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Genetics and pathophysiology of granulomatosis with polyangiitis (GPA) and its main autoantigen proteinase 3

Manfred Relle [a, *], Bernd Föhr [a], Federica Fasola [a, b], Andreas Schwarting [a, b]

[a] Department of Internal Medicine, Division of Rheumatology and Clinical Immunology, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany
[b] ACURA Rheumatology Center, Bad Kreuznach, Germany

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ABSTRACT

Granulomatosis with polyangiitis (GPA) is a severe autoimmune disease and one of the small vessel anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides. Although its etiology and pathophysiology are still widely unknown, it is accepted that infections, environmental factors, epigenetic modifications, and a genetic predisposition provide the basis for this systemic disorder. GPA typically evolves into two phases: an initial phase characterized by ear, nose and throat (ENT) manifestations, such as chronic sinusitis and otitis, ulceration of the oral cavity and pharynx, as well as pulmonary nodules and a severe generalized phase, defined by the occurrence of rapidly progressive glomerulonephritis, pulmonary hemorrhage, and arthritis. ANCAs, directed against the neutrophilic enzymes proteinase 3 and myeloperoxidase, are present in up to 90% of the affected patients in the systemic phase. As the humoral immunity is predominantly directed against neutrophilic antigens, it is apparent that neutrophils play a critical role in GPA both as target and effector cells. Although GPA pathogenesis is not well known, some susceptibility genes and loci have been identified by candidate gene approaches, genome-wide association studies, and meta-analyses, as well as familial association studies. Such genes are *CTLA4*, *PTPN22*, *COL11A2*, *SERPINA1*, and the MHC class II gene cluster. This review highlights the clinical, pathophysiological, and genetic background of GPA and aims to give an overview of recent efforts to identify GPA susceptibility genes. We point out the genetic basis of the main autoantigen PR3 and why it is so difficult to establish a murine GPA model.

1. Clinical features of granulomatosis with polyangiitis

Granulomatosis with polyangiitis (GPA) (formerly known as Wegener's granulomatosis) is a severe autoimmune disease of unknown etiology with a poor prognosis in the absence of an aggressive immunosuppressive therapy. Together with microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA, formerly known as Churg–Strauss syndrome), GPA belongs to the group of anti-neutrophil cytoplasmic antibody (ANCA)-associated small-vessel vasculitides (AAV) [1].

ANCA are disease (auto)antibodies frequently associated with GPA [2]. They are directed against neutrophilic antigens [3], which can be proven by indirect immunofluorescence (IF) with two main patterns, perinuclear (p-ANCA) and cytoplasmic (c-ANCA). Using enzyme-linked immunosorbent assays (ELISAs), it is possible to detect the particular binding of ANCA to myeloperoxidase (MPO) or to proteinase3 (PR3). p-ANCA, directed against MPO, are usually associated with MPA, whereas c-ANCAs directed against PR3 are present in 80–90% of patients with GPA [4–12].

Globally, only 68,000 to 140,000 new cases of AAV are diagnosed per year, which corresponds to an annual incidence of about 10–20 patients per million [13]. The reported prevalence varies from 24 to 157 per million [14]. Although GPA occurs worldwide, the disease has a greater incidence in high-latitude areas than in low-latitude areas and predominantly affects patients of Caucasian origin [15]. The prevalence of the disease in Japanese and African Americans is very low [16]. There are no gender-specific characteristics and every age group can potentially be affected. The peak of the disease, however, is between 40 and 65 years of age and children are rarely affected. Risk factors are medicines [17], infections [18], environmental factors [19], and a genetic predisposition (see section 4).

* Corresponding author. Department of Internal Medicine, University Medical Center Mainz, Langenbeckstrasse 1, D-55131 Mainz, Germany.
E-mail address: manfred.relle@unimedizin-mainz.de (M. Relle).

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Typical features of GPA are granulomatous changes (granulomas) in the upper and lower respiratory tract, vasculitis, and glomerulonephritis. The most typical and often the initial clinical manifestations of the disease (70–100%) are granulomatous ear, nose, and throat (ENT) lesions with sinusitis, chronic otitis media crusting rhinitis, saddle-nose deformity, or nasal septum perforation. A pulmonary involvement is observed in two-thirds of patients with parenchymal nodules and/or alveolar hemorrhage. In 40–100% of patients, rapidly progressive glomerulonephritis occurs, being the third main manifestation of GPA. Other typical clinical manifestations are caused by peripheral and central nervous system involvement with mononeuritis multiplex, sensorimotor polyneuropathy, or pachymeningitis. Less common but still to be mentioned are skin lesions with palpable purpura or necrotic papules and a cardiac involvement [14]. If untreated, the 1-year survival of the disease is 18% [20].

The immunosuppressive combination of corticosteroids and cyclophosphamide is used as classical standard therapy for remission induction, while azathioprine and methotrexate are the therapeutic options for remission maintenance. Nowadays rituximab, a chimeric monoclonal antibody targeted against the pan-B cell marker CD20, is considered a valid alternative for patients with severe GPA. Patients with localized GPA (involving only one or two of ENT, lungs, and kidneys) can be treated with corticosteroids and methotrexate [14]. Over the past 20 years, the survival of GPA patients has improved considerably owing to more effective diagnosis and recent trends in the management of GPA and its complications. By means of the current therapy, remission can be obtained in more than 90% of patients [20,21].

Even if ANCA support the diagnosis of AAV, approximately 10% of GPA patients have no detectable ANCA. Patients with ANCA-negative AAV could be positive for autoantibodies, which cannot be detected using the current methods and/or could generate ANCA of so far unknown specificity. Moreover, the differentiation of the ANCA status cannot be used to identify GPA versus MPA owing to a significant overlap of the ANCA patterns between the different forms of AAV. PR3-ANCAs are detected in only 70–80% of the GPA patients, whereas about 10% of the GPA patients present MPO-ANCAs, which are more characteristic of MPA [22]. Furthermore GPA, MPA, and EGPA have clinical and histological similarities, so that the classification of the AAV remains controversial, which in turn makes it difficult to investigate the diseases independently of each other. Schirmer et al. demonstrated in a recent paper that GPA associated with MPO-ANCA is a clinically distinct subset within AAV [23]. From their results they concluded that the classification of GPA patients according to ANCA specificity may improve the evaluation of relapse risk. For a deeper understanding and better differentiation of these diseases, several candidate gene approach studies have been performed over the years.

The PR3-ANCA-specific epitopes of PR3 have been detected [24,25], and it could be shown that their glycosylation status can affect the binding of the autoantibodies [26]. In addition, it could be demonstrated that modifications of the C-terminal end of PR3 have no effect on antigen recognition [27]. The diagnostic value of ANCA titers for disease activity is still a controversial issue. Although absolute titers correlate only weakly with disease severity [28] and a meta-analysis showed that they probably do not correlate with disease activity [29], more studies are necessary to confirm these results. It could be shown that disease activity is a cycle of 7.6 years [30], which in turn suggests environmental factors. This periodicity could not be detected in MPA.

Furthermore, it was ascertained that AAV patients exhibit a higher level of circulating PR3 in the blood plasma than healthy people [31]. It was also noted that PR3 transcription in leukocytes of AAV patients is upregulated [32] and the number of immune suppressive regulatory B-cells is reduced but not functionally deficient [33]. A membrane-bound proteinase 3 (mPR3) was found on neutrophils, which binds the PR3 ANCA of AAV patients [34]. Thus, an increased level of mPR3 on neutrophils seems to be a risk factor for vasculitis [35]. The balance between PR3 and its natural inhibitor α1-antitrypsin (1AT) also plays a role in GPA pathogenesis because PR3-ANCA inhibits the PR3-1AT complex formation, which correlates with disease activity [36].

ANCA and their antigen PR3 may also play an influential role in other diseases, such as a chronic obstructive pulmonary disease [37], polyarteritis nodosa (PAN), systemic lupus erythematosus (SLE) [38], myeolodysplasia [39], cystic fibrosis [40], and chronic inflammatory bowel disease [41]. However, in contrast to many other autoimmune diseases, like multiple sclerosis (MS), SLE, or rheumatoid arthritis (antigens listed in Ref. [42]), the spectrum of autoantigens is greatly reduced in GPA. This makes it an ideal candidate for studying autoimmune diseases, especially with regard to the pathological role of autoantibodies.

2. The main autoantigen PR3 and its gene PRTN3

The *PRTN3* gene encodes for PR3 and is located in humans on the short arm of chromosome 19 (19p13.3) [43,44]. It is closely related to the *AZU1* and the *ELANE* genes, which code for azurocidin and neutrophil elastase, respectively. These three genes are probably originated from duplication events and are located side by side together with the gene for complement factor D. Together they represent a functional group of serine proteases, which is called the APEA gene locus (Fig. 1) [45]. Using a transgenic approach, it could be shown that the human *ELANE* promoter is silenced, if integrated in an inappropriate chromatin environment, and that the high promyelocytic expression of this gene may come from DNase I hypersensitive sites of the APEA gene locus, which coincide with cis-transcriptional elements [45]. Obviously, epigenetic alterations play a crucial role in the (coordinated) expression of this gene cluster. The complex coordinated expression of these genes could also be demonstrated for mice: an enhancer sequence of the P*rtn3* gene, which is activated by Sp1, GABP, and Ets transcription factors, stimulates the *Elane* promoter [46].

Human *PRTN3* extends over 7 kb and is interrupted by four introns (Fig. 1) [47]. The promoter activity is myeloid-specific and contains a PU.1-binding site and a cytidine-rich CG element, both of which are important for the expression [48]. PR3 is synthesized as a preproenzyme [44,49,50]. Whereas the first exon codes for the first 20 amino acids of the amino-terminal leader peptide, the remaining five amino acids and the activation dipeptide are coded by exon 2. The dipeptide is removed once it is transported to a post-Golgi vesicle [50]. This safety mechanism is necessary because the premature activation of PR3 would seriously harm the cell by its protease activity. This together with the “sticky” characteristic of this basic protein make it almost impossible to overexpress recombinant PR3 in larger quantities. In the first intron there are tandem sequences [51] and Alu repeats, which are specific for primates, as well as an alternative promoter driving the expression of a cytosolic isoform of PR3 [52]. The second intron is small (266 bp) and exons 2 and 3 are therefore close to each other. Remarkably, intron 2 together with exon 1 (including the 5-noncoding region) has the highest GC-content of all exons and introns of the *PRTN3* gene. Another special feature of the *PRTN3* gene lies in the fact that the polyadenylation signal is duplicated and that the usage of the second copy may be associated with AAV [53].

A single nucleotide polymorphism (SNP) is a point mutation that has been enriched via inheritance to more than 1% in a gene pool. SNPs may have an impact on the amino acid sequence of a peptide, and thus on the function of a protein, if they are located in

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