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Review Article

The pathogenesis and treatment in antineutrophil cytoplasmic antibody associated vasculitis

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Abstract: Anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV) is a systemic autoimmune disease characterized by leukocytoclastic inflammation of small blood vessels. Commonly detected autoantibodies include anti-protease 3 (PR3) and anti-myeloperoxidase (MPO). Although cell necrosis plays an important role in the production of autoantibodies and the pathogenesis of AAV, the correlation between their titers and disease activity remains elusive. As improved detection techniques facilitate early diagnosis, a satisfactory efficacy can be achieved in patients with mild to medium severe AAV treated with glucocorticoids and immunosuppressants. However, resistant and relapsing AAV, sometimes life-threatening, do exist in clinical practice. In-depth understanding of pathogenesis of AAV may lend novel insight into the mechanism responsible for its formation and help find effective targeted therapies for refractory patients.

Keywords: Antineutrophil cytoplasmic antibody (ANCA) associated vasculitis, microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA), pathogenesis, targeted treatment

Introduction

Anti-neutrophil cytoplasmic antibodies (ANCA) associated vasculitis (AAV) refers to a group of systemic autoimmune diseases with protean manifestations [1]. The histopathologic feature is leukocytoclastic vasculitis, and renal biopsy often shows a pauci-immune necrotizing and crescentic glomerulonephritis. To date, several subtypes of AAVs have been identified including microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA) and eosinophilic GPA (EGPA), etc. [2]. Indirect immunofluorescence reveals two types of ANCA targeting myeloperoxidase (MPO) in perinuclear area (p-ANCA) and protease 3 (PR3) in cytoplasm (c-ANCA). In most cases, GPA is associated cANCA and MPA with pANCA [3]. Since EGPA differs from GPA and MPA with regards to histopathology and clinical features, it is not discussed in this review [4].

The pathogenesis of AAV is multifaceted and numerous factors may be involved, including the ANCA-induced activation of cytokine-primed neutrophils, the formation of neutrophils extracellular traps (NETs) and the activation of alternative complement pathways. Thus, neutrophils are not only effector cells for endothelial injury, but also as targets of innate immunity [5]. Aberrant functions of T and B cells, two major types of adaptive immunity cells, may break the tolerance and cause tissue damage. Their deregulation induces the activation of the neutrophils with subsequent inflammation and necrosis of the wall of small blood vessels, eventually progressing to AAV [6, 7]. Although in most patients with AAV, remission can be achieved by glucocorticoids (GCs) combining with immunosuppressants, treatment of resistant and refractory AAV remains a tremendous challenge and an unmet medical need. Intensive and extensive studies are being conducted to unveil the pathogenesis of AAV and to evaluate the efficacy of some investigative drugs. In this review, we summarized recent progresses in these respects, and discuss future directions of the AAV research.

Methods

PubMed was searched for combinations of the following indexed subject headings [MeSH]: Antineutrophil cytoplasmic antibody (ANCA) as-

sociated vasculitis, microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA), autoimmunity, pathogenesis, targeted treatment. Eosinophilic GPA (EGPA) was excluded from this review.

Pathogenesis

The genetic, epigenetic and environmental factors may be involved in the pathogenesis of AAV. Major histocompatibility complex II (MHC-II) related genes are closely related to AAV formation. GPA and MPA are associated with HLA-DP and HLA-DQ, respectively [8]. In addition, epigenetic regulation such as histone modification, DNA methylation and micro-RNA regulation, may play a role in their pathogenesis [9]. Further, infectious factors [10], airborne particles [9], certain medicines and drug addiction also contribute to the development of AAV [11]. Despite these findings, the underlying mechanism for AAV is not completely understood.

Neutrophils in the AAV formation

The basic mechanism for injury of vascular endothelial cells

Neutrophils, an important cell type in innate immunity, can phagocytize invading pathogens, causing degranulation, increased cytokine production and the formation of neutrophil extracellular traps (NETs) [12]. When neutrophils are stimulated by pro-inflammatory factors, especially tumor necrosis factor alpha (TNF-α) or C5a, MPO and PR3 migrate from the cytoplasm to the cell membrane where ANCAs bind to these specific antigens. This process initiates with the binding of antigen to the Fc portion of antibodies with involvement of β2-integrin, followed by the binding of the antigen to the Fab portion of ANCAs on neutrophil surface. Such binding induces respiratory burst and degranulation of neutrophils [5], and regulates the expression of tissue factors in neutrophils and endothelial cells, which results in hypercoagulability in AAV patients [13]. ANCAs further increase the production of proinflammatory cytokines by primed neutrophils. At the same time, reactive oxygen species (ROS) and lytic enzymes are generated or released [14]. These processes cause damage of vascular endothelial cells, leading to formation of vasculitis. In a mouse model of MPO-ANCA vasculitis, neutrophil depletion prevented disease progression, confirming the pivotal role of neutrophils in AAV [15]. Moreover, a previous study showed that synthesis of PR3 was dysregulated in mature neutrophils from GPA patients [16]. As PR3 may cause neutrophil apoptosis and the expression of PR3 was upregulated on the membrane of the apoptotic neutrophils, GPA patients have a decreased apoptosis of neutrophils [17, 18]. PR3-expressing apoptotic neutrophils may act as a danger signal and increase production of proinflammatory cytokines, chemokines and nitric oxide (NO) via IL-1R1/MyD88 signaling pathway. Similarly, PR3 membrane anchorage on apoptotic cells may activate macrophages with a subsequent inflammatory response. While after phagocytosis of apoptotic cells, PR3 destroys the anti-inflammatory reprogramming of macrophages, which decreases production of the anti-inflammatory factors and leads to an amplification loop of sustained inflammation [19].

Complements, NETs and ANCA

Formation and degradation of NETs

NETs are lattice-like structures containing extracellular DNA, histones, and neutrophil granule proteins such as MPO and PR3. As an integral part of the innate immune system, NETs are critical to host defense. When stimulated by the pathogens, immune complexes and chemical compounds, they can be released, and capture and kill the pathogens [20, 21].

The formation of NETs may result from excessive activation of neutrophils [20], or from degeneration and necrosis of these cells [22]. Carmona-Rivera et al. have demonstrated that adenosine deiminase 2 (ADA2) can decrease the concentration of extracellular adenosine in inflammatory sites, and the deficiency of ADA2 (DADA2) may activate the NF-κB pathway in macrophages with subsequent increase in the production of TNF-α. Patients with DADA2 often develop vasculitis. These findings suggest that increased concentrations of extracellular adenosine may promote NET formation [23]. Through a nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase (NOX)-dependent or -independent mechanisms, upstream mediators induce neutrophils to release NETs, and these mediators include ANCA, C5a, TNF-α, anti-lysosomal membrane protein 2 antibodies (anti-LAMP-2) and high mobility group box pro-

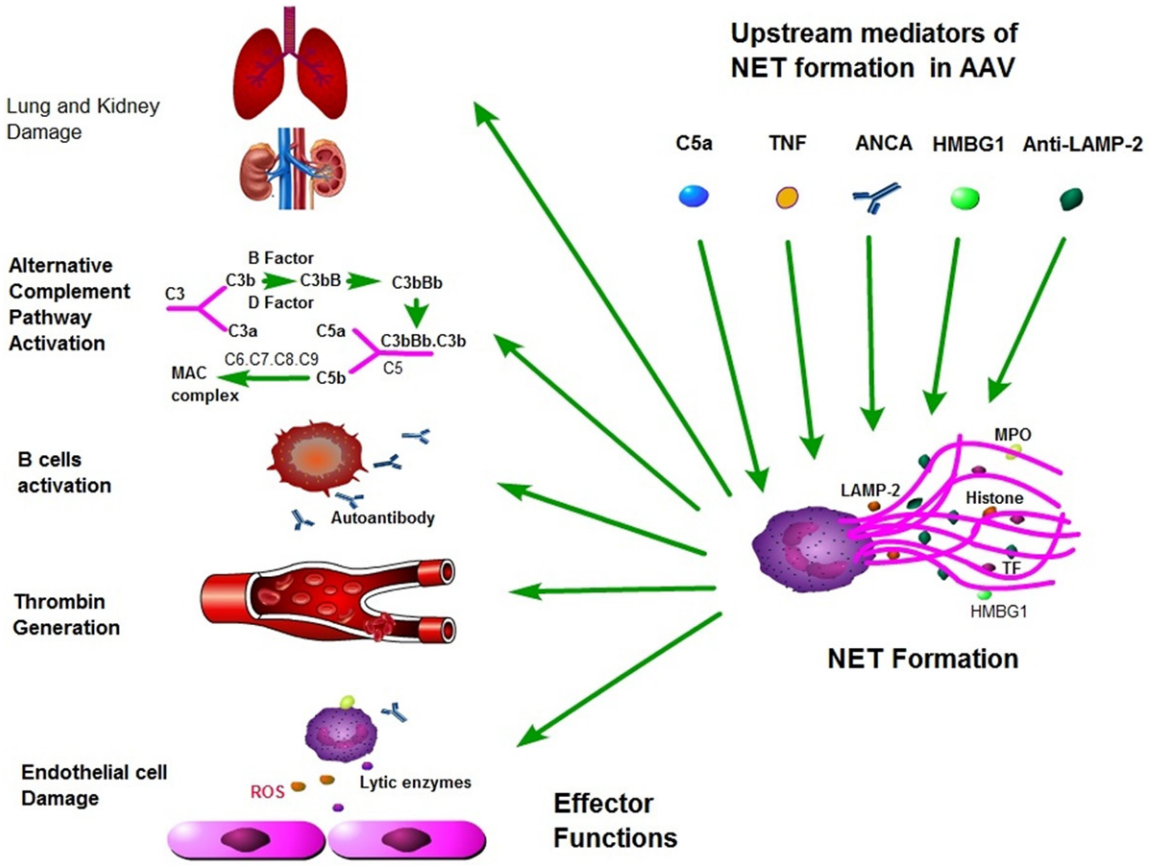

Figure 1. The formation and effector functions of NETs. Through a NADPH oxidase dependent mechanism, upstream mediators such as ANCA, C5a, TNFα, anti-LAMP-2 and HMGB1, prime neutrophils towards NET release. Bioactive proteins on NETs including MPO, LAMP-2, and HMGB1 cause alternation of cytoskeleton, generation of thrombin and activation of the alternative complement pathway, production of autoantibodies by B cells, and necroptosis of endothelial cells and damage of adjacent tissues.

tein 1 (HMGB1). It is plausible to postulate that different bioactive proteins on NETs may cause different disease phenotypes [24]. Neutrophil elastase may not only change the cytoskeleton of neutrophils, but also translocate to the nucleus where it works together with MPO to cause chromatin decompensation and the NET formation [25]. Generally speaking, the formation of lytic NETs relies on the activity of the NADPH oxidase. Upon stimulation, arginine residues are converted to citrulline residues by PAD4, resulting in histone deamination, the loss of positive charge and chromatin decompensation [26, 27]. An *in vitro* study showed that anti-LAMP-2 antibodies activated neutrophils to release NETs with autoantigens and antimicrobial peptides. NETs from AAV patients also express LAMP-2 and inhibition of autophagy can reduce anti-LAMP-2 mediated NET release [28]. These NETs may be released from neutrophils of peripheral blood and bronchoalveolar lavage in active AAV patients, inciting the generation of thrombin and activation of alternative complement pathway [13]. In addition, NETs activate plasmacytoid dendritic cells and autoreactive B cells in a TLR9-dependent manner, resulting in the production of ANCAs [20]. Further, they cause the death of endothelial cells through histone-dependent cytotoxicity [29], as evidenced by necrotizing lesions detected by immunofluorescent microscopy [30]. Since overexposure to NETs can cause tissue damage such as angiopathy, in physiological conditions, the formation and breakdown of NETs are tightly regulated [31]. NETs increase the uptake of ANCA antigens by myeloid dendritic cells (mDCs) [32], and cause the breakdown of tolerance to ANCA antigens [33], leading to vasculitis in lung and kidney in naïve mice immunized with NET components of mDCs [32] (**Figure 1**).

As NETs are mainly degraded by DNase1 in serum, the absence of DNase1 causes persistent presence of the NETs, leading to blood vessel obstruction and organ damage [34]. Compared to healthy individuals, the serum DNase1 activity and the ability to degrade NETs are significantly lower in the MPA patients [33]. The observations made in mouse model showed that pretreating NETs with DNase1 was critical for the production of ANCA, suggesting the formation of MPO-ANCA and PR3-ANCA requires the NETs-derived DNA [32]. Of note, DNase-1 treatment can prevent autoimmune response and pathogenesis of necrotizing crescentic glomerulonephritis in mice [31]. In addition, NETs induce the formation of autoreactive B cells causing persistent ANCA production. Collectively, antigen presentation, structural change of autoantigens and incomplete degradation of NETs result in NETosis and ANCA production.

Previous study has shown that ANCA-induced NETs formation is mainly mediated by receptor-interacting protein kinase-3 (RIPK1/3) and phosphorylation of the pseudokinase mixed-lineage kinase domain-like (MLKL) proteins, two major mediators of necroptosis pathway [31]. In addition, NETs activate alternative, instead of classical complement pathway, which incites endothelial cell damage [31]. These studies suggest that NETosis is a critical process to link neutrophil activation, complement production and endothelial injury.

Both circulatory and glomerular depositions of NETs can be detected in AAV patients, and may contribute to anti-MPO-induced renal injury [20, 35]. In the serum of MPA patients, anti-NET antibodies were detected, and IgG deficiency resulted in increased NETs degradation [36]. Nevertheless, observations with respect to the relationship among the serum level of NETs, the titers of ANCAs and the disease activity of AAV are inconsistent, often times, controversial [33, 35, 37-39]. Some studies demonstrated a potential correlation between NETs and clinical disease activity [33, 35], while other studies suggested that excessive NETs formation was independent of the presence of ANCAs [38, 39]. Lack of gold standard to quantify NETs *in vivo* may be responsible for such discrepancy. Further studies are warranted to establish a reliable and reproducible method to assess the effects of NETs on the disease activity of AAV.

The role of complement system

Complement system is the important player in innate immunity through elimination of foreign cells and microorganisms, induction of adaptive immune response, formation of immune complexes, cell apoptosis and tissue inflammation. Observations made from animal models and clinical studies indicate that the activation of complement system, especially, alternative pathway, plays a pivotal role in the development of AAV [40]. The deposition of alternative complement pathway components on NETs, as mentioned above, is also found *in vitro* [41].

C5a is a major activator for neutrophils [40]. It is also a potent chemoattractant able to recruit different adaptive immune cells including T cells. As T cells express C5a receptors on the surface, they can migrate to activation sites [42]. In addition, C5a participates in the activation of phagocytes and the release of enzymes and oxidants from granulocytes. In mouse models of MPO-ANCA vasculitis, complement activation is critical for AAV formation [43]. Further, C5a was detected in the supernatants of ANCA-activated neutrophils, causing neutrophil activation and ANCA-mediated glomerulonephritis by binding to neutrophil surface receptors [44].

Two downstream molecules, namely, sphingosine-1-phosphate (S1P) and high mobility group box 1 (HMGB1), are important players in the C5a activation of neutrophils [40]. S1P is a bioactive sphingolipid metabolite released from neutrophils and can increase the expression of C5aR on the surface of neutrophils to activate C5a, forming a vicious cycle. The S1P receptor antagonist inhibits C5a-induced neutrophil migration and ANCA-induced respiratory burst and degranulation [45]. In physiological condition, HMGB1 locates in the nuclei and acts as a pro-inflammatory mediator after release. In the C5a primed neutrophils, HMGB1 is released from the cytoplasm, and its exocytosis increases the translocation of ANCA antigens. The serum HMGB1 level correlates with the disease activity of AAV, and the release of NETs is enhanced when HMGB1 interacts with TLR2, TLR4 and RAGE in a NOX-dependent manner [46]. The activation of neutrophils is further

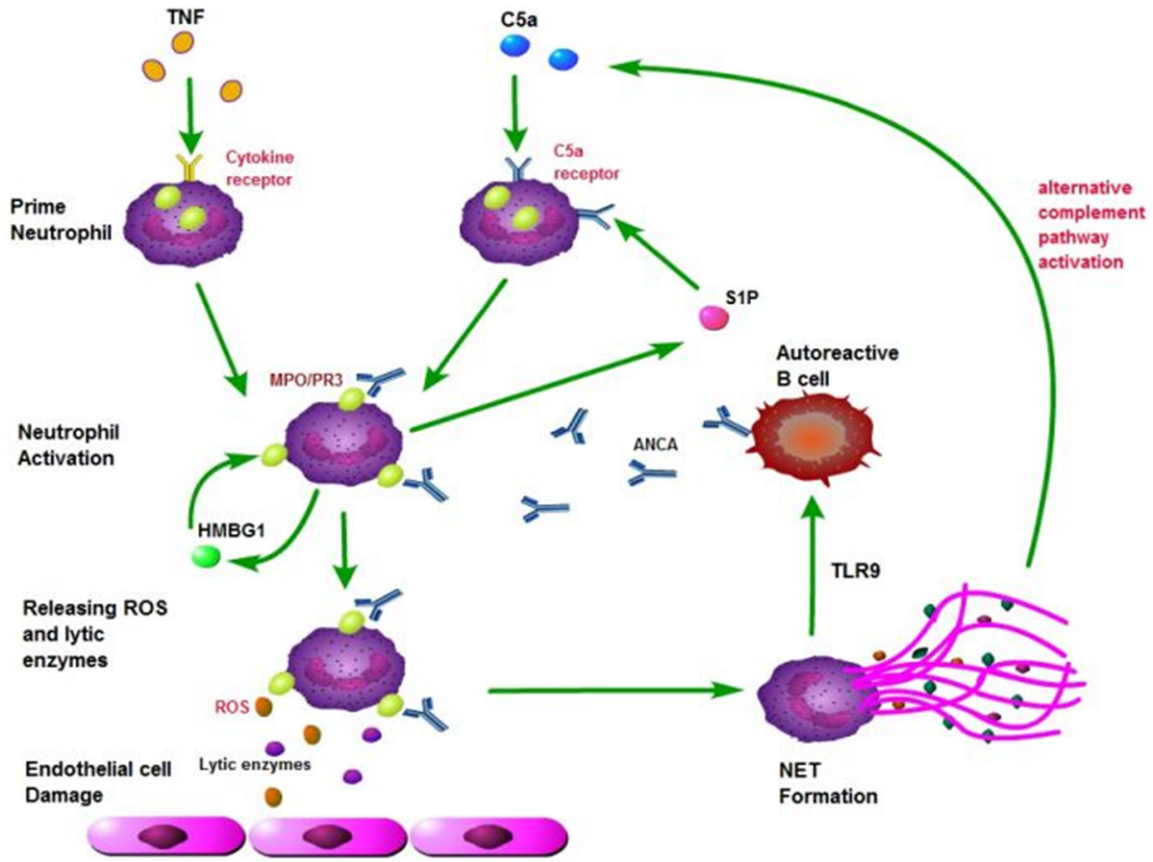

Figure 2. An amplification loop consisting of neutrophils, NETs and complements. Upon stimulation by pro-inflammatory factors, MPO and PR3 migrate from the cytoplasm to the cell membrane where ANCAs bind to them. ANCAs in turn increase the secretion of proinflammatory cytokines. Meanwhile, ROS and lytic enzymes are generated and NETs is released. NETs stimulate autoreactive B cells to produce autoantibodies in a TLR9-dependent manner and activate the alternative complement pathway to induce the production of C5a. S1P and HMGB1 are also released from neutrophils. S1P upregulates the expression of C5aR on the surface of neutrophils and HMGB1 increases the translocation of ANCA antigens. Finally, neutrophils are further activated and endothelial cells are injured in the process.

reinforced by C5a [47]. It is tempting to speculate that interactions between S1P, HMGB1 and C5a play an essential role in ANCA-mediated activation of neutrophils.

In summary, in the presence of ANCA, neutrophils and complement alternative pathway build up an amplification loop, eventually leading to the development of AAV [41, 48] (**Figure 2**).

Adaptive immunity-the role of T cells and B cells in AAV

Interactions between T cells and B cells

A previous study shows that the number of constitutively activated T cells is increased in AAV patients, which is positively correlated with disease severity [49]. Similar findings have been made regarding the role of B cells in AAV. B cells can not only present antigens to autoreactive T cells and provide the required co-stimulation signals for T cell activation, but also repress the anti-inflammatory activity of regulatory T cells (Tregs) [50].

T cells: Aberrant regulation and function of T cells have been well established in AAV patients. TH17 cells are a subset of T helper cells that can be detected in renal lesions of AAV patients. These cells promote the initiation and recruitment of neutrophils, and induce the production of autoantibody. Thus, they have detrimental effects on parenchymal cells at the site of inflammation [51]. In AAV, these cells produce a large amount of proinflammatory

cytokines IL-17 and IL-23. Importantly, the differentiation of some Tregs into TH17 cells and impaired suppressive functions of Tregs have been noticed in these patients [52-54]. While total Tregs with weak suppressive activity are higher, especially, $CD4^+CD25^+FoxP3^+$Tregs, the percentages and numbers of activated Tregs are lower in AAV patients [55]. Effector T cells are amplified in AAV and persistently activated. The balance of T cell immunity is disrupted because of increased percentage of the FoxP3-expressing Treg cells, increased number of effector $CD4^+$ T cell and dysfunctional co-inhibitory PD1/PDL1-axis [56-58]. Moreover, the transcriptional characteristics of $CD8^+$ T cells are associated with the frequency of recurrence in AAV patients [59]. These findings indicate a pivotal role of T cells in AAV pathogenesis and progression.

B cells: B-cell Activating Factor (BAFF), a cytokine of the TNF-α ligand superfamily, is of great importance in B cell homeostasis and interaction with activated T cells, causing the release of IFN-γ and amplification of autoimmune effects [60]. Elevated BAFF concentrations correlate with disease activity and ANCA titers in MPA and GPA patients [61, 62]. Besides, an *in vitro* study suggested that ANCAs may activate TNF-α primed neutrophils to release BAFF and improve the survival of B cell [61]. Of note, tertiary lymphoid structures (TLS) with a germinal center containing follicular dendritic cells and the highly proliferative B cells have been identified in glomerulonephritis lesions. It provides an essential source for antigen-presenting cells to promote T cell maturation at the periphery [63, 64]. In addition, $CD25^{high}TGF\text{-}\beta^{high}$ regulatory B cells (Bregs) can inhibit the differentiation of naïve T cells into Th-1 or Th-17 and produce different cytokines, mainly IL-10, which can inhibit or enhance autoimmune responses [6]. Bregs can also induce the apoptosis of activated T cells and enhance Tregs differentiation via intercellular contact and TGF-β secretion [65, 66]. The abnormal distribution of Bregs in active AAV patients has also been reported [67]. As there is no consensus on the production of IL-10 in AAV, more studies are needed to characterize Bregs phenotype and the effect of B cell on AAV [68, 69] (**Figure 3**).

Cytokine mediated regulation

High levels of circulating cytokines including IL-6 and TGF-β have been detected in the AAV patients, which skew in a fraction of naïve T cells towards Th17 cells in inflammatory sites [70]. B cell-derived IL-6 also promotes the proliferation of B cells and activates macrophages with subsequent increase in the production of other cytokines. It has been showed that serum IL-6 concentrations correlated with the disease activity in AAV and may rapidly decline once remission achieved [71]. TGF-β produced by Bregs is an anti-inflammatory cytokine able to modulate the activity of T cells [65]. Another anti-inflammatory cytokine produced by Bregs, e.g. IL-10, suppresses pathological immunity and induces CTLA4 and FoxP3 expression in T cells by direct cell-cell contact between Bregs and T cells mediated by CD40-CD40L interaction [72]. IL-17 can stimulate macrophages to secret TNF-α, resulting in upregulated expression of PR3 on neutrophils and the activation of NADPH oxidase in neutrophils and amplified immune response and persistent inflammation in AAV patients [52, 73]. IL-17 can cause direct damage to renal tubular cells, podocytes and endothelial cells, which results in further release of chemokines, loss of E-cadherin expression and lysis of the cytoskeleton with increased apoptosis, eventually progressing to renal injury [51].

Treatment

Significant improvement in the prognosis of AAV patients has been witnessed in the past few decades. Treatments of AAV consist of remission induction and maintenance. Induction is to rapidly suppress inflammation to prevent permanent organ damage. Glucocorticoid (GCs) and cyclophosphamide (CTX) are most commonly used medicines in remission induction. When complete remission is achieved, usually in 3-6 months, a longer remission maintenance lasting about 2 years is required to prevent relapses. The main drugs to maintain remission include methotrexate (MTX), azathioprine (AZA), mycophenolate mofetil (MMF) and leflunomide (LEF). Adverse effects of these drugs should be monitored carefully and periodically. When stable remission is achieved, the drugs can be discontinued [74-77]. However, some refractory or resistant patients do not respond well to these treatments. It is imperative to develop new medicines, preferably, steroid-sparing drugs, for these patients. Some agents targeting B cells, BAFF, T cells, cytokines and complements are under inten-

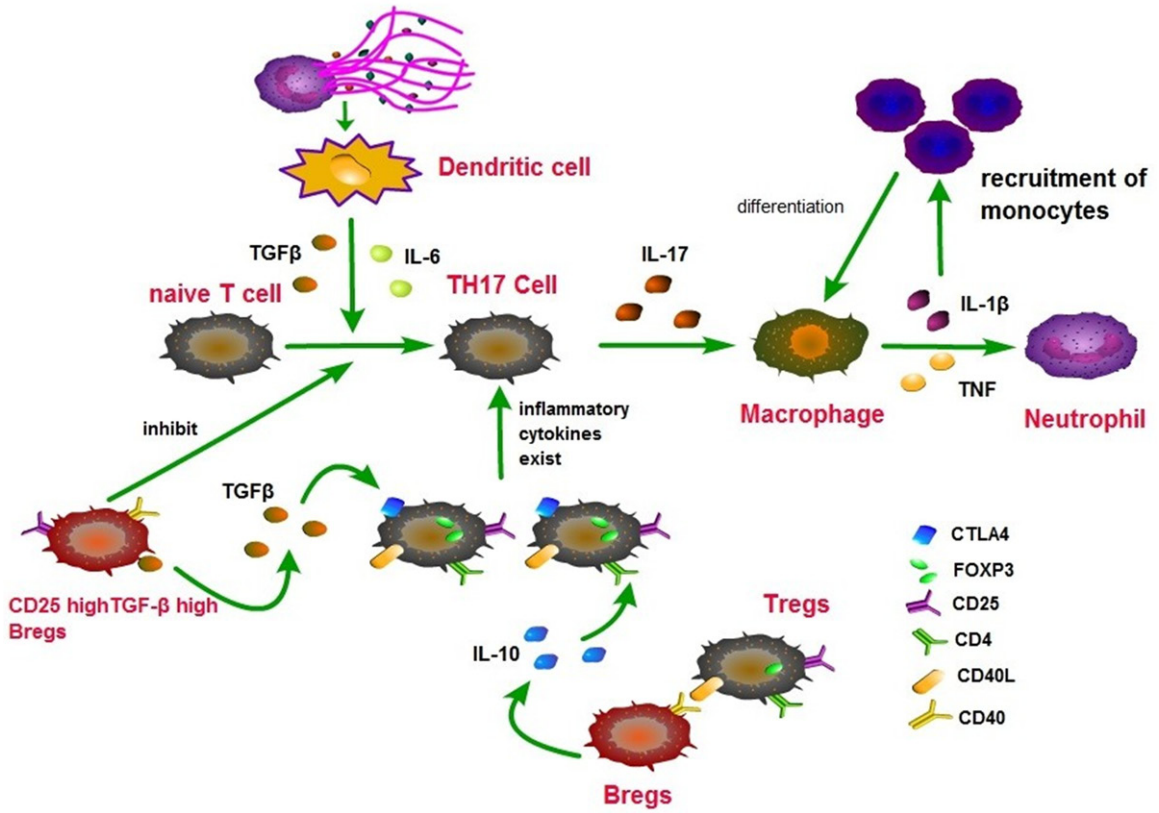

Figure 3. The role of T cell and B cell in AAV. NETs activates dendritic cells to produce TGF-β and IL-6, which skew in a fraction of naïve T cells towards Th17 cells in inflammatory sites. TH17 cells release IL-17, which stimulates macrophages to secret TNF-α and IL-1β, resulting in activation of neutrophils and recruitment of monocytes to differentiate into macrophages. While Bregs inhibit the differentiation of naïve T cells into Th-17 and produce different cytokines, mainly IL-10, which induces CTLA4 and FoxP3 expression in Tregs via direct cell-cell contact between Bregs and Tregs mediated by CD40-CD40L interaction. Bregs also enhance Tregs differentiation via TGF-β secretion.

sive clinical studies (**Table 1**), and more evidence is needed for their wide clinical applications. Based on the critical role of B cells in AAV pathogenesis, the B-cell-targeted therapies have been investigated extensively and intensively. Rituximab (RTX), a chimeric monoclonal antibody (mAb) against CD20, is the best studied biological agent for induction in patients with both new or relapsing AAV patients [89]. Major clinical trials associated with RTX for vasculitis are listed in the **Table 2**. Compared to conventional treatment, most targeted drugs are correlated with a reduction in relapse rate and earlier withdraw of immunosuppressants and glucocorticoid [79, 84, 88, 94, 95]. Several lines of evidence demonstrate that the administration of targeted drugs may be much safer and more cost-effective although impaired immune response and the infection-related adverse effect may increase [82, 87, 96, 97]. More studies are warranted to determine the optimal duration of treatment, the efficacy of cumulative targeted drugs therapy, and long-term outcomes after discontinuation.

Discussion

In this review, we summarized the recent progress regarding pathogenesis and novel treatments of AAV. It is now well accepted that ANCA is a main player in AAV formation, and neutrophils have a pivotal role in vasculitis. In addition, neutrophils, NETs and complement systems constitute an inflammatory circulatory amplification pathway in the course of disease progression. We hypothesize that during this process, the plasticity of neutrophils may be reduced, and their capability to adapt morphologically to arterioles is impaired, which causes their sequestration in small vessels. Neither T cells nor B cells can be also ignored, regardless of the production of cytokines or the induction

Table 1. Major targeted drugs for AAV

Medicine	Classification	Property	Effect
Rituximab	B cell-targeted agents	Chimeric anti-CD20 monoclonal antibody.	Superior to CTX/AZA regimens in inducing remission and reducing relapse.
Ofatumumab [78]	B cell-targeted agents	humanized anti-CD20 monoclonal antibody.	As a substitute for RTX.
Bortezomib [79, 80]	B cell-targeted agents	cell-permeable, reversible, selective proteasome inhibitor.	Improve disease activity in refractory AAV patients.
Belimumab [81]	BAFF-targeted therapies	humanized monoclonal antibody against soluble BAFF.	Used for maintenance therapy.
Alemtuzumab [82]	T cell-targeted agents	Humanized anti-CD52 monoclonal antibody.	Used for refractory AAV with high adverse events.
Abatacept [83, 84]	T cell-targeted agents	fusion protein bound to CD28 on T cells and CD80 or CD86 on antigen presenting cells.	It has steroid-saving capabilities and be well tolerated.
Tocillizumab [85]	Cytokines-targeted agents	humanized anti-IL-6 receptor antibody.	There is not enough evidence of treatment in AAV.
Etanercept [86, 87]	Cytokines-targeted agents	soluble TNF-α receptor combined with an IgG1 Fc portion.	It does not improve stable response rates and refers to tumorigenesis.
Avacopan [88]	Anti-complement therapies	C5a receptor antagonist.	Replace steroid treatment with low incidence of adverse reactions.

Table 2. Major randomized controlled trials associated with RTX for AAV

Trial and Year	Rationale/Question Behind Study	Conclusion
RAVE, 2010 [90]	RTX for remission induction in AAV.	RTX was not inferior to daily CTX treatment for induction of remission in severe AAV and more effective in relapsing disease.
RITUXVAS, 2010 [91]	RTX-CTX regimen for remission induction in AAV.	The RTX-based regimen was not superior to CTX for severe ANCA-associated vasculitis and had nothing to reduce early severe adverse events.
MAINRITSAN, 2014 [92]	Low-dose RTX for maintenance in AAV.	Fixed-interval RTX is superior to AZA for prevention of disease relapse and more accessible to acquire sustained remission.
MAINRITSAN-2, 2018 [93]	Remission maintenance with RTX dosing based on criteria of relapse.	AAV relapse rates did not differ significantly between individually tailored and fixed schedule rituximab regimens.
Rituximab Vasculitis Maintenance Study (RITAZAREM)	Whether repeating RTX stops vasculitis returning, how long patients remain well after the repeated RTX treatments are stopped, and if repeated RTX is safe.	It is ongoing (https://clinicaltrials.gov/ct2/show/NCT01697267).

of ANCAs. Their dysfunctions, changes in number and distribution have an indelible effect on the initiation of neutrophils and the establishment of an inflammatory circulatory amplification pathway. It is through this vicious cycle that the adhesion of accumulating neutrophils to endothelial cells is enhanced and a link is established between neutrophils initiation, vascular inflammation and the coagulation cascades, which eventually causes damage to vascular endothelial cells.

In the past decades, long-term survival rates have improved significantly due to the use of immunosuppressants including CTX, MTX and AZA. Simultaneously, novel targeted therapies are more commonly used in clinical practice, and numerous clinical trials are ongoing to test the efficacy of some new drugs for AAV.

The low incidence of AAV and the long duration of clinical trials make it difficult to recruit patients. In addition, due to the differences in assessment approaches and accuracy, no standardized protocol is available, causing inconsistency when interpreting the results.

Several critical questions are not yet answered. Firstly, it is not clear why the deposited complexes are pauci-immune segmental, whether the type of disease or ANCA is a major determinant of clinical outcomes in AAV patients. Secondly, what is the best method to quantify NETs accurately and detect complement activation *in vivo* routinely. Thirdly, controversy remains regarding the predictors of relapsing vasculitis including ANCA titer, serum B cells level and NETs concentration. Fourthly, several targeted drugs, such as Tabalumab, Atacicept and Eculizumab, have not been demonstrated their clinical efficacy for AAV. Finally, despite the significance of NETs in AAV formation, no targeted drugs are available to prevent the formation and degradation of NETs, and to regulate the process of neutrophil apoptosis. Great endeavors to find effective drugs targeting NETosis will be needed in future studies.

Conclusions

In summary, AAVs are complex diseases with the involvement of many factors. Different components of the immune system interact with each other to form a vicious cycle causing persistent inflammation. The prognosis of AAV has greatly improved in the recent years. More comprehensive studies are warranted to determine the predictors of relapse, and to develop effective drugs for refractory patients.

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Disclosure of conflict of interest

None.

Abbreviations

ANCA, antineutrophil cytoplasmic antibody; AAV, anti-neutrophil cytoplasmic antibodies associated vasculitis; MPA, microscopic polyangiitis; GPA, granulomatosis with polyangiitis; EGPA, eosinophilic GPA; MPO, myeloperoxidase; PR3, protease 3; NETs, neutrophils extracellular traps; TNF-α, tumor necrosis factor alpha; C5a, complement 5a; ROS, reactive oxygen species; ADA2, adenosine deiminase 2; DADA2, deficiency of ADA2; NADPH, nicotinamide adenine dinucleotide phosphate; LAMP-2, lysosomal membrane protein 2; HMGB1, high mobility group box protein 1; TF, tissue factor; PAD4, peptidylarginine deiminase IV; TLR, toll-like receptors; mDCs, myeloid dendritic cells; RIPK3/MLKL, receptor-interacting protein kinase-3/mixed lineage kinase domain-like; S1P, sphingosine-1-phosphate; NOX, nicotinamide adenine dinucleotide phosphate oxidase; Tregs, regulatory T cells; IL-, interleukin-; FoxP3, forkhead box P3; BAFF, B-cell activating Factor; IFN, interferon; Bregs, regulatory B cells; TGF, transforming growth factor; CTLA, cytotoxic T lymphocyte associated antigen; GCs, glucocorticoid; CTX, cyclophosphamide; MTX, methotrexate; AZA, azathioprine; MMF, mycophenolate mofetil; LEF, leflunomide; RTX, rituximab.

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