The role of monocytes in ANCA-associated vasculitis

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Abstract

The anti-neutrophil cytoplasm antibody (ANCA)-associated vasculitides (AAV) are a heterogeneous group of diseases causing inflammation in small blood vessels and linked by the presence of circulating ANCA specific for proteinase 3 (PR3) or myeloperoxidase (MPO). These antigens are present both in the cytoplasmic granules and on the surface of neutrophils, and the effect of ANCA on neutrophil biology has been extensively studied. In contrast, less attention has been paid to the role of monocytes in AAV. These cells contain PR3 and MPO in lysosomes and can also express them at the cell surface. Monocytes respond to ANCA by producing pro-inflammatory and chemotactic cytokines, reactive-oxygen-species and by up-regulating CD14. Moreover, soluble and cell surface markers of monocyte activation are raised in AAV patients, suggesting an activated phenotype that may persist even during disease remission. The presence of monocyte-derived macrophages and giant cells within damaged renal and vascular tissue in AAV also attests to their role in pathogenesis. In particular, their presence in the tertiary lymphoid organ-like granulomas of AAV patients may generate an environment predisposed to maintaining autoimmunity. Here we discuss the evidence for a pathogenic role of monocytes in AAV, their role in granuloma formation and tissue damage, and their potential to both direct and maintain autoimmunity. ANCA-activation of monocytes may therefore provide an explanation for the relapsing-remitting course of disease and its links with infections. Monocytes may thus represent a promising target for the treatment of this group of life-threatening diseases

ANCA-associated vasculitides

Anti-neutrophil cytoplasm antibody (ANCA)-associated vasculitides (AAV) are a group of rare systemic autoimmune diseases that affect small or medium-sized blood vessels. The term embraces three different nosologic entities: granulomatosis with polyangiitis (GPA, previously Wegener's granulomatosis), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA, previously Churg-Strauss syndrome), all of which are characterized by necrotizing vasculitic lesions with scanty or absent immune deposits and, for GPA and EGPA, by the presence of tissue granulomas, typically in the upper and lower respiratory tract [1]. Whilst there is significant clinical overlap between these diseases, each also demonstrates distinct pathological profiles. Thus, GPA is characterized by disrupting lesions of the upper respiratory

tract, lung involvement, and, in approximately 70% of patients, a pauci-immune necrotizing glomerulonephritis (GN) with extra-capillary proliferation [2]. In MPA, the kidney is affected in almost 100% of cases [2], with alveolar hemorrhage affecting one third of patients [3]. In contrast, in EGPA the kidney is not a main target of disease, whilst lung involvement is almost constant. EGPA is commonly associated with asthma and eosinophilia, with eosinophil tissue infiltration contributing to lesion pathogenesis [4].

Despite these clinical disparities, AAV patients are linked by their serological positivity for ANCA. These IgG antibodies target two main proteins in neutrophils and monocytes, namely proteinase 3 (PR3), a serine protease that on immunofluorescence assay shows a granular, cytoplasmic staining pattern (C-ANCA), and myeloperoxidase (MPO), which shows a peri-nuclear staining pattern (P-ANCA) [5]. PR3 seropositivity is typically found in GPA patients (at least in Caucasians) [6], whilst MPA and EGPA are predominantly associated with anti-MPO antibodies [5].

ANCA positivity is not completely diagnostic, and some patients clinically diagnosed with AAV are ANCA negative, while low levels of ANCA can be detected in some healthy individuals [7]. In EGPA only about 40% of patients have ANCA [2], typically those with renal involvement and more vasculitic features [4], compared to 75-95% of active GPA and MPA [2]. On the other hand, there is good scientific evidence to suggest that ANCA are closely linked to disease pathogenesis [8], and variations in ANCA titres are used as a rough tool for monitoring vasculitis activity in clinical practice. However, ANCA titres do not correlate well with disease activity in all reported series, but can be more useful in renal AAV [9].

Evidence for the pathogenicity of ANCA

Animal models of MPO AAV have been extremely useful and have demonstrated that, in mice and rats, IgG anti-MPO antibodies can cause necrotizing crescentic glomerulonephritis and systemic small vessel vasculitis [10, 11]. Attempts to establish an animal model of GPA by the direct injection of anti-PR3 antibodies into mice have been more problematic and have failed to induce any pathological changes, even after pre-treatment with LPS. Another approach using human-anti-PR3 antibodies in mice with a humanized immune system, generated mild pulmonary capillaritis and glomerulonephritis, but did not reproduce the GPA phenotype and, in particular, failed to induce granulomatous lesions [12].

In humans, PR3-ANCA may appear before sub-clinical and clinical GPA [13], and their production is also associated with the development of drug-induced systemic vasculitis [9]. There is a correlation between ANCA titre and specificity with disease activity and phenotype respectively. ANCA pathogenicity is also demonstrated by the clinical benefit observed in AAV with treatment strategies aimed at removing circulating antibodies, such as plasmapheresis [14], or at hindering antibody production by depleting B lymphocytes, eg. rituximab [15] , and this approach forms a valuable part of AAV treatment today. Direct evidence for the pathogenic nature of ANCA in

humans has also been provided by a sporadic case of transplacental MPO-ANCA transfer from mother to baby, with the development of pulmonary and kidney vasculitis in the newborn [8].

ANCA can activate neutrophils

The ANCA antigens MPO and PR3 are expressed in the azurophilic granules of neutrophils and the lysosomes of monocytes, but can also be expressed on the cell surface. Expression can be up-regulated, at least in vitro, by priming with tumor necrosis factor α (TNFα) [16, 17].

TNFα-primed neutrophils respond to ANCA stimulation by releasing their granules and reactive-oxygen-species (ROS), producing pro-inflammatory cytokines and increasing the expression of adhesion molecules on their surface, and they have been shown to activate and damage endothelial cells [18]. Moreover, ANCA induce neutrophils to release DNA, histones and proteins into the extracellular space as neutrophil extracellular traps (NETs), a phenomenon known as NETosis. This is primarily a mechanism of defense against infections, but could also be implicated in tissue injury and in the onset of autoimmunity in AAV, through PR3 and MPO presentation to antigen presenting cells [19]. The response of neutrophils to ANCA and their role in AAV has been discussed in many excellent reviews [19]. For the purpose of this review we will concentrate on the role of monocytes in AAV, an area of research which, to date, has received significantly less attention.

Monocytes in inflammation and autoimmune diseases

As one of the cell types expressing ANCA antigens, monocytes are prime candidates for mediating many of the systemic inflammatory effects seen in AAV. Their potential role in disease pathogenesis is only beginning to be elucidated, but a number of key observations suggest that these cells have an important role to play in AAV. Monocytes represent approximately 10% of human leucocytes in the blood stream and originate from the bone marrow. Although they have traditionally been regarded as the progenitors of tissue macrophages, recent studies have revealed that, at least during homeostasis, many tissue-resident macrophage populations are maintained by a process of self-renewal [20]. In conditions of tissue insult, these cells undergo further rounds of proliferation, but their numbers are also boosted by the recruitment of monocytes from the circulation. Once recruited, monocytes can differentiate into macrophages and are able to mediate a wide range of immunologic processes [20].

Monocytes are now recognized as a heterogeneous population, and can be divided into three sub-types, according to the level of expression of two cell surface antigens, CD14 (the co-receptor for Toll-Like-Receptor (TLR)-4), and CD16 (FcγIIIR). Thus, monocytes are described as classical ($CD14^{++}/CD16^{-}$), intermediate ($CD14^{++}/CD16^{+}$), or non-classical ($CD14^{dim}/CD16^{+}$) [21].The distinctions between these groups are not completely defined, and they may represent a maturation continuum rather than clear-cut divisions [22]. Nevertheless, these sub-divisions are

backed-up by differences in phenotype, in cytokine production at homeostasis or in response to infections, in phagocytosis capability, in the internalization and presentation of antigens, in the ability to differentiate, and in trafficking and migration [23, 24].

Monocyte subsets in the context of human disease have been explored, and recent evidence has highlighted the possible role of the intermediate population in autoimmune disorders such as rheumatoid arthritis [25]. Observations from Tarzi et al [26] in our laboratory have also revealed differences in ANCA antigen expression between monocyte subsets in AAV patients and healthy controls, showing that classical and intermediate monocytes display the highest levels of PR3, whereas MPO is more highly expressed on intermediate monocytes. In AAV patients, both PR3 and MPO expression correlate with expression of CD14. This observation suggests that distinct monocyte subsets may have different roles in the pathogenesis of AAV.

ANCA activation of monocytes

Challenge of monocytes with anti-PR3 and anti-MPO immunoglobulins elicits the production of the chemoattractant protein-1 (MCP-1) [27], and of a range of pro-inflammatory cytokines and prostanoids including TNFα, IL-1β, IL-8, IL-6 and thromboxane A2, [16]. In TNFα-primed cells, ANCA can evoke an increase in ROS production [17]. This allows monocytes to participate in leucocyte recruitment and the development of local inflammation. With regard to the mechanisms that lead from ANCA-antigen interaction to the effects observed in monocytes, it has been speculated that the recognition of surface PR3 or MPO by the antigen binding site of the autoantibodies may be sufficient for responses such as ROS production [17] or CD14 up-regulation [18], as demonstrated by a preserved or reduced response to the Fab_2 fragment of ANCA. However, for cytokine release concurrent cross-linking of surface Fcγ receptors may be necessary [28].

Monocytes in AAV – changes in monocyte physiology

A number of studies have described significant changes in the phenotype and activity of circulating monocytes in AAV patients. GPA patients show an increase of neopterine levels, (a soluble marker of monocyte/macrophage response to interferon (INF)-γ), and a correlation of neopterine and interleukin (IL)-6 levels with disease activity [29]. Circulating monocytes in GPA patients also show increased expression of membrane molecules such as CD63 (a degranulation marker), and CD64 (a high affinity Fcγ receptor, crucial in innate immunity) [29]. Moreover, higher levels of integrins, namely CD29, CD18, CD11a, CD11b, that are essential for leucocyte-endothelium interaction and leucocyte diapedesis, have also been demonstrated [30], suggesting that monocytes in AAV could be more prone to cause tissue damage through an increased ability to interact with activated endothelial cells [31]. Soluble monocyte activation markers, such as MCP1α and VEGF, that reflect systemic chemotactic and inflammatory activity and endothelial activation, show significantly higher levels in patients with acute disease [32]. Monocyte CD11b up-regulation has been shown to persist in quiescent phases of disease [32], and recent studies have demonstrated that monocytes from patients with active AAV, as well as from PR3 positive patients in remission,

express increased levels of CD14, suggesting that these cells may be more susceptible to TLR4-mediated activation [26]. It could therefore be argued that a certain degree of monocyte activation persists during remission, which may account for the relapsing-remitting character of GPA.

Monocytes in AAV-histological observations

ANCA-associated glomerulonephritis

Kidney involvement in AAV is a frequent event and is characterized histologically by focal necrosis of glomerular capillaries with extra-capillary proliferation and pauci-immune-staining on immunofluorescence [2]. Leucocyte infiltration of the tubulo-interstitial area is often present and, especially in the proximity of the glomeruli, it can be organized to form pseudo-granulomatous structures where giant cells can be found [33].

CD68+ monocytes/macrophages are the dominant infiltrating cell type in early biopsies of ANCA-associated glomerulonephritis (ANCA GN), where they infiltrate apparently normal glomeruli, as well as being present in well-developed lesions (**Figure 1)** [34]. Accordingly, monocyte chemoattractant protein-1 (MCP-1), an important chemokine for monocyte recruitment, is increased in kidney biopsies of AAV and its levels in the urine correlate with glomerular disease activity [35].

The early presence of these cells suggests that they may be important in the initiation of kidney damage, as they are considered relevant in the promotion of fibrinoid necrosis, cellular crescent development, and for recruiting the T lymphocytes, which, together with macrophages, dominate the later stages of ANCA GN [36]. CD68+ cells are also present in kidney biopsies of AAV patients with established disease, where they localize preferentially to the sites of active glomerular lesions, including crescents, areas of fibrinoid necrosis, peri-glomerular infiltrates and granulomas [33]. Moreover, it was recently shown that renal infiltrating CD68+ cells in ANCA GN display positivity for MPO and can produce extracellular-traps containing MPO, further contributing to tissue damage and MPO antigen presentation [37]. CD68+ cells recruited to the kidney during vasculitic inflammation are thus closely associated with damage initiation and progression.

Monocytes/macrophages in ANCA GN display an activated phenotype, showing MHC Class II up-regulation and intense calprotectin expression when compared to healthy controls [33]. The latter molecule is expressed by macrophages in active proliferative GN of different etiologies [38]. In AAV kidney biopsies, calprotectin is a marker of acute glomerular lesions, and thus associated with better renal prognosis in treated cases [39]. Calprotectin is also a key molecule for the amplification of inflammatory processes. Pro-inflammatory stimuli promote calprotectin translocation to the cellular membrane and its systemic release from neutrophils, monocytes and early macrophages. In turn, calprotectin induces cytokine and chemokine release, and tissue damage, generating an autocrine inflammatory response [40]. Serum levels of calprotectin are

raised in active AAV and remained elevated after treatment in patients that relapse [39]. Consistent with this observation, it has recently been demonstrated in an animal model of nephrotoxic nephritis that calprotectin may induce renal injury through its effects on macrophages [40].

Monocytes and granulomas in AAV

While in other granulomatous diseases, such as tuberculosis, granuloma formation represents a mechanism to isolate and contain the infection, the pathophysiology of granulomas in GPA is still unclear. Cells inside the granuloma are organized in a complex structure that favors contact and communication between cells of the adaptive and innate immune systems. In the GPA granuloma, PR3 containing cells (neutrophils and monocytes) are surrounded by antigen presenting cells, T cells, and B cells, organized in a pseudo-follicular manner as in lymphoid tissues [41]. The typical architecture of a granuloma consists of an external layer of T lymphocytes and an inner layer of macrophages, which eventually fuse to form multi-nucleated giant cells (MNGC) [42].

Monocytes may play a key role in orchestrating the immunologic response in this setting. They can interact with ANCA and be activated in the blood stream. Following chemotactic signals, they can be recruited to the granuloma site, where they can interact with T cells and differentiate into macrophages [42]. Once in the granuloma, macrophage-derived molecules such as osteopontin may perpetuate the mobilization of new monocytes from the peripheral blood, thus generating a destructive cycle of cell recruitment and activation [42]. Indeed, MNGCs with an osteoclast–like phenotype have been demonstrated within GPA lesions, and are thought to further mediate tissue destruction by the production of cathepsin K and tartrate-resistant acid phosphatase (TRAP) [43].

The peculiar microenvironment created by the granuloma may therefore act as a tertiary lymphoid organ, capable of skewing both the local and systemic immune response on a short or long term basis, and it has been suggested that this is the site where tolerance is broken [44]. Thus, monocytes in granulomas may be critical in shaping the local and systemic T cell response in AAV.

T cells in AAV and their interaction with monocytes

Abnormalities of circulating T cells have also been described in GPA patients. In particular, GPA patients show an expanded memory T cell population, a deficiency in Tregs and persistent T cell activation. Of particular interest is the demonstration that the Th17 population in GPA is expanded in both active disease and in remission [45], and autoantigen-specific memory T cells producing IL-17 are elevated in AAV in remission [46].

The role of Th17 cells in autoimmunity is supported by an increasing body of evidence demonstrating their pro-inflammatory effects, their ability to promote innate immune activation,

and their function, together with their product IL-17, in mediating local damage and the formation of auto-reactive germinal centers in granulomas [47].

Th17 T cells are described in GPA granulomas [48] and their generation may occur within the granuloma environment where monocytes and CD4+ T cells are in close proximity. Indeed, monocytes activated by TLR ligands or derived from non-lymphoid inflammatory sites have been shown to induce Th17 commitment of T cells in rheumatoid arthritis [49], suggesting that a similar event may occur in GPA granulomas. The ability of activated monocytes to interact with T cells, promote their recruitment and polarization, and modulate their activation, has also been demonstrated in rheumatoid arthritis [50].

The hypothesis of granuloma-mediated breakdown of tolerance is consistent with the natural history of GPA, where disease often begins as granulomatous lesions of the respiratory tract and may later evolve into systemic vasculitis. The environment within the GPA granulomas may therefore be the power house where monocytes redirect the T cell response [42], and push it towards pro-inflammatory Th17 cell production.

Do infectious agents link monocytes and AAV?

A significant body of evidence suggests that there is a link between AAV and infections. The antibiotic cotrimoxazole, for example, is successful in reducing the relapse rate in GPA [51], infections can trigger the acute phase of vasculitis, and nasal carriers of *S. Aureus* are more prone to relapse [52]. These *in vivo* findings are boosted by a significant amount of *in vitro* data examining the effect of pro-inflammatory cytokines, DAMPs, PAMPs and ANCA on monocyte activation.

Thus, TNFα, as produced in infectious diseases, has been shown to up-regulate MPO and PR3 expression on the monocyte cell surface, sensitizing them for interaction with ANCA [17]. Monocyte exposure to PR3-ANCA markedly increases their response to microbial antigens (**Figure 2**). Hattar et al. [52] for example, found that pre-incubation of monocytes with anti-PR3 antibodies increased the production of IL-8, TNFα and IL-6 in response to LPS, and of IL-8 upon stimulation with lipoteichoic acid (LTA), constituents of the Gram-negative outer membrane and Gram-positive cell wall respectively. This effect seems to depend on the ANCA-mediated up-regulation of the TLR4 co-receptor CD14 [52], an observation recently confirmed *in vivo* by Tarzi et al [26] who described raised levels of CD14 on circulating monocytes from PR3 patients both during active disease and in remission. Moreover CD14 expression correlated with ANCA antigen expression on monocytes of AAV patients, showing a stronger correlation with PR3 than MPO. The increased synthesis of CD14 in response to ANCA of both specificities (MPO and PR3) was also noted in another study, where it was concomitant with increased expression of the integrin CD18 [18]. Some, but not all [52], workers have shown that TLR and NOD (1 and 2) expression may be up-regulated in PR3-ANCA challenged monocytes and in monocytes from AAV patients, particularly in *S. Aureus* nasal carriers [53, 54].

The results described above suggest that both LPS stimulation during infections, and ANCA challenge in AAV, may use CD14 as a common mechanism of monocyte activation. The persistent up-regulation of CD14 on monocytes in PR3-AAV patients in remission may reflect a long-lasting activation of monocytes in GPA sustained by persistent nasal/upper airway infection, thereby predisposing GPA patients to more frequent relapses than in MPA [26]. Increased monocyte CD14 levels may also have particular relevance to the recently described increased level of the TLR4 ligand, calprotectin, in AAV patients [39]. Calprotectin is necessary to promote auto-reactive IL-17 producing CD8+T cell development in a mouse model of systemic autoimmune disease, suggesting that its presence favours the onset and amplification of auto-reactivity [55]. The LPS, TNFα, IL-1β, and INFγ, produced during infections, or in inflammatory states such as AAV, may therefore be responsible for driving calprotectin release and contributing to persistent autoimmunity in this disease [40].

In summary, monocytes in GPA patients may display a state of pre-activation, due to PR3-ANCA mediated sensitization, that amplifies their response to secondary stimuli with microbial antigens. The increased production of TNFα during infectious events leads to a rise in PR3 surface antigens, which exposes monocytes to the effect of anti-PR3 ANCA, and triggers the recruitment of other inflammatory cells, local damage and amplification of the immune response. Infectious agents also up-regulate calprotectin, which participates in the vicious circle of uncontrolled inflammation and auto-reactivity. Moreover, local inflammation/infection and the ensuing monocyte activation that persists in the remission phase of GPA may have a role in determining the predisposition to relapse (**Figure 2).**

Would therapeutic targeting of monocytes be beneficial in AAV?

Taken together, there is a significant body of evidence to suggest that monocytes act at different levels in the pathogenesis of AAV, and that they could provide a promising target in the development of new therapeutic strategies **(Table 1)**. However, significant depletion of the monocyte pool would severely affect innate immunity with a corresponding increase in susceptibility to sepsis. Targeting of specific monocyte subsets has been proposed, but a clear pathogenic subgroup has not yet been delineated in AAV, and our understanding of the plasticity and consequent dynamic phenotype of monocytes is still in its infancy [24]. A fascinating therapeutic development in this direction could consist of “reprogramming” monocytes, possibly via epigenetic mechanisms. Recent insights into natural immunity suggest that modification of histone methylation or acetylation in monocytes may form the biochemical basis for the specific memory of innate immunity and for the shaping of monocyte phenotype. Although the clinical use of “epigenetic drugs” is to date mostly restricted to hematology and oncology [56], histone deacetylase inhibitors have proved beneficial in several animal models of inflammatory diseases [57], and could perhaps be used in AAV to modulate surface ANCA antigen expression and down-regulate monocyte activation molecules during remission.

Treatments designed to hinder the persistent activation of monocytes in AAV could also provide significant benefit for maintaining remission in AAV patients. CD14 and calprotectin are involved in the crosstalk between immune responses to infections and autoimmunity, and may play a pivotal role in the amplification of uncontrolled inflammation in AAV. Both of these molecules remain up-regulated in AAV in remission [26, 39]. Anti-CD14 monoclonal antibodies (MAb) treatments, alone or coupled with complement inhibition, are being investigated in the intensive care setting as a method of reducing the uncontrolled inflammatory response and its detrimental effects in severe sepsis [58, 59, 60] and their effect in AAV may be worth investigating. On the other hand, quinoline-3-carboxyamide, an inhibitor of the calprotectin subunit S100A9, has proved effective as an immune-modulator in some animal models of autoimmune disease, and preliminary clinical data are available with regard to its use in diverse autoimmune diseases, such as multiple sclerosis, and systemic lupus erythematous [61, 62]. This approach could also have clinical applicability in AAV.

With regard to granulomas, MNGC are a source of degrading enzymes and tissue damage in AAV. Identifying the molecular mechanisms involved in monocyte-macrophage multi-nucleation and MGGC formation would be required to inform the development of therapies designed to prevent the development of disrupting lesions in GPA. An example is the gene Kcnn4, recently characterized in rat macrophages, inhibition of which proved beneficial for renal lesions in a model of nephrotoxic nephritis [63].

Hampering monocyte migration to the target organs could be useful for the treatment of early stage ANCA GN. Inhibition of the chemokine CCL2/MCP-1 or its receptor CCR2 has already been tested in animal models of renal diseases, including crescentic GN, where reduced monocyte/macrophage infiltration was coupled with better histology and clinical parameters [64, 65]. Various inhibitors of the CCR2-CCL2/MCP-1 axis have been proposed for clinical trials in different diseases, but not yet for ANCA GN [66]. Inhibition of leucocyte integrins or adhesion molecules is an alternative strategy [65]. For example neutralizing antibodies to LFA-1/ICAM-1 [65], or VLA-4 (CD49b/CD29) reduced renal injury in crescentic GN in rats [67].

Lastly, targeting monocyte pro-inflammatory products is a feasible strategy. IL-6 is increased in active AAV patients' sera and at the sites of active lesions (ANCA GN and skin vasculitis) and the inhibition of IL-6 receptor with a humanized monoclonal antibody, tocilizumab, was effective in patients with allergy to or refractory to standard therapy [68]. Both IL-1 and TNFα have a pathologic role in AAV. Activated monocytes/macrophages in kidney biopsies of ANCA GN stain positive for TNFα and IL-1 [33]. Both of these cytokines were demonstrated to mediate glomerular damage in experimental models of crescentic GN, where disease was prevented or halted by means of IL-1 receptor antagonists [69, 70] or TNFα blockade [71]. Inhibiting TNFα action would also prevent monocyte (and neutrophil) priming. Different anti-TNFα monoclonal antibodies could therefore have utility in the induction phase of treatment and in refractory cases, although attention has to be paid to the risk of serious infections [72, 73, 74]. Several IL-1 blockers, such as

anakinra, canakinumab (anti-IL1β neutralizing antibody), or rilonacept (soluble decoy receptor) have been approved for clinical use in several autoinflammatory and autoimmune disorders, and could represent a potential field of investigation for use in AAV [75].

Monocyte-macrophage derived IL-23 is a recognized pro-inflammatory mediator in autoimmunity, promoting Th17 differentiation. In GPA patients IL-23 is found in granuloma biopsies and IL-23 serum levels correlate with disease severity [76]. A drug targeting the p40 subunit of IL-23, (common to IL-23 and IL-12), has already been approved for psoriasis treatment, and is under investigation for use in other autoimmune diseases. Development of new molecules more specific for IL-23 inhibition is under way [77].

To conclude, over the last decade there has been a growing awareness of the pivotal role of monocytes in the pathophysiological mechanisms of AAV. Further investigation of monocytes, to understand their regulatory mechanisms, better characterize the different subsets, and determine their role in inflammatory/autoimmune diseases, is warranted to develop new, more specific, therapeutic targets for AAV.

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Figure legends

Figure 1. ANCA-associated glomerulonephritis (ANCA GN)
Hematoxylin and eosin staining and CD 68 immunohistochemistry of kidney biopsies from two patients with ANCA GN.
CD68+ monocytes/macrophages are the dominant infiltrating cell type in patients with early ANCA GN, where they infiltrate apparently normal glomeruli and early lesions **(A).**
CD68+ cells are also present in biopsies of patients with established ANCA GN, where they localize preferentially to the sites of active glomerular lesions, including crescents, areas of fibrinoid necrosis, peri-glomerular infiltrates and granulomas **(B).**

Figure 2. Monocytes at the axis between infectious diseases and AAV
The amplification loop in AAV leads to raised production of ROS, an increased recruitment of inflammatory cells, (mediated by the release of MCP-1 and IL-8), and a rise in the production of inflammatory cytokines, that may perpetuate and spread the phlogistic process. Other cytokines may be involved in the commitment of Th17 cells, mediating inflammation, local damage and promoting auto-immunity.
(1) Monocytes in homeostatic conditions express low levels of ANCA antigens.
(2) During infections, PAMPs together with pro-inflammatory cytokines and DAMPs activate monocytes, raising CD14 levels and 'priming' monocytes by inducing increased surface expression of ANCA antigens. In response to these stimuli, monocytes also produce calprotectin and TNFα that amplify in an autocrine manner their activation.
(3) ANCA interaction with ANCA antigens and Fc receptors on monocytes causes further activation with increased CD14 expression.
(4) Monocytes expressing increased ANCA antigens and CD14 respond to ANCA and CD14-dependent PAMPs, producing increased pro-inflammatory cytokines. Chemokine production (MCP-1, IL-8) attracts additional leucocytes, thereby perpetuating the cycle of monocyte activation and pro-inflammatory mediator production and amplifying the inflammatory process.
The alternative pathway of complement is also activated, further contributing to the inflammatory response (omitted for clarity).

Abbreviations: ANCA, anti-neutrophil cytoplasm antibody; TNFα, tumor necrosis factor alpha; PAMPs, pathogen associated molecular patterns; LPS, lipopolysaccaride; DAMPs, danger associated molecular patterns; TNFαR, tumor necrosis factor alpha receptor; TLR, toll-like receptor; ROS, radical oxygen species; IL-1, interleukin 1; IL-6, interleukin 6; MCP-1, monocyte chemotactic protein 1; IL-8, interleukin 8; Th 17, T helper 17 cells.