Chapter

117

New Insights in Glomerulonephritis

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Unexpectedly, understanding of the processes of glomerular damage are turning out to have much wider public health implications than previously thought. The aim of this paper is to introduce some new simplifications and new understandings, and plant thoughts about how this knowledge may affect future medical practice.

THE GLOMERULUS

A glomerulus is a bundle of highly specialized capillary loops, in which three major cell types participate. The glomerular basement membrane (GBM) contains some unique or nearly-unique components, in that several standard basement membrane molecules have glomerulus-specific isoforms. For example type IV collagen, which is in most tissues comprised of $\alpha 1$ and $\alpha 2$ chains, is in the GBM made up of $\alpha 3$, $\alpha 4$ and $\alpha 5$ chains. Similarly laminin and proteoglycan molecules in the GBM are tissue-specific. But these differences in composition probably do not lead directly or alone to the unique properties of the glomerular filter. In fact the fibrillar network structure of the GBM seems to be quite open even to molecules as large as antibodies. Anti-GBM antibodies in the circulation can bind to GBM antigens, and antibodies can penetrate as far as the podocytes on the outer surface of the glomerular capillary.

ENDOTHELIAL CELLS

Glomerular endothelial cells are specialized microvascular endothelial cells. They are very thinly smeared over the inside of the GBM and fenestrated, i.e they have windows, through which circulating molecules have fairly free access to the GBM itself. Interestingly the formation of these pores seems to be dependent on the production of vascular endothelial growth factor (VEGF) by glomerular epithelial cells on the outside of this unit. One condition where VEGF signalling appears to be deranged is in pre-eclampsia. Endothelial cells are damaged in the microangiopathies such as HUS, TTP, and may be the direct target in small vessel vasculitis.

THE PODOCYTE IS A KEY CELL

Podocyte is the name given to the highly specialised epithelial cells that interdigitate as they wrap around the outside of capillary loops. They form a characteristic structure just adjacent to the glomerular basement membrane known as the slit diaphragm. There is now substantial evidence that this is the final molecular size barrier. It is made up of filaments of a protein Nephrin, the fingers of which interlock like the teeth of a zip. In the autosomal recessive disease Finnish Congenital Nephrotic Syndrome, deficiency of this protein leads to such a severe nephrotic syndrome that it is sometimes treated by bilateral nephrectomy. It is striking that almost all the known causes of congenital or infantile nephrotic syndrome are conditions affecting the podocyte. Only one of these conditions concerns a basement membrane protein.

Podocytes are important for another reason. They seem to have limited ability to divide and replace themselves, and so if they die, they may leave an area of GBM exposed, causing it to adhere to Bowman's capsule or the epithelial cells covering it, and this may progress from an adhesion to a segmental scar in a process beautifully illustrated by Wilhelm Kriz.

It follows that things that damage podocytes seem likely to cause proteinuria, and things that damage them

severely will lead to segmental scars, the disease we call focal segmental glomerulosclerosis, FSGS. Is this the case? In animal models the drug puromycin, and in humans the bisphosphonate pamidronate when used in high doses, cause proteinuria, FSGS and renal failure. Other mechanisms for causing more definitely specific injury to podocytes give similar results, supporting that hypothesis.

MESANGIAL CELLS

Mesangial cells sit in the middle of the glomerulus and have some properties of muscle cells, they are contractile cells that seem to hold the capillary loops together, and some properties that make them seem somewhat macrophage-like. Increased mesangial matrix deposition is a feature of many diseases. Because they are the easiest cells to grow and study in tissue culture systems, these cells have received a possibly disproportionate amount of attention in the last 20 years, so we may be over-neglecting them now.

THE GLOMERULUS AS A UNIT

Histology freezes cells in a moment and we tend to imagine them as stationary bodies. It is likely that the glomerulus is much more dynamic. This would help to explain a number of puzzles, but one is, if the slit diaphragm is the final barrier to large molecules, why doesn't it clog? It may be helped by some retardation of large molecules as they approach the slit diaphragm, through the mucopolysaccharide overlying the endothelial cell surface and filling the fenestrae, or through the substance of the GBM itself, or both. But clearly these permit large amounts of albumin to pass when there is proteinuria. This static structure cannot explain the properties of the filter.

Another series of observations show that this is not simply a static seive. Injecting lipopolysaccharide into mice leads to an immediate increase in glomerular permeability to albumin, and it has been suggested (without much evidence) that this might be a physiological response to rid the body of toxic mediators. But before and since then a proliferation of other influences on this barrier have been characterized, and it is clear that it is under the control of a number of influences. These include molecules that are targets of drugs that we already know to be successful, mentioned later.

FEATURES OF GLOMERULAR INJURY

Hematuria requires breaks in the glomerular basement membrane. This can be caused by a structurally abnormal basement membrane, as in the inherited type IV collagen disorder Alport syndrome. More commonly though, it is caused by destructive damage to the GBM caused by inflammation. These diseases are characterized by infiltration by cells of the immune system and by attempts to heal the inflammatory injury proliferation of glomerular cells.

Proteinuria requires damaged or unhappy podocytes. This can be produced in two ways. First, immune attack on podocytes, or podocyte toxins, will cause proteinuria and pure nephrotic syndrome without inflammation. Second, the barrier to protein requires preservation of the very complex architecture of these structures. Damage by scarring after inflammation, or by depostion of extra material in the glomerulus as in amyloidosis or diabetes, will cause proteinuria. Inflammation may also cause direct damage to podocytes.

Glomerular injury can therefore be seen as a spectrum that has at one end the podocyte-damaging and architecture-altering diseases that are non-inflammatory, and at the other end the inflammatory diseases that cause breaks in the GBM. Note that all these latter diseases are likely to show some proteinuria, though in the most acute ones, it may be relatively little until scarring has developed. All the glomerular diseases can be positioned somewhere along this spectrum.

THE CAUSES OF GLOMERULAR INJURY

Besides the deposition and architecture-altering diseases already mentioned, most glomerulonephritis is autoimmune. The evidence for this comes from direct evidence of immune involvement such as deposition of antibodies, from investigations in model systems attempting to replicate and explain human disease, and from observing the effects of treatment.

There is space to discuss only very briefly three rather remarkable examples of glomerular disease where there has been real progress in understanding initiation, pathogenesis, or possible treatment.

Membranous nephropathy is one of the common causes of nephrotic syndrome in adults, and in the two thirds of cases where it is not secondary to drugs or infection it is a difficult disease with a significant rate of progression to ESRD. The course can sometimes be modified by aggressive immunosuppression, at some risk. it has long been suspected from animal experiments that podocyte damage is caused by an autoantibody developing to a cell surface molecule Neutral Endopeptidase on the podocyte. In rats, autoimmunity to the protein Megalin reproduces almost all of the features of

human membranous nephropathy, but humans do not express this protein on their podocytes. Pierre Ronco from Paris recently identified a remarkable series of cases in neonates that helped to confirm that the animal evidence is right. Mothers who lack a specific podocyte surface molecule are apparently normal. If they become sensitised to this protein through childbirth they may generate an antibody response just as in Rhesus incompatibility. It crosses the placenta and fully reproduces the findings of membranous nephropathy in their infants. As antibody levels fall after birth, the kidneys may slowly recover, depending on the severity of the damage. This does not appear to be the specific target in idiopathic membranous, but clearly narrows down the search.

Anti-GBM disease is an example from the very opposite end of the spectrum where very rapidly progressive destructive disease damages glomeruli. Antibody is central to this disease too, but acts in concert with cell-mediated immunity. Here the target is one of the GBM-specific type IV collagen chains. As in membranous nephropathy, there is a remarkable human model in Alport post-transplant anti-GBM disease, when a small minority of Alport patients who are transplanted may develop immunity to the GBM antigen in their transplanted kidney that they themselves lack. Recently the molecular details of how tolerance to this autoantigen may be broken in humans, and how it may be prevented or treated in animals, have been demonstrated, but these are the subjects of a paper on their own. It means that we are in an extraordinarily privileged position of being able to investigate the detailed mechanisms of initiation of an autoimmune process in man. That should help us to understand how to treat or prevent it in other contexts.

Alport syndrome, mentioned above, is an inherited condition in which basement membrane structure deteriorates with time and leads to glomerulosclerosis and renal failure, because of absence of a tissue-specific type IV collagen isoform (usually a5 which is X-linked, occasionally a3 or a4). It also causes deafness because of a similar degeneration of the cochlear basement membrane, and it is the second most common inherited cause of renal failure after polycystic kidney disease. In 2006 two groups reported that in a mouse model of this disease, bone marrow transplantation from a normal donor could reduce the severity of the renal disease. Donor cells in the location of podocytes appeared to be synthesizing the protein that the animal's own kidney cells lacked. This is a surprizing result, as above it was mentioned that there is evidence that podocytes have limited ability to be replaced. Could they be replaced or repaired by stem cells? In view of the centrality of podocytes to a number of renal diseases, this could be more important than simply to Alport syndrome.

LONG-TERM CONSEQUENCES OF GLOMERULAR DAMAGE

Finally the area that is probably the most important for our health and wealth.

It is a common observation that a patient with glomerular disease who is acutely saved from death or dialysis by excellent medical care, or good luck, experiences a long term slow decline in kidney function that may in some lead to later development of end stage renal disease. A huge body of evidence has led to the conclusions which are summarised in the next two paragraphs.

The risk of deterioration is higher the greater the original damage. Hypertension is associated with progression, and proteinuria has an even stronger impact. There appears to be a genetic and probably racial impact on the rate of deterioration too, although the nature of the genes or factors involved are not yet understood.

Treatment of hypertension is beneficial in patients with proteinuria. Use of angiotensin converting enzyme inhibitors offers benefits above those of other drugs that achieve the same blood pressure lowering effect, and can achieve remarkable stabilization of renal function in a proportion of patients, as has been shown for diabetes.

WHY IS ESRD INCREASINGLY COMMON?

The explosion of treatments for end stage renal disease in the developed world probably has two or three strands to it. The first is that people are living longer, so the long-term impact of renal injury early in life has longer to manifest itself. The second is probably atherosclerosis. The third is diabetes, though we may be seeing just the beginning of this.

THE CARDIOVASCULAR IMPLICATIONS OF RENAL DAMAGE

In all these groups, the predictors of progression listed above remain true, but renal services have been saved from submergence by the remarkable observation that proteinuria is very strongly associated with cardiovascular death. The risk is increased further if glomerular filtration rate is reduced. Population studies

show that only a small minority of patients with renal impairment develop end stage renal disease; most die of other causes, particularly cardiovascular disease. Not everyone with a raised creatinine could or should be seen by a nephrologist – only if it's deteriorating, or associated with substantial proteinuria, as these are the patients who may get end stage renal disease. The others will usually die first unless they stop smoking, take exercise, and attend to other cardiovascular risk factors. They should have good blood pressure control and probably take an ACE inhibitor.

HOW DO ACE-INHIBITORS WORK?

The standard explanation is that they reduce glomerular hypertension by lowering efferent arteriolar vasoconstriction. This helps to explain why they drop GFR in patients with renal artery stenosis, and predispose to acute renal failure in hypovolaemia or shock, but it is not enough to explain the complete disappearance of proteinuria in many patients receiving these drugs. The answer must lie in the podocyte. This has angiotensin receptors on it, but it also has many other receptors; as described above, it is a dynamic and responsive cell. Other drugs that may influence its control over protein filtration include the endothelin receptor antagonists, which are now undergoing clinical trials. But there are other targets and there will be more drugs aiming to achieve the benefits of ACE inhibitors, but better, or without hyperkalemia.

WHY IS PROTEINURIA ASSOCIATED WITH MORTALITY?

We don't know why this glomerular problem is so strongly associated with cardiovascular mortality. We do not know how direct the association is, or what the usual mechanism of proteinuria is, except that it is rarely active glomerulonephritis. Nor do we know whether treating the cardiovascular risk in these patients will lighten or worsen the epidemic of end stage renal disease. The full implications of glomerular damage are only beginning to get through to the medical community.