Coming of Age—CC Chemokine Ligand 18 in ANCA-Associated Vasculitis

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The management of pauci–immune focal necrotizing GN (FNGN) remains a challenge, with morbidity and mortality remaining stubbornly high. Well powered controlled clinical trials have optimized older treatments and introduced new ones, but substantial numbers of those affected still die of active disease or from the toxicity of the drugs used to treat it.1,2 Current strategies for tailoring therapy to disease activity are clearly inadequate because of the lack of biomarkers that accurately reflect the underlying pathogenesis and can be used to anticipate clinical relapses. The need for new approaches provided the starting point for a study from the laboratory of Stahl and colleagues in this issue of JASN.3 Brix et al.3 suggest that the CC chemokine ligand 18 (CCL18) contributes to the pathogenesis of ANCA–associated crescentic GN and that its serum concentration may be a clinically relevant marker of disease intensity.

It was originally assumed that assays for autoantibodies to myeloperoxidase (MPO) and proteinase 3 (PR3) would be ideal biomarkers for ANCA–associated disease activity. Unfortunately, although positive assays for anti-MPO and anti-PR3 have a >90% sensitivity and specificity for diagnosing pauci–immune FNGN and in vitro studies, experimental models, and genetic studies provide a compelling case for their pathogenicity,4 recent evidence from controlled trials as well as meta-analyses show that this is not the case.5–7 Indeed, assays for ANCA and autoantibodies to MPO or PR3 reflect disease activity poorly and cannot be used to predict disease relapses in those treated with either traditional immunosuppressive drugs or rituximab. Alternative biomarkers currently being explored include developing more pathogenetically relevant assays for antibodies to MPO and PR3,8–10 monitoring titers of more recently described autoantibodies in ANCA associated vasculitis,11 and monitoring injury using targeted circulating or urinary biomarkers.12,13 Although promising, none of these have yet led to the development of a robust biomarker; hence, there is interest in the different strategy by Stahl and colleagues.3

Brix et al.3 optimized methods for extracting mRNA from paraffin–embedded renal biopsies before using array technology to analyze the genes expressed in renal biopsies from a representative group of 30 patients with ANCA–associated crescentic GN. Brix et al.3 identified just over 1300 gene transcripts that were robustly overexpressed, whereas 342 were downregulated in patients’ biopsies compared with healthy control kidneys. Brix et al.3 then focused on chemokine genes, because they were among the most highly expressed and because of their known importance for renal injury by recruiting and activating specific leukocyte subsets.14 Of the many chemokine genes that were upregulated in patients’ biopsies, CCL18 was the most highly expressed of all, with approximately 100-fold higher levels than in normal kidneys. The source of the CCL18 was shown by immunohistology to be a subset of CD68–positive mononuclear phagocytes (that is, macrophages or dendritic cells). These were far more frequent in the patients’ biopsies and clustered around glomeruli and in foci of interstitial inflammation. Both the abundance of CCL18 mRNA and the number of CCL18-positive cells correlated with cellular crescents and the extent of interstitial inflammation but not fibrosis of tubular atrophy. As a group, the serum CCL18 concentration in patients’ sera was significantly raised at the time of the biopsy and decreased toward normal after immunosuppressive drugs were started, but it was again increased during disease relapses. Interestingly, the group of patients who relapsed had significantly higher concentrations of CCL18 at presentation. Thus, CCL18 expression reflected acute injury in ANCA associated vasculitis, and it is important to put it into the context of what is currently known about CCL18 and its involvement in disease.

The chemokine CCL18 is present in relatively high concentrations in human serum, and it is one of a group of cytokines that has a role in tissue homeostasis and inflammation.15 CCL18 has no murine equivalent, probably because it arose from a gene fusion event that occurred after the evolutionary separation of rodents and primates.16 It was described independently by a number of groups in the late 1990s and given different names, reflecting the circumstances of its discovery:17 pulmonary- and activation-regulated chemokine, alternative macrophage activation–associated chemokine-1, dendritic cell chemokine-1, and macrophage inflammatory peptide-4. Macrophages and dendritic cells are the major sources of CCL1817,18; it is expressed constitutively by alveolar macrophages in the lung and to a lesser extent, dermal dendritic cells in the skin. Much still needs to be
learned about the cues that control CCL18 synthesis by macrophages, but expression is increased by either classic or alternative-activating stimuli; maturation of dendritic cells probably has a similar effect. The receptor for CCL18 is CCR8, but this was only identified relatively recently, which is one of the main reasons why data about the cells that respond to it are so fragmentary. However, responding cells include T cells recruited into the skin during contact sensitivity and monocyte-derived macrophages infiltrating inflamed joints in rheumatoid arthritis. Early studies showing increased CCL18 expression in allergic diseases, such as asthma and contact hypersensitivity, led to the idea that CCL18 had a particular role in Th2 responses and immune modulation. However, subsequent studies that showed that it is also overexpressed in inflammatory diseases, including rheumatoid arthritis and giant cell arteritis, suggest that this is not the case.

Thus, one of the principal uncertainties is whether CCL18 has a pathogenic role in inflammatory disease or conversely, is part of a negative feedback loop that damps down over exuberant inflammation. The second part of the study by Brix et al. provides important new data on this question not available elsewhere, and this is, perhaps, the most novel aspect of the study.

Knockout mice are one of the most powerful tools for analyzing cytokine function, but this is not feasible for CCL18, which lacks a mouse homolog. However, CCR8 provides a partial solution to the problem, because it is found in both mice and humans. In humans, CCR8 is the receptor for CCL1 and CCL18, whereas in mice, it binds CCL1 and CCL8. Accordingly, Brix et al. took advantage of the availability of genetically deficient mice to study the influence of CCR8 in nephrotic nephritis (NTN)—a model for crescentic GN caused by T cell–mediated (Th1 and Th17) immunity in mice.

Brix et al. first established that expressions of the two CCR8 ligands, CCL8 and CCL1, were both increased 50- to 100-fold as injury developed and that CCR8 expression was modestly increased as well. Brix et al. then induced NTN in CCR8-deficient mice and showed that injury was less severe as judged by, functionally, BUN or morphologically, crescent score and interstitial inflammation. The number of mononuclear phagocytes was specifically reduced, whereas T cell numbers were unchanged, showing that CCR8 was critical for recruiting (and possibly, activating) the infiltrating monocytes rather than the T cells responsible for the underlying immunopathology. However, the severity of injury was identical in wild-type and CCR8 deficient mice, into which normal monocytes had been adoptively transferred. However, this result must be regarded as tentative, because Brix et al. did not have enough CCR8 deficient mice to be able to perform the control experiment to confirm that adoptive transfer of CCR8-deficient monocytes was ineffective—more work needs to be done here.

These results establish a pathogenic role for CCR8 and presumably, its ligands in NTN. Extrapolation from preclinical models to humans always carries dangers, but specific caveats must be made in this case. First, NTN is a highly reproducible model of crescentic nephritis but not an analog of ANCA-associated disease, and it will be important to confirm the results in one of the models of FGN induced by autoimmunity to MPO. Second, the studies tested the effects of CCR8 deficiency and not those of its ligands (murine CCL1 and CCL8) or the human equivalent (CCL18). Third, in humans, CCL18 has been reported to have inhibitory effects beyond its interaction with CCR8 (for example, by directly and indirectly inhibiting other chemokines from binding to their cognate receptors); potentially, these could attenuate the inflammatory effects of CCL18 in the human kidney.

None of these should detract from the value of the new information provided by Brix et al. They clearly document the involvement of CCL18 in ANCA–associated crescentic GN for the first time and provide the best evidence yet that CCR8 ligands, such as CCL18, have a pathogenic rather than a protective role in Th1/Th17–mediated inflammatory diseases. Like all good studies, the data raise many new questions: one obvious one would be whether CCL18 expression was equally raised in the granulomatous and nongranulomatous forms of ANCA-associated vasculitis, because it has recently been shown to be dichotomously expressed in the tuberculous (granulomatous) and lepromatous (nongranulomatous) forms of leprosy. Additional studies will be needed to determine whether circulating CCL18 has sufficient predictive power to be a clinically useful biomarker.

DISCLOSURES
None.

REFERENCES


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Myeloid Cell HO-ming in AKI

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Heme oxygenase 1 (HO-1) is a readily inducible enzyme that converts highly reactive free heme molecules into carbon monoxide, iron, and biliverdin. Through this action, HO-1 influences reactive intermediate production, modulates the immune system, and affects cell survival in multiple patho- genic situations. In humans, HO-1 deficiency is associated with severe hemolysis, dysregulated inflammation, renal abnormalities, and early death. After stress or pharmacologic manipulation, HO-1 is upregulated in numerous tissues including the renal vasculature, tubular epithelial cells, and renal interstitial...