

Granuloma in ANCA-Associated Vasculitides: Another Reason to Distinguish Between Syndromes?

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Published online: 29 September 2013
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Abstract The 2012 renewed Chapel Hill Consensus Conference (CHCC) officially named three clinicopathological entities, i.e. granulomatosis with polyangiitis (GPA), eosinophilic granulomatosis with polyangiitis (EGPA), and microscopic polyangiitis (MPA), as major variants of anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV). Recent genetic and cohort studies revealed the need for further differentiation between the entities, for example regarding differences in outcome. As well as ANCA reactivity, upper and lower airway disease were found to be differentiating factors for AAV variants, improving prognostic ability regarding relapse prediction and associated clinical features. Extravascular granulomatosis, or “granuloma”, which describes both clinically relevant granulomatous manifestations and histopathologically documented granulomatous inflammation, is characteristic of localized and systemic GPA, but not MPA. This review summarizes new knowledge regarding granuloma in the head and neck region of AAV, its histomorphological equivalents in the upper and lower respiratory tract, and evidence for a granulomatous phenotype of a persistent localized GPA variant. This comprises the development of disease activity and damage scores for extravascular lesions in the ear, nose, and throat (ENT) regions, and imaging techniques. In addition, findings linking extravascular manifestations to granulomatous inflammation are described. We hypothesize that, as for ANCA, necrotizing granulomatous inflammation and its clinical manifestations are discriminators,

assisting subclassification of AAV and/or GPA subphenotypes which will be useful both for designing clinical trials and for treating patients successfully.

Keywords ANCA-associated vasculitides · Granulomatosis with polyangiitis · Genome-wide association studies · Granuloma · Classification · Subclassification · Phenotypes

Introduction

The 2012 revised Chapel Hill Consensus Conference (CHCC) defined microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA, formerly WG), eosinophilic granulomatosis with polyangiitis (EGPA, formerly CSS), and single-organ manifestations (e.g. renal-limited forms) as the major clinicopathological variants of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV) [1]. They belong to the category of vasculitides predominantly affecting small vessels, have a pauci-immune vasculitis, and are probably pathogenetically associated with either proteinase 3-ANCA (PR3-ANCA) or myeloperoxidase-ANCA (MPO-ANCA) [2]. Because the diseases are rare and there is some overlap between them, most clinical trials have recruited a mixture of AAV patients, or at least of both GPA and MPA patients [3]. AAV patients commonly have signs or symptoms of systemic vasculitis in the lungs and kidneys, and have a poor prognosis if left untreated [4]. In recent years management has improved, and even life and/or organ-threatening AAV is today a treatable condition [5, 6]. European Vasculitis Society (EUVAS) randomized controlled trials (RCT) defining all AAV patients as belonging to a single category reported reduced survival, mainly caused by adverse events and/or chronically relapsing disease progression [7–9]. However, small and large cohorts comprising only GPA patients had better outcomes, with fewer deaths and reduced relapse [10, 11].

This article is part of Topical Collection on *Vasculitis*

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The need to recognize different clinical phenotypes observed within AAV [12] and to distinguish between different forms of one entity, e.g. GPA, has been discussed before [13]. Recent genome-wide association (GWAS) data, cohort studies and further improvement of diagnostic tools have provided support for the hypothesis that MPA, GPA, and otherwise defined entities should be studied separately [14]. New diagnostic or classification criteria that enable more accurate discrimination between different entities (or courses) have been acknowledged, suggesting that documentation of both ANCA specificity and granulomatous manifestations improves prognostic ability regarding clinical outcomes [15, 16].

Extravascular granulomatosis featuring necrotizing granulomatous inflammation, or “granuloma”, which describes both clinically relevant granulomatous manifestation and a histopathological characteristic, is symptomatic of both localized and systemic GPA. Granulomatous inflammation of the ENT region is associated with tissue and bone destruction [17] and, similarly to PR3-ANCA, upper and/or lower respiratory tract involvement seems to be associated with frequent relapse [18]. Interestingly, GPA relapses, especially those involving the upper airways, were reduced after treatment with cotrimoxazole [19, 20]; this topic has recently been reviewed [6]. The main way in which MPA differs from GPA is that extravascular granulomatosis is, by definition, absent, but vasculitis symptoms and renal disease are more prominent. The most important predictors of renal outcome are creatinine level at diagnosis, sclerotic lesions, and the number of normal glomeruli in the kidney biopsy [21]. EGPA is the least common of the major AAV entities. It seems to progress in stages similar to those of GPA, but extravascular features differ histomorphologically and clinically. Hypereosinophilia and asthma are central clinical manifestations, the latter usually preceding vasculitis by some years and being associated with symptoms of allergic rhinitis [22, 23]. Necrotizing granulomatous inflammation of the ENT tract with cartilage or bone destruction is not observed. Extravascular (eosinophil-rich) interstitial inflammation with involvement of the myocardium is associated with bad prognosis [23, 24]. In contrast with MPA, the time of testing is critical to identifying ANCA-positive EGPA patients [25]. As well as the idiopathic phenotypes listed above, ANCA-associated vasculitis can have other etiologies; for example, it may be drug or infection-induced (reviewed in Ref. [26]).

Herein, we summarize evidence that ANCA specificity can be used to distinguish between AAV varieties, and argue that extravascular manifestations, especially granuloma, can be used for the same purpose. The strongest argument for granuloma’s differentiating function comes from the description of a localized GPA variant that did not develop into full-blown disease, which was reported by two independent studies [27•, 28•]. By analogy with the use of ANCA to discriminate

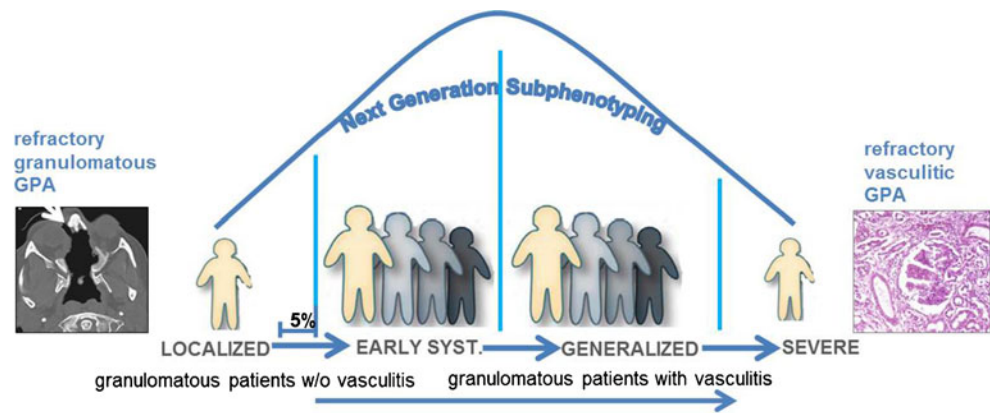
between AAV phenotypes, we discuss the potential use of granuloma for future subgroup differentiation.

ANCA Specificity is a Discriminating Factor in AAV

Genetic Studies: Single Nucleotide Polymorphisms and Alleles are Associated with MPO-ANCA or PR3-ANCA

Initial data generated from candidate genes revealed heterogenic backgrounds for GPA, EGPA, and MPA. In an extended association screening of apoptosis-linked genes, a haplotype surrounding the RXRB gene (encoding the retinoid X receptor β) on chromosome 6q21.3 was highly significantly associated with GPA [29]. This haplotype includes the HLA-DPB1 gene, which had previously been associated with chronic beryllium disease, another granulomatous disease. This association was subsequently confirmed for an independent sample, with impressive effect size (odds ratio 3.38). The association with this allele (HLA-DPB1*0401) was only observed for ANCA-positive GPA [30]. Kelley and co-workers [31] observed IgA and IgG ANCA engagement of Fc receptor genetic variants, which might also affect the outcome of GPA. A strong association of EGPA with IL-10 promoter polymorphisms was detected [32]. In the search for genetic links in AAV, a major collaborative GWAS study organized by the EUVAS group combined single nucleotide polymorphisms (SNP) screening with candidate gene screening. The results confirmed previous associations between GPA and the HLA-DP region, SERPINA1, and PR3; risk of MPA is associated with the HLA-DQ region. Unexpectedly, the strongest associations with risk genes were observed for ANCA subtypes, i.e. PR3-ANCA or MPO-ANCA [33••], revealing the relevance of autoimmune mechanisms and the pathogenic function of ANCA. However, a warning was given not to convert the data immediately into “direct clinical implications in terms of disease classification or diagnosis”, because of inherent limitations in the first GWAS study [34] and, for example, a lack of functional consequences of the PR3 promoter SNP (rs62132295) had already been described some time previously [35]. A second GWAS study, organized by the VCRC group in the US, re-confirmed the strong association between GPA and the HLA-DP allele [36]. Interestingly, polymorphisms in the toll-like receptor 9 gene also differed, being either PR3-ANCA⁺ and MPO-ANCA⁺ AAV or GPA and MPA, respectively [37]. Transcription profiles of different cellular subsets identified for vasculitis and for individual entities, including GPA [38, 39], could help to identify (bio)markers and assist separation of subphenotypes. One could also envisage future genetic studies analyzing extreme subphenotypes, on the basis of cohort studies resembling GPA studies already performed [27•, 28•] (Fig. 1), to determine whether specific risk genes or molecular profiles can be identified.

Fig. 1 Definition of GPA according to the EULAR/EUVAS scheme [3], revealing that improved understanding of disease variations has led to higher resolution with regard to identification of subphenotypes and transitions. *Left side:* computed tomographic scan of the head with a destroyed orbital wall (white arrow) (from Ref. [73]; reprinted with permission from the Massachusetts Medical Society; copyright 2005)



Cohort Studies: ANCA Specificity is Useful for Prognostic Subclassification

Confirming and extending previous results [40–42], a recent study has found ANCA to be superior to CHCC and European Medicines Agency classifications for predicting relapse. PR3-ANCA⁺ patients were almost twice as likely to relapse as those with MPO-ANCA. Moreover, it was observed that PR3-ANCA was prevalent in patients with granulomatous inflammation and that almost all patients with destructive lesions of the upper airways were PR3-ANCA⁺ [43•]. Two cluster analyses of GPA and MPA patients yielded five classes associated with different outcomes. The cluster model which included ANCA specificity was regarded as more accurate and simple to use [44]. With regard to ANCA negativity, there are probably true and false ANCA-negative variants in AAV. A recent study provided an intriguing explanation of why MPO-ANCA positivity is likely to be missed [45].

Altogether, and despite limitations, genetic and cohort studies strongly support the use of ANCA specificity for subclassification of AAV. Thus, because of the evidence summarized above indicating the usefulness of ANCA subspecificities in classification and differences in outcome [14], future clinical study cohorts incorporating GPA, MPA and EGPA should add the prefix PR3-ANCA, MPO-ANCA, or ANCA-negative [1]. This also applies to variants of GPA and EGPA without clinically overt signs of vasculitis; in most cases, these are retrospectively recognized as localized variants and usually develop into systemic vasculitis within a variable time frame [3, 46]. The 2012 CHCC consensus is to “add the prefix MPO-ANCA, PR3-ANCA or ANCA-negative”.

Granuloma as Another Differentiating Factor in AAV

Extravascular granulomatosis is a major factor differentiating MPA from GPA [1], and in EGPA extravascular lesions are usually not associated with necrotizing granulomatous inflammation [22]. We summarize findings on clinical manifestations

in the ENT region that are likely to be induced by or associated with granulomatous inflammation. We assume that, for example, destructive or expanding tumor-like granulomatous manifestations not only follow different pathogenic pathways from vasculitis manifestations but also enable differentiation of additional disease subphenotypes, at least for GPA.

The Neglected Granulomatous Manifestation and its Clinical Phenotypes

Similarly to PR3-ANCA, involvement and/or disease of the ENT region has been recognized as a predictor of relapse in AAV [38, 39]. Despite this, granulomatous manifestations in the head and neck region are sometimes neglected, because they have been regarded as mild and can be occult, at least in the beginning. However, nasal blockage, nasal bridge collapse, chronic sinusitis, hearing loss and subglottic stenosis are severe manifestations suggestive of GPA and commonly contributing to the vasculitis damage index (VDI) score. A survey of 701 North-American GPA patients revealed that, after fatigue and joint pain, symptoms in the ENT region were the predominant presenting features at diagnosis [47]. Further, a small study of GPA (24 patients) reported sinusitis and subglottic stenosis as the most commonly observed head and neck manifestations at diagnosis [48]. Regarding disease-related damage the upper airways were prominent—secondary to kidney involvement in a Turkish cohort of 50 GPA patients [49]. A cross-sectional study of 283 AAV patients comparing VDI and combined damage assessment (CDA) scores reported high incidence of ENT-related organ damage [50]. This is complemented by a study using EUVAS RCT data from 183 patients and revealing an association between ENT disease at baseline and subsequent ENT damage [51]. This must be taken into account, especially in fully-developed disease with life-threatening systemic vasculitis when granulomatous manifestations may seem of minor importance. When remission is induced, “grumbling” disease should be re-evaluated by means of an interdisciplinary approach, e.g. by ENT specialists, and using new imaging techniques to assist in finding “occult” granulomas. Comprehensive

overviews of airway manifestations in adults and children have been published [52, 53], revealing the destructive potential of GPA-related granulomatous inflammation in the head and neck region.

Subglottic stenosis of the trachea is the most severe manifestation in the ENT region, because it can turn into a life-threatening condition and requires increased dilatations, open airway reconstruction, and other local management procedures [54, 55]. In our experience, activity-adapted immunosuppression combined with moderate intervention may be a promising strategy. Orbital masses, also called “granuloma(-tous) masses”, are a rare and treatment-resistant granulomatous manifestation, which was observed in 5 % of a GPA cohort of 1,142 patients [56]. In this study nearly half the patients were refractory to highly potent immunosuppression, approximately three-quarters had visual impairment, and approximately every fifth patient suffered from blindness. Similar observations were documented for a French AAV cohort of 1,286 patients, among whom GPA was the most frequent diagnosis; 6.6 % of these patients had orbital inflammatory tumors [57]. Promising new therapy, for example rituximab, does not always lead to remission or response, especially for refractory GPA and if granulomatous manifestations, e.g. retro-orbital granulomas, are present [58–60]. Localized GPA, especially with involvement of the upper respiratory tract, has been successfully treated with co-trimoxazole, which is recommended (either alone or with glucocorticoids) for induction of remission and for maintenance therapy [6].

The need for improved diagnosis and therapy for granulomatous manifestations is revealed by an EUVAS-based analysis of long-term patient survival [8]. New disease activity scores for granuloma-induced ENT signs have recently been developed, with better sensitivity and specificity than the scores currently used in outcome studies. The proposed scoring system is designed to complement the Birmingham vasculitis activity score (BVAS) but has yet to be adapted for reliability and intraobserver variation [61, 62]. There is also a recognized lack of a best practice biomarker and/or global imaging procedure for quantifying the volume of the granuloma mass and its distribution and progression, including the degree of scarring. However, magnetic resonance imaging can detect early subglottic stenosis, differentiate between active and inactive subglottic inflammation, recognize early proptosis, and monitor the response of treatment-resistant retro-orbital granulomatous masses [51, 63, 64]. In future, when studying treatment-resistant or refractory GPA caused by subglottic or orbital involvement, imaging procedures should supplement AAV scoring systems for disease activity and for damage.

The Granulomatous Phenotype of Persistent Localized GPA

Localized GPA usually is regarded as transition stage, developing over time into the generalized and sometimes

immediately dangerous stages of early systemic, generalized, severe and refractory disease (Fig. 1) which include organ and/or life-threatening complications, for example alveolar hemorrhage and/or nephritis [3, 6]. However, a rare variant of persistent localized GPA was described by two newer and comparable studies [27, 28]. This persistent localized GPA variant is characterized by granulomatous inflammation confined to the ENT region, i.e. without identifiable evidence of systemic vasculitis. In the first study, long-term persistent localized GPA was observed in ~5 % of a German monocentric cohort (1,024 GPA patients). Nearly half the patients with localized GPA remained ANCA-negative and long-term survival was excellent, but frequent relapse and damage in the ENT region were observed which tended to be refractory to standard treatment. Nasal cartilage erosion and lysis, septum perforation, destruction of the inner nose (e.g. loss of conchae), and retro-orbital granulomatous masses were observed. Major clinical consequences were severe pain and organ damage with functional loss, i.e. blindness or airway obstruction. Many parts of the ENT tract can be affected by obstruction, e.g. stenosis of the subglottic region and trachea, the Eustachian tube, or the lacrimal duct. In the second study, the French Vasculitis Study Group observed that 3.2 % of 494 GPA patients had disease localized to one organ. Clinical manifestations were of the granulomatous type, for example orbital granulomas, ENT disease, or isolated lung nodules. These patients remained in the localized stage throughout follow-up. Patients with long-term localized GPA have—according to the 2012 CHCC definition—“no identifiable evidence of systemic vasculitis but when they exhibit clinical and pathological changes identical to those seen in GPA respiratory tract involvement and, especially if they are ANCA-positive, they should be included in the GPA category” [1]. In EGPA extravascular manifestations can occur, but compared with GPA they are less frequent, rarely

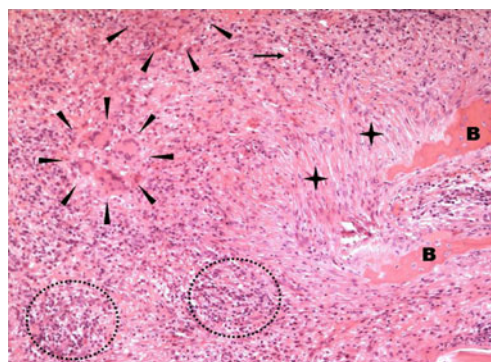


Fig. 2 GPA in sinonasal mucosa (H&E, ×100): granulomatous inflammation comprising the characteristic ill-defined Wegener's granuloma with epithelioid cells and several giant cells (*arrowheads*) on the left side, inflammatory destruction of adjacent bone (*B*) by pannus-like tissue (*asterisks*) on the right side, and typical dense lymphoplasmocytic inflammatory background with beginning of the formation of follicular structures (*dotted circles*) below (from Ref. [70], by permission of Oxford University Press)

granulomatous and/or destructive, and affect different organs [22–24]. Definition of a persistent “localized EGPA variant” seems to be difficult, and is the subject of current research [46].

Association of Necrotizing Granulomatous Inflammation with Extravascular Manifestations

Granulomatous and nongranulomatous extravascular inflammation is common in GPA and has a variety of morphologies. During the early phase, neutrophilic microabscesses with scattered multi-nucleated giant cells are prevalent. In later phases, degenerating neutrophils and necrotic debris form a central, irregularly outlined necrotic zone (geographic necrosis), which is surrounded by poorly defined granulomatous inflammation with palisades of elongated macrophages and scattered multi-nucleated giant cells. The mixed inflammatory infiltrate includes monohistiocytic epithelioid cells and giant cells and a large and heterogeneous population of other cells, including clusters of T and B lymphocytes with partial follicular organization, activated and follicular dendritic cells, plasma cells, PR3-expressing neutrophils, scattered eosinophils, mast cells, debris-laden macrophages, and high endothelial venules suggestive of ectopic lymphoid-like tissue neof ormation. All characteristic symptoms of GPA can be found in nasal biopsies of active disease [65]. We believe that elucidating molecular and cellular patterns within the granulomatous inflammation, although time-consuming, is worthwhile because it may result in better understanding of the pathogenesis and possibly also in identification of prognostic markers. Interestingly, B cells derived from granulomatous inflammation of an ANCA-negative patient with localized GPA produced autoantibodies directed against a transmembrane protein, but not PR3 [66, 67]. Whether this is caused by a lack of PR3-ANCA⁺ B lymphocytes in the subphenotype of ANCA-negative localized GPA or by difficulty in finding local PR3-ANCA-producing cells remains to be investigated. If pathogenic ANCA are evolving from dysregulated natural homeostatic autoimmunity and exogenous factors have an effect [2], there must still be some local site(s) where the dysregulation is initiated and either occurs or does not. Somatic mutations and clonal expansion of immunoglobulin genes derived from granulomatous inflammation (in the ENT region) indicate local ongoing immune responses in GPA [66, 68, 69], which in most, but not all, cases may lead to or favor the formation of pathogenic PR3-ANCA [70]. Of note, on the transcriptional level the primary (nasal) tissue in GPA had a different profile from that of other inflammatory disorders, with involvement of the nasal mucosa [71]. Increased heterogeneity was observed in the GPA transcription patterns, which might also be indicative of subphenotypes. It was recently revealed that fibroblasts lining bone or cartilage, sometimes in a pannus-like pattern (Fig. 2), contribute to destruction of bone or cartilage, for example via expression

of matrix metalloproteases [72•]. These results support the theory that granulomatous inflammation actively participates in the development of clinically relevant extravascular manifestations, including midline destruction or ulcerative lesions described above. Because not all GPA patients seem to have lesions resulting in destruction of the nose or eye, these features might indicate specific subphenotypes of GPA.

Conclusions

Whereas systemic vasculitis is common to all AAV, extravascular manifestations seem to differ between the main subtypes of AAV. On the basis of the association with relapse, identification of a persistent localized GPA variant, and indications of a mechanistic link between granulomatous inflammation and ENT destruction, we propose that granuloma might be another differentiating factor in AAV, or at least in GPA. Confirmation of this theory will require identification of appropriate surrogate markers for the granuloma, similar to the ANCA, which, for example, are suggestive of a destructive disease course, specific extravascular manifestations, or treatment resistance.

Acknowledgments The work of the authors is supported by the German Research Society (DFG), Clinical Research Unit (CRU) KFO170. The authors would like to thank Claudia Möck and Stefanie Dörner for helping with preparation of the manuscript. This review is dedicated to Professor emeritus Wolfgang Müller-Ruchholtz on the occasion of his 85th birthday to recognize his valuable contributions to and thoughtful advice on immunological research.

Compliance with Ethics Guidelines

Conflict of Interest Antje Mueller, Konstanze Holl-Ulrich, and Wolfgang L. Gross declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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