LAMPs and NETs in the Pathogenesis of ANCA Vasculitis

Xavier Bosch
Department of Internal Medicine, Hospital Clinic, University of Barcelona, Barcelona, Spain

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Strong evidence from in vitro studies, animal models, and clinical observations suggests that anti-neutrophil cytoplasmic 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 ANCs 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 ANCs. 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 microneighborhood–related mechanisms.9,10

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 P 41 to 49 epitope was 100% homologous with amino acids 72 through 80 of mature FimH, an adhesin located at the tip of type 1 fimbriae and essential for attachment of Gram-negative pathogens such as Escherichia coli, Klebsiella pneumoniae, and Proteus mirabilis to host epithelia.

Reciprocal inhibition experiments showed that autoantibodies recognizing human LAMP-2 from individuals with FNGN, specifically the P 41 to 49 epitope, cross-react with FimH. Rats immunized with recombinant FimH fusion protein

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Correspondence: Dr. Xavier Bosch, Department of Internal Medicine Hospital Clinic, Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain. Phone: 34 93 227 5539; Fax: 34 93 227 9236; E-mail: xavbosch@clinic.ub.es

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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, with 15 to 31% of rat glomeruli affected by crescents; two rats also developed hemorrhagic pulmonary vasculitis. Data not shown reported that nine (69%) of 13 patients with FNGN had a microbiologically confirmed diagnosis of infection by FimH-expressing bacteria (mainly *E. coli*) in the 12 wk before presentation. Furthermore, autoantibodies in these patients bound the human LAMP-2 region containing the cross-reactive P 41 to 49 epitope.²

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 neutrophil-related cell death.¹¹ Ultimately, the gummous DNA web sticks to endothelia and causes tissue damage during sepsis,¹² similar to neutrophil-induced inflammation of capillaries in AAV. The stimuli leading to the release of NETs are dependent on the neutrophil respiratory burst,¹³ which is activated by ANCA binding to PR3 or MPO on the neutrophil surface.

Kessenbrock *et al.*³ also observed strong NET formation in patients with AAV but not in healthy donors after priming neutrophils with TNF-α and incubating them with purified IgG. After 180 min, 23% of neutrophils incubated with ANCA-IgG produced NETs compared with 11% of control IgG-treated neutrophils. The authors also induced NETs with a PR3-specific mouse mAb and demonstrated that both PR3 and MPO localized with extracellular chromatin fibers that interacted directly with NET DNA.

Looking for *in vivo* evidence of NET formation, they found typical NET components in apposition to neutrophil infiltrates 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 ANCAps perpetuate a vicious circle of 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 auto-antibodies 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.

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.² 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 also present on NETs and can interact directly with NET DNA, with the subsequent detection of circulating LAMP-2–DNA complexes. Studies to assess whether the suppression of NET formation through scavenging of reactive oxygen species could improve AAV and halt chronic autoimmunity in these patients also are a more distant goal.

DISCLOSURES

None.

REFERENCES

The Development of Urinary Biomarkers for Kidney Disease
Is the Search for Our Renal Troponin

Lynda Anne Szczech
Department of Medicine, Division of Nephrology, Duke University Medical Center, Durham, North Carolina

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The practice of nephrology is long recognized to treat and comfort on the basis of a blend of science and art. As nephrologists, we do an amazing job of monitoring kidney function with rather rudimentary measures such as serum creatinine and urine output. When patients ask what are the “normal” values for each of these measures, we all answer in a similar and somewhat nonspecific manner reflecting the “art” of interpretation: What is normal creatinine? Answer: It is like golf: High is bad, low is good. What is a normal amount of urine? Answer: Depends on how much you drank and ate.

The new field of biomarkers for kidney injury or disease is an exciting and rapidly advancing scientific area. Recent developments in the study of several urinary biomarkers of kidney injury have focused on NGAL, NAG, and KIM-1. Two articles in this issue of JASN are part of a growing literature that will shift the balance between the art and science of understanding—shifting toward new evidence that anticipates changes in kidney function more reflective of better science. As we read this rapidly growing literature and seek out our “renal troponin,” we can take a lesson from our cardiology colleagues: Our ultimate goal is to identify patients with a clinical syndrome earlier to initiate promptly a therapy that ultimately affects outcomes in a more positive way. It is these last two points that will be essential in prioritizing our future research agenda.

The new study by Siew et al. provides data to support the use of urinary NGAL as a predictor of acute kidney injury (AKI) in the intensive care unit. More than 400 critically ill patients in intensive care were enrolled, had urinary NGAL measured, and were followed prospectively to identify those with AKI defined as an increase in serum creatinine of >0.3 mg/dl or 50%. Median values of urinary NGAL were significantly higher on enrollment in patients who experienced AKI during the subsequent 48 h (190 versus 57 ng/mg; P < 0.001).

The new study by Paragas et al. also provides data to support the ability of urinary NGAL to discriminate among different types of kidney disease in patients with HIV infection. Patients with HIV-associated nephropathy had significantly greater values of urinary NGAL than those with HIV without chronic kidney Disease (CKD), those with HIV and CKD (not HIV-associated nephropathy), and those with a variety of non-HIV glomerular lesions. The choice of multiple comparison groups allows the reader to consider the effect of HIV infection, CKD, both, or neither and the multiple types of kidney diseases in relation to the level of urinary NGAL.

Although these new studies will play a foundation role in the future validation of these biomarkers, they are not without limitations. Minor methodologic issues can be discussed, such as the use of a dichotomized outcome of AKI. Although the definition is widely accepted and common in the literature, arguably, AKI exists on an exaggerated spectrum from the damage of a single tubular cell to acute cortical necrosis. Under scoring this continuum is a recent article examining NGAL and other urinary biomarkers after cardiac surgery. In that study, regardless of whether the patient was considered to have or have not experienced AKI (using the same definitions used by Siew et al.), a rise from the baseline value with subsequent fall back to baseline was seen over time. Stated another way, even patients without any significant change in creatinine experienced a rise and fall in their urinary NGAL. In the study in this issue of JASN, there were also statistical differences in mean peak concentrations of NGAL between those with and without AKI. Furthermore, it is clear that considerable overlap exists in the ranges of NGAL for groups with and without


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Correspondence: Dr. Lynda Anne Szczech, Duke University Medical Center, Box 3646, Durham, NC 27710. Phone: 919-668-8008; Fax: 919-668-7128; E-mail: szcze001@mc.duke.edu
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