderly individuals along with similar prevalence in both genders suggests a role for environmental triggers, which would supplement the genetic risk factors.[11] The effect of environment is further strengthened by seasonal variations in incidence of disease, which might be contributed by infectious triggers or UV radiation due to latitude differences.[11] Such an infection theory is also strengthened by the observations in a recent study showing cyclic occurrence of PR3+ AAV.[16]

Various bacterial infections have been shown to precede disease onset and also recurrence, indicating a role of infections in the pathogenesis of AAV. The association with disease onset implicates that infections could be a trigger (cause) of disease events, while the association with disease recurrence could implicate the same or be a side effect of the immunosuppressive therapies (consequence). As seen in Figure 2, both bacterial and viral infections have been implicated in AAV. Presence of *Staphylococcus aureus* in nasal carriage has been shown to be correlated with relapse in PR3+ AAV,[17] but there are contradictory studies as well. This heterogeneity in association between presence of bacterium and disease development can be partly explained by genetic differences in the isolates of *S. aureus* in different groups of patients. This theory is partly supported by a recent study showing differences in isolates from PR3+ AAV patients and MPO+ AAV as compared to controls, indicating a possible role for pore forming leucocidins in PR3+ AAV.[18] Additionally, *S. aureus* is known for expressing several so called superantigens, which are capable of activating T cells in a HLA-independent fashion by interacting directly with the T-cell receptor and which has been suggested to at least partly explain the association with relapse.[17] Still, this feature does not explain the association with HLA-DP. An alternative scenario could be the phenomenon of molecular mimicry as complementary PR3 identified as autoantigen (see further below in the autoreactivity paragraph) has been shown to be similar to translatable peptides from Ross river virus,[19] *S. aureus* [20] and *Entamoeba histolytica*.[21] Two recent studies showed a lower microbiome diversity in upper respiratory tract of GPA patients,[22,23] but differences in presence of *S. aureus* were only observed in the European study [22] and not in the US study.[23]

Another viral infectious entity often discussed in conjunction with AAV is human cytomegalovirus (HCMV). A recent study showed that HCMV exposure results in generation of virus-specific CD4+ CD28 null T cells, which exhibit Th1 phenotype and express endothelium homing markers. This was also independently associated with increased arterial stiffness.[24] A clinical trial to test outcome of the anti-viral

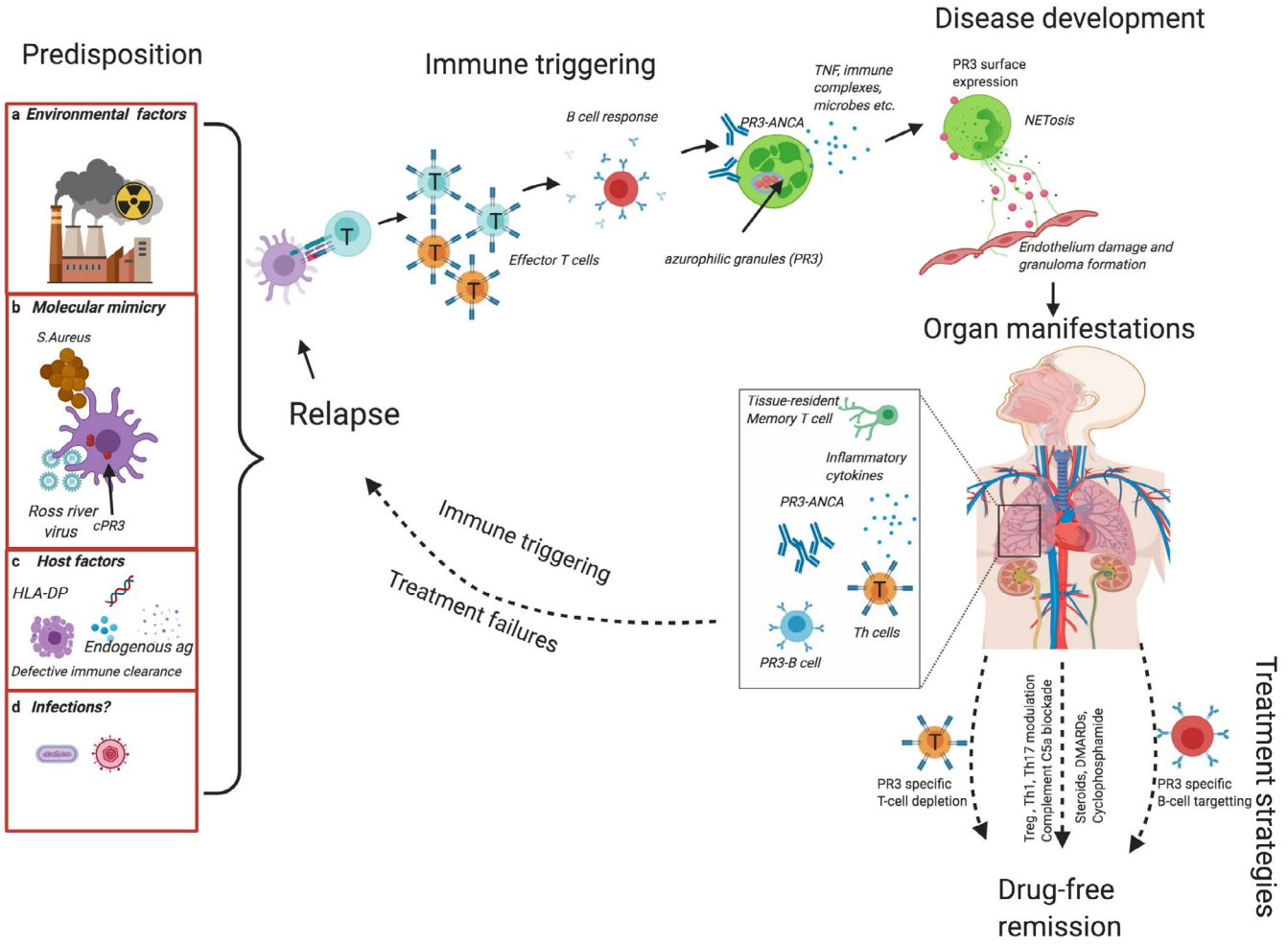

FIGURE 2 Pathogenesis of PR3+ AAV—plausible mechanisms:
Predisposition is likely a multifactorial process, influenced by (A) environmental factors, for example exposure to smoke, dust and radiations and/or (B) molecular mimicry due to exposure of antigens from bacteria, for example *S. aureus* derived cPR3 peptides and/or (C) genetic factors including HLA-DPB1*04:01 and endogenous exposure of autoantigens by increased NETosis and defects in clearance of apoptotic bodies and/or (D) other infections due to known/unknown agents. Immune triggering includes activation of CD4+ T cells following recognition of cPR3/PR3/other antigens presented on APCs complexed with HLA-DP. Such T cells can differentiate into either tissue-resident memory cells or effector cells (Th1, Th17, TFH) and perform various effector functions, including helping B cells in generating cPR3/PR3-specific antibodies. Disease development: Neutrophils can be activated and release endogenous PR3 (stored in azurophilic granules) to their membrane or by generating net-like structures to engage the neighbouring endothelial cells. This membrane form of PR3 has been proposed to be a major antigenic target in the disease. Dying cells can trigger different levels of disease manifestations ranging from tissue damage to inflammation and granuloma formation. Organ manifestations include ear, nose, throat, upper respiratory tract, lungs, cardiovascular system or kidneys, and affected sites can have cell infiltrates containing pathogenic T and B cells as well as innate immune cells secreting pro-inflammatory cytokines. Treatment strategies: The clinical goal is drug-free remission. Higher relapse rates associate with specific organ manifestations and PR3-ANCA titres. DMARDs or cyclophosphamide can potently reduce inflammation, and complement system component C5a blocking is a new pathway under investigation. To completely avoid relapse, a strategy to eradicate the disease-specific immune memory would be warranted, for example by targeting the autoreactive cells. However, these cells are still incompletely explored

drug valaciclovir to prevent CMV-mediated adverse immune responses is ongoing,[25] and data are awaited.

4 | AUTOREACTIVITY TO PROTEINASE 3 (PR3)

Proteinase 3 (PR3) is the *PRTN3* gene encoded elastase-related serine protease present in neutrophil granules and on neutrophil cell membrane. Other enzymes in this subfamily include cathepsin G, N-elastase and neutrophil serine protease. PR3 is the most abundant protease in this family and is contained in azurophilic granules within neutrophils and monocytes. The other ANCA target MPO, despite also being present in the same granules, is not a member of this protease family. An immunological difference between PR3- and MPO-positive diseases also becomes evident from the demonstration that MPO-ANCA injection resulted in induction

of glomerulonephritis and vasculitis in mice,[26] whereas injection of PR3-ANCA did not.[27] There are however differences between human and mouse PR3 in terms of substrate specificity, enzyme kinetics, expression profile and charge distribution, which likely has hampered the development of an animal model of PR3+ AAV.[28] In context of PR3+ AAV, the membrane form (mPR3) is believed to be a major antigenic target in.[29] The activity of PR3 is regulated by α-1-antitrypsin, which also has been shown to be a genetic risk factor in GWAS studies, altogether strongly putting the PR3-α-1-antitrypsin axis in the centre of autoimmunity in PR3+ AAV disease pathogenesis.

In context of autoimmunity, both neutrophils and monocytes expressing PR3 have been shown to be clustering with T,[30] B and plasma cells [31] in the local inflammatory infiltrates [32] in PR3+ AAV. These infiltrates can act as local follicles of lymphocytes, so called ectopic lymphoid-like tissues (ELT),[32] and have also been shown to be a source of PR3+ B cells [33] implicating an active role of these structures in perpetuating autoimmunity. In addition to PR3, a complementary peptide (cPR3, 105-201) (anti-sense coding peptide from fragmented PRTN3 gene) has also been shown to react with PR3-ANCA in a mouse model.[34] Further, PR3-cPR3 antibodies form an idiotypic pair, meaning that antigen-binding region of anti-cPR3 antibody can be recognized as antigen by PR3-ANCA.[34] This has led to the hypothesis that PR3-ANCA can originate from an immune response to cPR3 antibodies. Interestingly, as previously mentioned various microbes have proteins similar to cPR3, thereby suggesting molecular mimicry as a potential mechanism in induction of disease (Figure 2). Different types of triggers including such antibodies, bacterial or fungal infections leading to cytokine release may further activate and/or prime neutrophils. NETosis, the process of DNA expulsion, will lead to more bioavailability of autoantigen (PR3) and more autoimmune responses, manifesting as inflammatory activity in different organs including ENT, lungs, kidney or cardiovascular system (Figure 2). In a clinical context, PR3-ANCA alone or in combination with other factors has been strongly associated with relapse in PR3-AAV.[35-38] Recently, alterations in levels of several PR3-binding apoptosis-related proteins including calreticulin and annexin-A1 were demonstrated in AAV patients.[39] This implicates that defective immune clearance of PR3 can also contribute to maintaining, or re-instating autoimmune reactions (Figure 2C).

5 | T-CELL SUBSETS AND THEIR EFFECTOR FUNCTION IN DISEASE PATHOGENESIS

As already mentioned, the HLA-DP association implicates CD4+ T cells in PR3+ AAV. Indeed, T cells have been identified since long in granulomatous lesions, vasculitic regions and in glomerulonephritis.[40] Consequently, there have been several studies exploring the phenotype and proportion of different CD4 T-cell subsets in AAV. Pro-inflammatory Th1 cells have been identified in nasal mucosal tissues, bronchoalveolar lavage and peripheral blood in AAV.[41,42] Both PR3- and MPO-specific Th17 responses were recently demonstrated in AAV patients using ELIspot assay,[43] which is interesting in the context of *S. aureus* exposure as the bacterium is a classical Th17 inducer. A skewed PR3-specific Th17 response was also demonstrated in AAV patients in remission.[44] Altogether, these studies indicate that an inflammatory milieu may override immune regulation. Indeed, CD4+ $CD25^{hi}$ regulatory T cells were not able to suppress proliferation of responder T cells in GPA patients.[45] This was subsequently demonstrated to be due to an increase in CD4+ $FoxP3^{low}$ non-Treg cells, which secrete pro-inflammatory cytokines.[46] Another study demonstrated a skewing from a more inflammatory response (Th17) in the active phase of GPA towards Treg and Th2 responses in remission.[47] So far, antigen-specific Tregs have not been studied in AAV, but Tregs are known to also function in a bystander fashion. Importantly, immune homeostasis is a multifactorial and coordinated process and the transition towards an anti-inflammatory response is likely to involve both adaptive and innate immune processes. Nonetheless, identification of tolerogenic antigen-specific T cells can be a way forward for immunological homeostasis induction strategies.

Another set of T cells extensively studied in AAV in both peripheral blood and site of inflammation is the terminally differentiated so called CD4+ CD28null T cells (as reviewed in Martinez et al [48]). These cells have been shown to have Th1 profile with higher levels of CCR5 in cells found in granulomatous lesions [49] and have been associated with CMV infection and increased risk of infection and mortality.[50,51] Recently, expansion of these cells was shown to correlate with concomitant CMV and Epstein-Barr virus positivity.[52] Importantly, such CD28-negative T cells have been demonstrated to display cytotoxic effector functions in other disease settings and may also in the context of AAV promote local tissue destruction and inflammation. So far, these cells have not been linked to autoreactivity though.

Given the central position of the ANCA autoantibodies, T-follicular helper cells (TFH), the specialized CD4+ T cells that provide help to B cells in germinal centres have gained a lot of interest and have been shown to be reduced in patients on BCD therapy.[53] Another outcome from BCD would be a lessened antigen presentation by autoreactive B cells. Today, these two scenarios (the relative importance of autoreactive TFH versus autoreactive B cells as APC) have not been fully addressed. A recent study has attempted to re-classify AAV on the basis of differential immune phenotypes and showed association of phenotype with differential organ involvement.[54]

Three distinct immune signatures were proposed based on i) antibody production, ii) cytotoxicity and iii) neutrocytosis/lymphocytopenia. This indicates that these processes can serve as distinguishers from pathogenesis point of view. For instance, patients with more autoantibody features may be more prone to relapse. This study however reported similar signatures for PR3+ and MPO+ patients, perhaps due to their relatively small sample size. Nonetheless, it will be interesting to validate these clinical phenotypes and immune signatures in larger and well-characterized groups of patients.

6 | AUTOIMMUNITY AND RELAPSE

A significant clinical challenge in PR3+ AAV is disease relapse and relatively poor understanding of relapse predictors. There has been evaluation of various clinical parameters as predictors of relapse including a combined increase in anti-PR3 antibodies, CRP titres and number of neutrophils in a 6-month period,[55] anti-PR3 antibodies alone,[35,37] cardiovascular involvement,[7] lung and upper respiratory involvement [56] and presence of HLA-DPB1*04:01 genotype.[57] Further, positive titres of PR3-ANCAs at the time of switching from induction therapy (cyclophosphamide) to maintenance therapy (azathioprine) were associated with relapse,[38] whereas negative ANCA levels were correlated with a reduced risk of relapse.[36] All these studies bring the presence of PR3-ANCA into a central position, and at the same time maintain that like other autoimmune diseases, PR3+ AAV is also a multifactorial disease. The presence of autoantibodies depends either on persistence of autoreactive plasma cells or continuous activation of short-lived plasma blasts. Here, the latter is the most likely explanation given the transient nature of PR3 autoantibodies and their resolution following BCD. It also points towards an active role for B-cell helpers, that is TFH subpopulation in the relapse, but this remains to be studied in detail.

Also, both the disease itself and the immunosuppressive therapies given to severe AAV patients may infer sensitivity to infections and possible triggering of a relapse. This means, for example, that PR3+ AAV patients with lung involvement may be more susceptible to develop a severe COVID-19 disease after SARS-CoV-2 infection as complement activation and pseudovasculitis have been reported in severe cases.[58,59] Today, data on COVID-19 in AAV are however lacking.

7 | THERAPIES AND CONCLUDING REMARKS

As stated before, present-day therapy for PR3+ AAV relies on generalized immunosuppression using high dose of steroids, or disease-modifying anti-rheumatic drugs (DMARDs), cytotoxic drugs such as cyclophosphamide or BCD therapy. There is a plethora of biological therapies and clinical trials going on. Several second-generation anti-CD20 drugs with improved functions including different epitope specificity, induction of apoptosis and cytotoxicity are in development phase across the globe. A promising candidate that needs mention here is a fully humanized anti-CD20 mAb (obinutuzumab), which exerts more antibody-dependent cytotoxicity and direct B-cell killing as compared to its predecessors and which is in a clinical trial in lupus nephritis.[60] On T-cell side, use of abatacept (binding CD80/CD86) is rare. Chimeric antigen receptor T cells (CAR-T cells), that is cytotoxic cells finding their target by expressing an antibody construct on their surface, are in use for tumour settings. A newer generation of 'killer cells' is chimeric autoantibody receptor T cell (CAAR-T), which instead have autoantigen (epitope) on their surface in order to interact with, and kill, autoreactive B cells reacting to the same antigen. So far, their use has been demonstrated in mice.[61]

On innate immune system side, complement component C5a has been thought to be a promising target for controlling inflammation and the outcomes of clinical trial targeting C5a in AAV patients are currently awaited.[62] Collectively, ongoing clinical trials and translational studies are trying different ways of modulation of innate and adaptive immune responses. Antigen-specific therapies in this context both by targeting autoreactive T and B cells and by inducing antigen-induced tolerizing therapies can be the way ahead for a sustained drug-free remission. A better understanding of the relative contribution of autoimmune versus inflammatory immune responses in disease triggering versus relapse and at different sites of inflammation will help pave the way for these specific interventions.

Drug-free remission is the aspiration in PR3+ AAV just as it is in many different chronic inflammatory and/or autoimmune diseases. The relative success of rituximab greatly favours further hunt for more specific therapeutic alternatives, including possibly antigen-specific intervention. This would implicate the possibility of finding and eliminating the underlying autoimmune components, for example the antigen-specific CD4+ T cells. So far, T-cell epitopes on PR3 have not been identified, perhaps due to T-cell expansions in AAV primarily being assigned to superantigen responses. Therefore, it still needs to be established whether there are autoreactive T-cell expansions in this disease and whether such memory cells increase the risk for relapse. With new technology, cells can today be precisely interrogated also in biopsy material, and PR3+ AAV represents a disease entity that has the potential to greatly benefit from such endeavours.

ACKNOWLEDGMENT

The authors would like to acknowledge Christina Gerstner for internal review of the figures.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors participated in discussing, drafting and finalizing the manuscript.

ORCID

Ravi K. Sharma https://orcid.org/0000-0002-2658-0002
Iva Gunnarsson https://orcid.org/0000-0002-4514-7706
Vivianne Malmström https://orcid.org/0000-0001-9251-8082

REFERENCES

1. Schirmer JH, Wright MN, Herrmann K, et al. Myeloperoxidase–antineutrophil cytoplasmic antibody (ANCA)–positive granulomatosis with polyangiitis (Wegener's) is a clinically distinct subset of ANCA-associated vasculitis: a retrospective analysis of 315 patients from a german vasculitis referral center. *Arthritis Rheumatol*. 2016;68:2953-2963.
2. Mahr A, Specks U, Jayne D. Subclassifying ANCA-associated vasculitis: a unifying view of disease spectrum. *Rheumatology*. 2019;58(10):1707-1709.
3. Nakazawa D, Masuda S, Tomaru U, Ishizu A. Pathogenesis and therapeutic interventions for ANCA-associated vasculitis. *Nat Rev Rheumatol*. 2019;15:91-101.
4. Alba MA, Jennette JC, Falk RJ. Pathogenesis of ANCA-associated pulmonary vasculitis. In. Proceedings of the Seminars in respiratory and critical care medicine: Thieme Medical Publishers, 2018:413-24.
5. Hutton HL, Holdsworth SR, Kitching AR. ANCA-associated vasculitis: pathogenesis, models, and preclinical testing. In. Proceedings of the Seminars in Nephrology: Elsevier, 2017:418-35.
6. Lyons PA, Rayner TF, Trivedi S, et al. Genetically distinct subsets within ANCA-associated vasculitis. *N Engl J Med*. 2012;367:214-223.
7. Walsh M, Flossmann O, Berden A, et al. Risk factors for relapse of antineutrophil cytoplasmic antibody–associated vasculitis. *Arthritis Rheum*. 2012;64:542-548.
8. Holl-Ulrich K. L18. Granuloma formation in granulomatosis with polyangiitis. *Presse Medicale (Paris, France)*. 1983;2013(42):555-558.
9. Mackiewicz Z, Rimkevičius A, Petersen J, et al. Macrophages overloaded with tissue debris in Wegener's granulomatosis. *Ann Rheum Dis*. 2005;64:1229-1232.
10. Mahr A, Guillevin L, Poissonnet M, Aymé S. Prevalences of polyarteritis nodosa, microscopic polyangiitis, Wegener's granulomatosis, and Churg-Strauss syndrome in a French urban multiethnic population in 2000: a capture–recapture estimate. *Arthritis Care Res (Hoboken)*. 2004;51:92-99.
11. Watts RA, Mahr A, Mohammad AJ, Gatenby P, Basu N, Flores-Suárez LF. Classification, epidemiology and clinical subgrouping of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis. *Nephrol Dial Transplant*. 2015;30:i14-i22.
12. Xie G, Roshandel D, Sherva R, et al. Association of granulomatosis with polyangiitis (Wegener's) with HLA–DPB1* 04 and SEMA6A gene variants: evidence from genome-wide analysis. *Arthritis Rheum*. 2013;65:2457-2468.
13. Martorana D, Maritati F, Malerba G, et al. PTPN22 R620W polymorphism in the ANCA-associated vasculitides. *Rheumatology*. 2012;51:805-812.
14. Jagiello P, Aries P, Arning L, et al. The PTPN22 620W allele is a risk factor for Wegener's granulomatosis. *Arthritis Rheum*. 2005;52:4039-4043.
15. Carr EJ, Niederer HA, Williams J, et al. Confirmation of the genetic association of CTLA4 and PTPN22 with ANCA-associated vasculitis. *BMC Med Genet*. 2009;10:121.
16. Watts RA, Mooney J, Skinner J, Scott DG, MacGregor AJ. The contrasting epidemiology of granulomatosis with polyangiitis (Wegener's) and microscopic polyangiitis. *Rheumatology*. 2012;51:926-931.
17. Popa E, Cohen JW. The relation between Staphylococcus aureus and Wegener's granulomatosis: current knowledge and future directions. *Intern Med*. 2003;42:771-780.
18. Glasner C, de Goffau MC, van Timmeren MM, et al. Genetic loci of Staphylococcus aureus associated with anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitides. *Sci Rep*. 2017;7:1-9.
19. Davies D, Moran J, Niall J, Ryan G. Segmental necrotising glomerulonephritis with antineutrophil antibody: possible arbovirus aetiology? *BMJ (Clin Res Ed)*. 1982;285:606.
20. Stegeman CA, Tervaert JWC, Sluiter WJ, Manson WL, de Jong PE, Kallenberg CG. Association of chronic nasal carriage of Staphylococcus aureus and higher relapse rates in Wegener granulomatosis. *Ann Intern Med*. 1994;120:12-17.
21. Pudifin D, Duursma J, Gathiram V, Jackson T. Invasive amoebiasis is associated with the development of anti-neutrophil cytoplasmic antibody. *Clin Exp Immunol*. 1994;97:48-51.
22. Lamprecht P, Fischer N, Huang J, et al. Changes in the composition of the upper respiratory tract microbial community in granulomatosis with polyangiitis. *J Autoimmun*. 2019;97:29-39.
23. Rhee RL, Sreih AG, Najem CE, et al. Characterisation of the nasal microbiota in granulomatosis with polyangiitis. *Ann Rheum Dis*. 2018;77:1448-1453.
24. Chanouzas D, Sagmeister M, Dyall L, et al. The host cellular immune response to cytomegalovirus targets the endothelium and is associated with increased arterial stiffness in ANCA-associated vasculitis. *Arthritis Res Ther*. 2018;20:194.
25. Chanouzas D, Dyall L, Nightingale P, et al. Valaciclovir to prevent Cytomegalovirus mediated adverse modulation of the immune system in ANCA-associated vasculitis (CANVAS): study protocol for a randomised controlled trial. *Trials*. 2016;17:338.
26. Xiao H, Heeringa P, Hu P, et al. Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *J Clin Investig*. 2002;110:955-963.
27. Pfister H, Ollert M, Fröhlich LF, et al. Antineutrophil cytoplasmic autoantibodies against the murine homolog of proteinase 3 (Wegener autoantigen) are pathogenic in vivo. *Blood*. 2004;104:1411-1418.
28. Relle M, Föhr B, Fasola F, Schwarting A. Genetics and pathophysiology of granulomatosis with polyangiitis (GPA) and its main autoantigen proteinase 3. *Mol Cell Probes*. 2016;30:366-373.
29. Van Rossum AP, van der geld YM, Limburg PC, Kallenberg CGM. Human anti-neutrophil cytoplasm autoantibodies to

proteinase 3 (PR3-ANCA) bind to neutrophils. *Kidney Int*. 2005;68:537-541.
30. Capraru D, Müller A, Csernok E, et al. Expansion of circulating NKG2D+ effector memory T-cells and expression of NKG2D-ligand MIC in granulomaous lesions in Wegener's granulomatosis. *Clin Immunol*. 2008;127:144-150.
31. Voswinkel J, Mueller A, Kraemer JA, et al. B lymphocyte maturation in Wegener's granulomatosis: a comparative analysis of VH genes from endonasal lesions. *Ann Rheum Dis*. 2006;65:859-864.
32. Mueller A, Holl-Ulrich K, Lamprecht P, Gross W. Germinal centre-like structures in Wegener's granuloma: the morphological basis for autoimmunity? : Oxford University Press 2008.
33. Weppner G, Ohlei O, Hammers CM, et al. In situ detection of PR3-ANCA+ B cells and alterations in the variable region of immunoglobulin genes support a role of inflamed tissue in the emergence of auto-reactivity in granulomatosis with polyangiitis. *J Autoimmun*. 2018;93:89-103.
34. Pendergraft WF, Preston GA, Shah RR, et al. Autoimmunity is triggered by cPR-3 (105–201), a protein complementary to human autoantigen proteinase-3. *Nat Med*. 2004;10:72-79.
35. van Dam LS, Dirikgil E, Bredewold EW, et al. Proteinase-3-anti-neutrophil cytoplasmic antibodies (PR3-ANCAs) predict relapses in ANCA–associated vasculitis patients after rituximab. *Nephrol Dial Transplant*. 2020. https://doi.org/10.1093/ndt/gfaa066. [Epub ahead of print].
36. Morgan MD, Szeto M, Walsh M, et al. Negative anti-neutrophil cytoplasm antibody at switch to maintenance therapy is associated with a reduced risk of relapse. *Arthritis Res Ther*. 2017;19:129.
37. Thai L-H, Charles P, Resche-Rigon M, Desseaux K, Guillevin L. Are anti-proteinase-3 ANCA a useful marker of granulomatosis with polyangiitis (Wegener's) relapses? Results of a retrospective study on 126 patients. *Autoimmun Rev*. 2014;13:313-318.
38. Slot MC, Tervaert JWC, Boomsma MM, Stegeman CA. Positive classic antineutrophil cytoplasmic antibody (C-ANCA) titer at switch to azathioprine therapy associated with relapse in proteinase 3–related vasculitis. *Arthritis Care Res (Hoboken)*. 2004;51:269-273.
39. Everts-Graber J, Martin KR, Thieblemont N, et al. Proteomic analysis of neutrophils in ANCA-associated vasculitis reveals a dysregulation in proteinase 3-associated proteins such as annexin-A1 involved in apoptotic cell clearance. *Kidney Int*. 2019;96:397-408.
40. Gephardt GN, Ahmad M, Tubbs RR. Pulmonary vasculitis (Wegener's granulomatosis): immunohistochemical study of T and B cell markers. *Am J Med*. 1983;74:700-704.
41. Csernok E, Trabandt A, Müller A, et al. Cytokine profiles in Wegener's granulomatosis: predominance of type 1 (Th1) in the granulomatous inflammation. *Arthritis Rheum*. 1999;42:742-750.
42. Lúdvíksson BR, Sneller MC, Chua KS, et al. Active Wegener's granulomatosis is associated with HLA-DR+ CD4+ T cells exhibiting an unbalanced Th1-type T cell cytokine pattern: reversal with IL-10. *J Immunol*. 1998;160:3602-3609.
43. Martinez Valenzuela L, Draibe J, Quero M, et al. Exploring Frequencies of Circulating Specific Th17 Cells against Myeloperoxidase and Proteinase 3 in ANCA Associated Vasculitis. *Int J Mol Sci*. 2019;20:5820.
44. Abdulahad WH, Stegeman CA, Limburg PC, Kallenberg CG. Skewed distribution of Th17 lymphocytes in patients with Wegener's granulomatosis in remission. *Arthritis Rheum*. 2008;58:2196-2205.
45. Abdulahad WH, Stegeman CA, van der Geld YM, Doornbos-van der Meer B, Limburg PC, Kallenberg CG. Functional defect of circulating regulatory CD4+ T cells in patients with Wegener's granulomatosis in remission. *Arthritis Rheum*. 2007;56:2080-2091.
46. Reijnders TD, Stegeman CA, Huitema M, Rutgers A, Heeringa P, Abdulahad WH. Unraveling the identity of FoxP3+ regulatory T cells in Granulomatosis with Polyangiitis patients. *Sci Rep*. 2019;9:1-9.
47. Szczeklik W, Jakieła B, Wawrzycka-Adamczyk K, et al. Skewing toward Treg and Th2 responses is a characteristic feature of sustained remission in ANCA-positive granulomatosis with polyangiitis. *Eur J Immunol*. 2017;47:724-733.
48. Martinez Valenzuela L, Bordignon Draibe J, Fulladosa Oliveras X, Bestard Matamoros O, Cruzado Garrit JM, Torras AJ. T-lymphocyte in ANCA-associated vasculitis: what do we know? A pathophysiological and therapeutic approach. *Clin Kidney J*. 2019;12:503-511.
49. Lamprecht P, Bruhl H, Erdmann A, et al. Differences in CCR5 expression on peripheral blood CD4+ CD28− T-cells and in granulomatous lesions between localized and generalized Wegener's granulomatosis. *Clin Immunol*. 2003;108:1-7.
50. Slot MC, Kroon AA, Damoiseaux JGMC, et al. CD4+ CD28 null T Cells are related to previous cytomegalovirus infection but not to accelerated atherosclerosis in ANCA-associated vasculitis. *Rheumatol Int*. 2017;37:791-798.
51. Morgan MD, Pachnio A, Begum J, et al. CD4+ CD28− T cell expansion in granulomatosis with polyangiitis (Wegener's) is driven by latent cytomegalovirus infection and is associated with an increased risk of infection and mortality. *Arthritis Rheum*. 2011;63:2127-2137.
52. Kerstein A, Schüler S, Cabral-Marques O, et al. Environmental factor and inflammation-driven alteration of the total peripheral T-cell compartment in granulomatosis with polyangiitis. *J Autoimmun*. 2017;78:79-91.
53. Zhao Y, Lutalo PMK, Thomas JE, et al. Circulating T follicular helper cell and regulatory T cell frequencies are influenced by B cell depletion in patients with granulomatosis with polyangiitis. *Rheumatology*. 2014;53:621-630.
54. Matsumoto K, Suzuki K, Yoshimoto K, et al. Significant association between clinical characteristics and immuno-phenotypes in patients with ANCA-associated vasculitis. *Rheumatology*. 2020;59:545-553.
55. Hogan PC, O'Connell RM, Scollard S, Browne E, Hackett EE, Feighery C. Biomarkers predict relapse in granulomatosis with polyangiitis. *J Biomarkers*. 2014;2014:1-4.
56. Pagnoux C, Hogan SL, Chin H, et al. Predictors of treatment resistance and relapse in antineutrophil cytoplasmic antibody–associated small-vessel vasculitis: comparison of two independent cohorts. *Arthritis Rheum*. 2008;58:2908-2918.
57. Hilhorst M, Arndt F, Joseph Kemna M, et al. HLA–DPB1 as a Risk Factor for Relapse in Antineutrophil Cytoplasmic Antibody-Associated Vasculitis: A Cohort Study. *Arthritis Rheumatol*. 2016;68:1721-1730.

58. Magro C, Mulvey JJ, Berlin D, et al. Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: a report of five cases. *Transl Res*. 2020;220:1-13.
59. Varga Z, Flammer AJ, Steiger P, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet*. 2020;395:1417-1418.
60. Reddy V, Klein C, Isenberg DA, et al. Obinutuzumab induces superior B-cell cytotoxicity to rituximab in rheumatoid arthritis and systemic lupus erythematosus patient samples. *Rheumatology*. 2017;56:1227-1237.
61. Ellebrecht CT, Bhoj VG, Nace A, et al. Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease. *Science*. 2016;353:179-184.
62. Jayne DRW, Bruchfeld AN, Harper L, et al. Randomized trial of C5a receptor inhibitor avacopan in ANCA-associated vasculitis. *J Am Soc Nephrol*. 2017;28:2756-2767.

How to cite this article: Sharma RK, Lövström B, Gunnarsson I, Malmström V. Proteinase 3 Autoreactivity in Anti-Neutrophil Cytoplasmic Antibody–associated vasculitis—Immunological versus clinical features. *Scand J Immunol*. 2020;92:e12958. https://doi.org/10.1111/sji.12958