ANCAs Are Also Antimonocyte Cytoplasmic Autoantibodies

J. Charles Jennette and Ronald J. Falk

Names can be misleading. The term ANCA is a misnomer, because ANCAs recognize antigens that are in not only the primary granules of neutrophils but also, the peroxidase-positive lysosomes of monocytes, including both proteinase 3 (PR3) and myeloperoxidase (MPO).

The first published article on ANCA was in 1982 by Davies et al. (1), who reported eight patients with segmental necrotizing GN who all “had in their serum a factor that stained the cytoplasm of neutrophil leucocytes by indirect immunofluorescence” (1). This article was largely overlooked, and widespread recognition of ANCA awaited the publication in 1985 of an article in The Lancet by van der Woude et al. (2), which “found antibodies reacting with the cytoplasm of ethanol-fixed granulocytes and monocytes (anticytoplasmic antibodies, ACA)” in patients with active granulomatosis with polyangiitis (2). This report clearly showed that ANCAs react with both neutrophils and monocytes and used the more generic term anticytoplasmic antibodies (ACPAs) rather than ANCAs (2). For several years after the discovery of ANCAs, there were many advocates for using the term ACPA rather than ANCA; however, the term ANCA prevailed, and ACPA was relegated to becoming the acronym for anticitrullinated protein antibody (3).

Although ANCAs react with monocytes, they do not react with macrophages. Using indirect immunofluorescence microscopy assays, Charles et al. (4) observed that normal human peripheral blood monocytes have well defined cytoplasmic staining with MPO-ANCA and PR3-ANCA. However, monocytes that are cultured in vitro progressively lose reactivity with MPO-ANCA and PR3-ANCA as they differentiate into macrophages, which have no reactivity using this method. Patient alveolar macrophages obtained by bronchoalveolar gavage and peritoneal macrophages obtained during dialysis also do not react with MPO-ANCA or PR3-ANCA. These observations indicate that ANCA can directly interact only with monocytes before and shortly after activation but not with mature macrophages.

Initial in vitro studies of the pathogenic potential of ANCA showed that primed neutrophils can be activated by ANCA to undergo respiratory burst and degranulation (5). This has been confirmed by numerous additional in vitro studies (reviewed in ref. 6). A lesser number of in vitro studies clearly show that ANCA also can activate monocytes (6–10). Multiple animal models support a pathogenic role for ANCA in vivo (11). These studies have not delineated the respective pathogenic roles of neutrophils versus monocytes, although one study indicated that selective depletion of neutrophils was sufficient to prevent acute necrotizing glomerular injury in a mouse model of ANCA GN induced by anti-MPO antibodies (12).

In this issue of CJASN, Zhao et al. (13) show that, at the time of biopsy, monocytes/macrophages are the most frequent leukocytes in very early segmental necrotizing lesions in patients with ANCA GN, with lesser numbers of neutrophils. Zhao et al. (13) also identified increased numbers of macrophages in normal-appearing glomeruli in specimens with ANCA GN. Zhao et al. (13) conclude that activated macrophages are important in the induction of acute lesions and potential targets for therapy. A technical limitation of the study was the identification of neutrophils by histologic appearance alone, whereas monocytes and macrophages were detected by immunohistochemical markers (13). Zhao et al. (13) used CD68 as a marker for monocytes/macrophages, which may result in an overestimation of monocytes/macrophages and an underestimation of neutrophils, because CD68 is in not only the lysosomes or monocytes/macrophages but also, the primary granules of neutrophils. Nevertheless, the observations are valuable and warrant careful consideration.

The results of the work by Zhao et al. (13) are in accord with those reported in the work by Weidner et al. (14), which also showed that monocytes/macrophages and to a lesser extent, neutrophils were the predominant leukocytes in glomeruli in renal biopsies from patients with ANCA GN, with $4.7 \pm 7.4$ monocytes/macrophages compared with $3.2 \pm 7.4$ neutrophils per glomerular cross-section (14). Neither study identified substantial numbers of T lymphocytes or B lymphocytes (13,14). This latter finding differs from the study by Cunningham et al. (15), which reported $7.3 \pm 6.1$ macrophages, $3.7 \pm 2.5$ T lymphocytes, and $2.8 \pm 1.7$ neutrophils per glomerular cross-section in patients with pauci-immune crescentic GN. This discrepancy could be the result of the timing of the biopsy relative to the stage of the glomerular injury if there are more neutrophils and monocytes in earlier lesions and more macrophages and T lymphocytes in later lesions. Neutrophils are evanescent at sites of...
inflammation, because they undergo apoptosis, netosis, and necrosis within hours of activation (16). Interestingly, in 1976, Donald et al. (17), using electron microscopy, described intravascular lysis of neutrophils in the lesions of granulomatosis with polyangiitis. By electron microscopy, Zhao et al. (13) observed predominantly macrophages rather than neutrophils localized at perforations and attenuations of glomerular capillary basement membranes.

Monocytes are recruited to sites of acute inflammation by the same stimuli that recruit neutrophils, and their initial responses, including respiratory burst and degranulation, are similar to those of neutrophils (18). For example, both neutrophils and monocytes use a similar repertoire of receptors to become activated, use a similar set of receptors to marginate and accomplish diapedesis, and undergo similar respiratory burst and degranulation. Thus, it is no surprise that they both have MPO and PR3. However, neutrophils are quickly destroyed during the early phase of acute inflammation, whereas monocytes transform into macrophages and persist into the subacute and chronic phases of inflammation. Monocytes are one of the earliest arrivals at sites of inflammation and quickly transform into macrophages, which contradict the misconception that T cells must be involved to orchestrate the arrival of macrophages (19). In fact, the reverse is true: macrophages orchestrate the arrival of T cells at sites of inflammation (19).

Although the functional and physiologic classification of macrophages has proven to be much more complex than initially thought, at least conceptually, macrophages have been classified as M1 or M2 (19,20). In general, M1 macrophages direct T cells to produce Th1-like cytokines (e.g., IFN-γ) that stimulate specific cytolytic T cells and activate more M1 macrophages, whereas M2 macrophages stimulate T cells to produce Th2-like cytokines (e.g., IL-4 and TGF-β) and simulate matrix production and fibrosis (19). M1 macrophages are more involved in engaging the adaptive immune response, and M2 macrophages are more involved in orchestrating the innate immune response that can lead to either repair or scarring.

Zhao et al. (13) attempted to characterize the macrophages in ANCA GN as M1 or M2 using antibodies to CD68 as a marker for all monocytes/macrophages and antibodies to CD163 as markers for M2 macrophages. Zhao et al. (13) observed that CD68+CD163+ macrophages are the most numerous macrophages at sites of segmental fibrinoid necrosis, which supports their conclusion that M2 macrophages are important in the development of the early lesions of ANCA GN. However, there were some CD68+CD163− cells at sites of necrosis. These could be M1 macrophages; alternatively, they could be neutrophils, which also are CD68+ and CD163− (21).

M2 macrophages may be involved in not only acute glomerular injury but also, acute tubular injury. Palmer et al. (22) recently phenotyped the interstitial leukocytes in renal biopsy specimens showing acute tubular injury. Palmer et al. (22) used anti-CD68 as a panmacrophage marker, anti-CD163 as an M2 macrophage marker, and anti–HLA-DR as an M1 macrophage marker. Palmer et al. (22) concluded that the macrophages that accumulate around injured tubules after acute tubular injury are predominantly M2 macrophages.

The article by Zhao et al. (13) in this issue of CJASN serves notice that the ANCA misnomer should not impede consideration of the pathogenic potential of the antimonocyte specificity of ANCA. Additional careful evaluation of patient samples and sequential analysis of inflammatory events in animal models of ANCA GN are called for to follow up on this important report.

Disclosures
None.

References

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