LAMPS and NETs in the Pathogenesis of ANCA Vasculitis

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Strong evidence from in vitro studies, animal models, and clinical observations suggests that anti-neutrophil cytoplastic antibodies (ANCA) play a critical role in the vascular damage of ANCA-associated vasculitis (AAV).1 Understanding the key pathogenic mechanisms of AAV may provide a safer and more rational therapeutic approach. Although the pathogenic role of ANCA in AAV has been studied extensively, it is still not clear why ANCAs appear. Two recent papers in Nature Medicine2,3 provide a previously undescribed molecular explanation of the origin, development, and perpetuation of injury in AAV that may have important clinical implications.

In the first article, Kain et al.2 reported that infection by fimbriated bacteria triggers cross-reactive autoimmunity to a previously characterized ANCA antigen, lysosomal membrane protein 2 (LAMP-2), resulting in the production of autoantibodies that activate neutrophils and damage human microvascular endothelium in vitro and cause pauci-immune focal necrotizing glomerulonephritis (FNGN) in rats.2 In the second article, Kessenbrock et al.3 demonstrated that the formation of neutrophil extracellular traps (NETs), which are involved in neutrophil cell death during infection, trigger vasculitis, and perpetuate the autoimmune response against neutrophil components in patients with AAV.

It has long been suspected that infection plays a role in the development of ANCAs. They were discovered in 1982 while Davies et al.4 were studying antinuclear antibodies in serum from patients with FNGN, many of whom were infected with the Ross River virus. Several ANCA autoantigens are leukocyte proteins implicated in host defense against infectious diseases.5,6 Wegener’s granulomatosis may be an atypical cell-mediated immune response to an exogenous or endogenous antigen in the respiratory tract that results in granuloma formation and the development of humoral autoimmunity to proteinase 3 (PR3).6 One theory of PR3-directed autoimmunity involves the complementary peptide of PR3, which is encoded by the antisense strand of the PR3 gene. Exposure of the immune system to this peptide triggers the formation of antibodies that cross-react with PR3.7 DNA sequences complementary to the PR3 gene are found in microorganisms including Staphylococcus aureus, supporting the role of infectious agents as triggers of PR3 autoimmunity through molecular mimicry. Cotrimoxazole treatment reduces the frequency of relapses in patients with Wegener’s granulomatosis in remission, probably by eliminating or reducing S. aureus in the upper airways.8

Kain et al.2 suggested that molecular mimicry is also the primary mechanism in the development of pauci-immune FNGN in patients with ANCA; however, the antigen involved is not PR3 or myeloperoxidase (MPO) but LAMP-2, a heavily glycosylated type I membrane protein first reported as a target of ANCA in patients with active pauci-immune FNGN in 1995.9 LAMP-2 in neutrophils is expressed on the membranes of intracellular vesicles that contain MPO and PR3; it is also abundant on the surface of endothelial cells. LAMP-2 plays a role in antigen presentation and in the adhesion of peripheral blood mononuclear cells to the vascular endothelium and is involved in microbe clearance–related mechanisms.6,10

Kain et al.2 studied sera from 84 patients with active pauci-immune FNGN and found 38 patients had anti-MPO antibodies, 39 had anti-PR3 antibodies, and 70 (83%) had one or the other; however, autoantibodies to human LAMP-2 were found in 78 (93%) of 84 patients. These autoantibodies also bound epitopes on fully glycosylated LAMP-2 from human plasma membrane. When WKY rats were administered an intravenous injection of human LAMP-2–specific rabbit IgG, which cross-reacts with rat LAMP-2, all rats developed severe renal injury, with focal capillary necrosis in 22.2% of glomeruli after 24 h and crescents in 21.0% after 48 h.

By incubating H4B4, a mAb to human LAMP-2, Kain et al.2 also established that ANCA to LAMP-2 activate neutrophils and induce apoptosis of endothelial cells. The authors characterized two epitopes recognized by anti–LAMP-2 antibodies from patients with pauci-immune FNGN. Whereas neither of the human LAMP-2 epitopes were homologous for MPO or PR3, the P41 to 49 epitope was 100% homologous to host epithelia. Reciprocal inhibition experiments showed that autoantibodies recognizing human LAMP-2 from individuals with

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FNGN, specifically the P 41 to 49 epitope, cross-reacted with FimH. Rats immunized with recombinant FimH fusion protein developed antibodies to FimH that cross-reacted with human LAMP-2. FimH-immunized rats also developed antibodies to rat LAMP-2 and human-like pauci-immune FNGN, which caused pauci-immune FNGN in mice: The remaining triggers(s) need identification.

Kessenbrock et al.3 did not miss the infectious link, even though they found that NETs occur in AAV in the absence of infection. Active release of NETs, which trap and kill invading microbes extracellularly, characterizes a unique type of infection. Although reproduction of these experimental results is necessary, one clinical implication could be the development of a diagnostic test for anti–LAMP-2 antibodies to determine their specificity and sensitivity. Kain et al.2 found that >90% of patients with active pauci-immune FNGN had circulating anti–LAMP-2 antibodies, whereas only approximately half had anti-MPO and anti–PR3 antibodies. It would also be useful to study independent cohorts of patients with AAV for evidence of anti-FimH and anti–P 41 to 49 reactivity. In addition, the relationship between anti-MPO and anti–PR3 antibodies and anti–LAMP-2 antibodies should be clarified; Kain et al. did not comment on whether rats receiving anti–LAMP-2 antibodies subsequently developed anti-MPO and anti–PR3 antibodies; however, if it is proved that fimbriated bacteria with the relevant amino acid sequence of mature FimH trigger pauci-immune FNGN in individuals with the required host factor, then the therapeutic implications might be far-reaching: Treatment of relapses with proper antimicrobial agents could reduce the need for toxic immunosuppressants. It would also be interesting to determine whether LAMP-2–specific autoantibodies can trigger NET formation and whether LAMP-2 is present on NETs and can interact directly with NET DNA.

Looking for in vivo evidence of NET formation, they found typical NET components in conjunction with neutrophil infiltration in affected glomeruli from patients with AAV and acute worsening of kidney function. In these cases, NETs were prominent in specimens with strong neutrophil infiltration, suggesting that NET formation occurs mainly during active disease. Finally, by using MPO-specific capture and subsequent DNA-specific detection antibodies, they identified circulating MPO–DNA complexes in patients with AAV but not in control subjects. The authors speculated that ANCA-binding to MPO and PR3 antibodies can trigger NET production, maintaining the delivery of antigen–chromatin complexes to the immune system. Although their experiments did not involve infection, the propensity of neutrophils to form NETs in patients with AAV was enhanced by bacterial infection with S. aureus, which is known to induce NETs strongly and seems to be linked to relapses during AAV.

These two studies raise various questions. If anti-MPO antibodies cause pauci-immune FNGN in mice, then how is it that anti–LAMP-2 antibodies produce a similar effect? It may be that the two types of antibodies act synergistically to cause injury. Otherwise, anti–LAMP-2 antibodies might alter the function of LAMP-2 in the presentation of cytoplasmic antigens such as MPO and PR3, with the subsequent synthesis of antibodies against them. Kain et al.,2 by linking the LAMP-2 autoantigen with a mechanism for disease initiation, clearly showed that infection with fimbriated bacteria induces autoantibodies to human LAMP-2 through molecular mimicry and that these antibodies bind microvascular endothelium and cause injury; however, infection with fimbriated bacteria is not sufficient for the development of pauci-immune FNGN in mice: The remaining triggers(s) need identification.

References


Disclosures

None.


