Shining a LAMP on pauci-immune focal segmental glomerulonephritis

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Anti-neutrophil cytoplasm antibody (ANCA)–associated vasculitis frequently presents with renal involvement manifested by a focal segmental necrotizing glomerulonephritis, which is typically pauci-immune. Although considerable insight has been gained regarding potential mechanisms of organ damage, researchers have remained relatively ignorant of the initiating factors breaking immune tolerance. A recent report has provided evidence that molecular mimicry may be critical, with immune responsiveness toward a bacterial fimbrial protein inducing a cross-reactive autoimmune response toward lysosomal-associated membrane protein-2 (LAMP-2). Use of an experimental model demonstrates that this response generates ANCA and provokes pulmonary-renal disease, reminiscent of human ANCA–associated vasculitis. Greater understanding of the immune mechanisms underlying the development of ANCA should lead to more focused approaches to the treatment of small-vessel vasculitis.

Kidney International (2009) **76,** 15–17; doi:10.1038/ki.2009.123; published online 22 April 2009

KEYWORDS: ANCA; glomerulonephritis; LAMP-2; vasculitis

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Received 20 January 2009; revised 25 February 2009; accepted 3 March 2009; published online 22 April 2009

In their original report of a new autoantibody associated with pauci-immune glomerulonephritis, termed the 'anti-neutrophil cytoplasm antibody (ANCA)', Davies *et al.*[1] described eight patients who showed this antibody, of whom seven showed reactivity to Ross River virus. The authors suggested that the antibody resulted from an infection with this type of arbovirus. Subsequently, van der Woude[2] demonstrated a close association between ANCA and active Wegener's granulomatosis, establishing ANCA as a new biomarker of certain forms of small-vessel systemic vasculitis, and this was confirmed by Falk[3] in other cohorts of patients with pauci-immune glomerulonephritis (Figure 1). In the context of vasculitis, ANCAs were found to bind two key antigens found in neutrophil granules and monocyte lysosomes, proteinase–3 (PR-3)[4] and myeloperoxidase (MPO).[5] The diagnostic value of assays for ANCA is now widely accepted.[6]

The pathogenicity of anti-MPO antibodies *in vivo* and *in vitro* has been demonstrated, whereas only convincing *in vitro* effects have been found with anti-PR-3 antibodies.[7–9] More than 10 years ago a new ANCA target, lysosomal-associated membrane protein-2 (LAMP-2), was described, but its significance was unclear.[10] Other antigenic targets have subsequently been recognized, but these are not generally associated with small-vessel vasculitis, although certain pathological associations are known: for example, bactericidal/permeability increasing protein (BPI)-ANCA in patients with cystic fibrosis[11] especially those colonized by *Pseudomonas*, and atypical ANCA binding multiple target antigens,[12] which now include tubulin beta and bacterial FtsZ[13] in primary sclerosing cholangitis and inflammatory bowel disease. Interestingly, many antigenic targets are leukocyte proteins involved in host defense against infectious diseases. Indeed LAMP-2, along with LAMP-1, has been implicated as a critical molecule in maintaining the microbicidal activity of phagosomes[14] and regulating their maturation. LAMP-2, along with Toll-like receptors, also play a pivotal part in modulating autophagosome numbers and maturation, and deficiency of LAMP-2 leads to the accumulation of autophagosomes in the heart and skeletal muscles of mice, as well as in patients with Danon disease, who demonstrate LAMP-2 mutations. Thus, LAMP-2 is intimately involved in mechanisms regulating microbe clearance. Importantly, although the association between

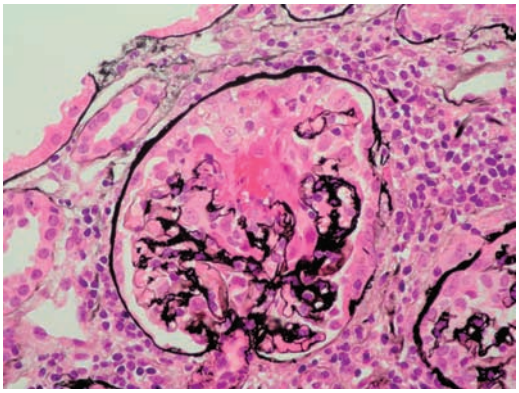

Figure 1 | Photomicrograph of a renal biopsy from a patient with active ANCA-associated vasculitis (AAV), demonstrating crescentic glomerulonephritis and fibrinoid necrosis in the glomerular tuft. Silver stain. Magnification $\times$ 400. Courtesy of Dr Candice Roufosse, Department of Histopathology, Hammersmith Hospital.

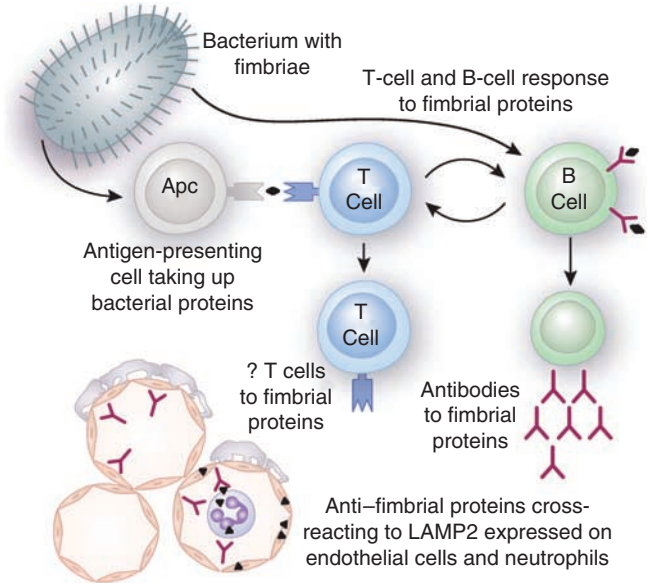

Figure 2 | Sketch demonstrating the proposed mechanism of molecular mimicry inducing immune responsiveness to bacterial fimbrial protein and in turn lysosomal-associated membrane protein (LAMP-2), resulting in activation of circulating neutrophils and damage to glomerular endothelial cells.

infections and autoimmunity has long been recognized, direct evidence for causality with regard to renal disease has mostly been lacking.[15,16]

Since the original descriptions of ANCA, we have come to understand more about the pathogenesis of ANCA-associated vasculitis (AAV), in particular many of the downstream events that occur once ANCAs are present, such as how ANCA interacts with leucocytes and endothelial cells, inducing their activation and effector functions.[17] What has remained unclear is how immune tolerance towards these ANCA antigens is broken in the first instance. It is therefore of considerable interest that a recent study by Kain *et al.*[18] has linked the antigenic target LAMP-2 described earlier, common to all forms of ANCA-associated glomerulonephritis,[10] with a mechanism for disease initiation. In particular, this study reiterated the importance of infectious agents in the etiology of autoimmune diseases and produced convincing evidence demonstrating cause and effect. Earlier clinical observations had implicated bacterial carriage of the Gram-positive coccus *Staphylococcus aureus* as a provoking factor for disease relapses in patients with Wegener's granulomatosis.[19] In addition, work on the proposed complementary peptide hypothesis demonstrated a similarity between amino-acid sequences in *S. aureus* and a complementary PR-3 peptide and, similar to the current study by Kain, implicated molecular mimicry as a mechanism of disease initiation.[20]

Kain *et al.*[10] earlier described LAMP-2, a glycosylated membrane protein contained within neutrophil granules along with PR-3 and MPO, as an ANCA target and found antibodies to LAMP-2 in 14/16 patients with necrotizing glomerulonephritis.[10] However, the importance of these antibodies has been recognized only now. This study shows that anti-LAMP-2 antibodies are found in patients with active AAV and have the potential to be pathogenic, activating neutrophils and causing microvascular endothelial cell injury *in vitro*, as well as inducing a focal segmental necrotizing glomerulonephritis (FSNGN) when injected into susceptible rats. In addition, anti-LAMP-2 antibodies recognized a nine-amino-acid epitope (P_{41-49}) with significant homology to a bacterial fimbrial protein FimH, found in various Gram-negative bacteria. Interestingly, this LAMP-2 epitope shares no homology with either MPO or PR-3, and anti-LAMP-2 antibodies do not cross-react with anti-MPO or anti-PR-3 antibodies, nor do they prevent them from binding to their respective antigens. However, through a series of inhibition experiments the authors demonstrate that sera containing anti-LAMP-2 antibodies are prevented from binding the P_{41-49} epitope by recombinant FimH protein or lysates from bacteria containing the FimH protein, but not by those that lack the FimH. Conversely, sera are prevented from binding FimH protein by LAMP-2.

Finally, to complete the picture, the authors immunized WKY rats with recombinant FimH protein (or P_{41-49}) and showed ANCA reactivity within the serum, anti-LAMP-2 activity and pauci-immune focal necrotizing glomerulonephritis (Figure 2). These data convincingly demonstrate that FimH proteins can induce anti-LAMP-2 activity, and that these antibodies can be pathogenic. Linking this with the human condition, the authors report that almost 70% of their patients with FSNGN (Figure 1) had recent evidence of an infection with a fimbriated organism and evidence of LAMP-2 antibodies, including reactivity to the dominant P_{41-49} epitope. By contrast, patients with fimbriated bacterial infections but no renal disease had antibodies to FimH, but neither anti-LAMP-2 antibodies nor antibodies to the

dominant epitope P_{41-49}. These findings are intriguing but slightly confusing, as they suggest that another critical factor is required to generate anti-LAMP2 reactivity, and that infection with fimbriated bacteria is not sufficient for development of pauci-immune FSNGN in patients. This is not really surprising as Gram-negative infections are considerably more common than pauci-immune FSNGN, and other triggers would be expected to be required for disease to develop. On the other hand, susceptible rats such as the WKY strain, which are used in many models of glomerulonephritis, may be more easily provoked into developing disease with a single stimulus.

Overall these findings are highly significant. These new data take the association with infectious agents one step further by describing a potential mechanism for disease initiation that requires more investigation. Independent cohorts of patients should be studied for evidence of anti-LAMP-2 antibodies, anti-FimH, and anti-P_{41-49} reactivity, and should in addition include larger cohorts of control subjects with other glomerular diseases. We should also investigate those ANCA-negative patients with pauci-immune FSNGN to observe whether they demonstrate anti-LAMP-2 reactivity, and explore the differences between ANCA-positive patients with limited disease and those with systemic features including renal involvement. The relationship between anti-MPO, anti-PR-3, and anti-LAMP-2 antibodies needs further investigation to understand why they co-exist and whether they play a role in inducing each other through, for example, leukocyte activation. Perhaps their interaction may help explain why ANCA titers do not always correlate with disease activity, and why discrepancies between the anti-MPO/PR-3 ELISA results and ANCA detection by indirect immunofluorescence on neutrophils are observed in patients. Additionally, one would predict that T-cell responses to LAMP-2 and indeed FimH should be detected at increased frequency in patients with active AAV, as they have been shown to react with PR-3 and MPO. One issue with the current study is that the effects of anti-LAMP antibodies were shown using rabbit or mouse derived antibodies, not human ones (because of the coexistence of anti-PR3 and anti-MPO antibodies in the serum samples). These effects should be confirmed with human anti-LAMP antibodies, as there may be differences in pathogenic potential between antibodies from different species even with similar antigenic targets.

Should we routinely screen for anti-LAMP-2 activity in all our AAV patients? Not yet, until the specificity and sensitivity of the antibody are verified, but perhaps we should be saving sera from such patients to make this analysis possible in the future. Should we alter our management based on these data? Again, not until we have more information regarding the relationship between disease activity and antibody titer, and the effect of immunosuppression on antibody positivity. Kain *et al.* may have finally shed some light on a mechanistic association between infections and autoimmune pathology that many have suggested but have not been able to prove.

DISCLOSURE

The authors declared no competing interests.

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