Immune and Inflammatory Glomerular Injury

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It is now over a century since the first animal model of glomerulonephritis was established by Lindemann [1] who injected rabbits with heterologous antiserum to rabbit kidney raised in guinea pigs. In recent years there has been increasing opposition to the use of animals for research with an escalation in damage to property and violent attacks on researchers by those opposed to such research. This has been a major problem in the UK and is an increasing problem in the US [2]. There is therefore a need for scientists who carry out animal research to demonstrate that the inevitable discomfort and harm which is suffered by experimental animals is reduced as far as possible by good experimental design and, most importantly, is justified by the benefits it brings in understanding and treating human disease. In this talk I will discuss models that have been used in studying immune and inflammatory glomerular injury and ask;

1. How representative they are of human disease
2. What are the key insights they have given into pathogenesis
3. How has this been translated into treatment
4. Are we in danger of being misled by reliance on animal models

Although other animals such as rabbits and sheep have been studied most experiments on glomerulonephritis have been performed in rats or mice. There is a clear advantage to performing experiments in mice because of the availability of gene targeted mice and therefore there is a need for good models of human disease that can be used in mice.
I will discuss the following models which I think are most useful in understanding human glomerular disease:

**Nephrotoxic nephritis**

This model is induced by the injection of a heterologous antibody raised against a preparation of glomerular antigen. Classically there is acute injury produced by binding of the heterologous antibody to the glomerular basement membrane followed by a second phase of injury in which there is an autologous immune response to the heterologous antibody which then acts as a planted antigen. This second phase can be accelerated by pre-immunising the animals with heterologous immunoglobulin. Typically the autologous phase is characterised by proliferative glomerulonephritis with crescent formation in susceptible animals. The heterologous phase closely resembles human anti-GBM disease but the autologous phase has more in common with an immune complex glomerulonephritis with the heterologous antibody acting as a planted antigen. Proliferative immune complex glomerulonephritis can also be produced by antigens planted by other mechanisms, for example, on account of their charge [3]

**Heymann nephritis**

This is a model of membranous glomerulonephritis originally induced in rats by immunisation with a suspension of renal cortex and adjuvant. Subsequently a passive form of the model was induced by the injection of heterologous antibody raised against tubular brush border antigens. It is now known that the model depends on a cross reaction of these antibodies with antigens including megalin on podocyte foot processes.

**Models of lupus nephritis**
There are several models of SLE in mice which are characterised by the generation of autoantibodies and the development of glomerulonephritis with immune complex deposition. In my opinion the model that most closely resembles human disease is that produced by crossing NZB and NZW mice but other models include the MRL/lpr mouse and the BXSB mouse. These models have been widely studied and have provided insights into genetic susceptibility to SLE, mechanisms by which tolerance is broken and have been used for elucidating the role of mediators of glomerular injury both by examining gene targeted animals and by administration of pharmacological agents.

**Models of ANCA-mediated glomerulonephritis**

A major step forward in our ability to model human glomerular disease was the demonstration by Jennette and co-workers that transfer to normal mice of splenocytes or serum from myeloperoxidase (MPO) deficient mice immunized with MPO led to a pauci-immune necrotising glomerulonephritis [4]. This was the first demonstration that anti-MPO antibodies were pathogenic and has led to further insights into the mechanisms of injury in ANCA-mediated glomerular inflammation.

**Dense deposit disease and thrombotic microangiopathy**

Pigs with a spontaneous deficiency of factor H and mice in which the factor H gene has been knocked out develop persistent activation of the alternative pathway of complement activation and a glomerulonephritis resembling human dense deposit disease [5]. If the Factor H deficient mice are then made transgenic for a truncated factor H protein they develop spontaneous thrombotic microangiopathy (Pickering et al submitted)

The following is a very selective discussion of what I consider the major insights that these models have given us into pathological mechanisms;
Neutrophils and macrophages

It is clear that these cells play a major role in causing inflammatory glomerular damage leading to proteinuria and also to capillary wall rupture with crescent formation. Neutrophils are the key cell in heterologous nephrotoxic nephritis and macrophages are of central importance in the autologous phase. Macrophage depletion greatly attenuates injury in these models [6;7]. In anti-MPO induced glomerulonephritis in mice neutrophil depletion ameliorates disease [8] and the anti-MPO response has been shown to be directed against MPO on neutrophils and macrophages [9]. Macrophages in proliferative glomerulonephritis have an activated phenotype [3] and there is interesting work suggesting that they become programmed when they enter the glomerulus [10]. It is likely that the balance between different activation states in macrophages may determine whether there is progression or resolution of glomerular injury. Understanding the signals that attract and activate macrophages is of major importance.

Fc receptors

Fc receptors on circulating leukocytes play a central role in the induction of glomerular injury in models of nephrotoxic nephritis [11;12] and lupus nephritis [13]. The class of immunoglobulin deposited in glomeruli affects which Fc receptors are activated and determines severity of inflammation [14] and interaction between activatory and inhibitory Fc receptors also influences the inflammatory response [15].

Complement

Perhaps surprisingly, complement activation does not appear to play a major role in immune complex mediated crescentic nephritis nor in lupus nephritis [16] although there is evidence that this is in part because of the opposing effects of the classical pathway in facilitating removal of immune complexes and the alternative pathway in
promoting inflammation. Complement is of central importance in the Heymann nephritis model of membranous gn and the alternative pathway has been shown to be critical in a mouse model of anti-MPO glomerulonephritis [17]. Alternative pathway activation has also been shown to be central in mouse models of dense deposit disease [18] and HUS. Antibodies directed at C5 have led to amelioration in models of lupus nephritis and dense deposit disease.

T cells

There is good evidence that as well as being important in controlling antibody synthesis T cells may have a direct effector role in glomerular injury in nephrotoxic nephritis in both mouse [19] and rat [20]. It is a challenge to define how important this mechanism may be in humans.

Genetics

Animal models have provided insights into genetic susceptibility to glomerular disease in models of lupus nephritis in the mouse [21] and in a model of crescentic glomerulonephritis in the rat [22]. In the latter model susceptibility was associated with a variation in copy number of the Fcgr3 gene and we then showed that there was also variation in copy number of the human FCGR3B gene that was associated with susceptibility to lupus nephritis.

This represents a very selective look at the insights that animal models have given into pathogenesis of glomerulonephritis and I believe that the examples I have chosen are likely to be of strong relevance to human disease. It is reasonable, therefore, to ask whether these insights into pathogenesis have led to advances in therapy for human glomerulonephritis. Disappointingly, I think there is very little in terms of treatment that has followed from work on these models. One exception is in the area of
complement inhibition. However, I think it is possible to speculate on where advances might come in future. I think promising areas for development of treatment include: inhibition of Fc receptor activation, modulation of macrophage activation, inhibition of complement, particularly the alternative pathway and C5 activation; inhibition of chemokines. It is also important to consider ways of targeting therapy specifically to the inflamed glomerulus and interesting strategies include the use of genetically modified macrophages [23] or of cytokines which are designed only to be activated at sites of inflammation [24].

It is inevitable that there will be differences between the way experimental animals respond to glomerular injury and the way humans do that may lead to misleading conclusions from animal experiments and, of course, this is one of the arguments that those opposed to animal experimentation rely on. However, there is one specific example of how animal experiments may be misleading that I would like to explore and this is not due to differences between species but to a failure to use appropriate controls. In 1999 we published data on a mouse in which serum amyloid P component (SAP) had been genetically deleted and which developed autoimmunity [25]. We concluded that SAP protected against autoimmunity and this has been widely quoted. However, the knock out had been created using embryonic stem cells derived from the 129 strain of mouse which were then transferred to C57BL/6 mice. This means that even when the knock out mice were subsequently back crossed to the C57BL/6 strain the genetic material around the SAP locus on chromosome 1 was of 129 origin and we subsequently showed that producing a congenic mouse with a 129 segment of chromosome 1 on a C57BL/6 background led to similar autoimmunity even when SAP was present [26]. This shows the need for tremendous caution when interpreting results with genetically manipulated animals.
In conclusion, there are a number of reproducible animals models of glomerulonephritis which, I believe, have led to insights into pathogenesis that are highly likely to be of relevance to human disease. However, it is disappointing that there is very little to show for all the work that has been done on these models in terms of rational development of new therapies.

Reference List


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