Nephrotoxic nephritis and glomerulonephritis: animal model versus human disease

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ABSTRACT

Glomerulonephritis (GN) encompasses a range of immune-mediated disorders that cause inflammation within the glomerulus of the kidney. The pathogenesis of GN is complex. Intricacy arises from factors such as autoimmunity, cancer and structural abnormalities within the kidney. Studies using animal models have highlighted crucial interaction between inflammatory cells and cells intrinsic to the kidney, both of which are fundamental to the pathogenesis of GN. This review aims to provide insight on a 'suitable' model for nephrotoxic nephritis and glomerulonephritis (NTN GN) and relate its experimental validity to humans. The BALB/c NTN murine model and Wistar Kyoto (WKY) rat have held experimental validity in the study of GN in humans. The chemokine receptor CXCR3 also mediates renal T-cell recruitment and subsequent tissue injury in NTN. It is noteworthy to consider CXCR3 blockade in Th1-mediated renal inflammation as future therapeutic options for patients with GN and subsets thereof. Currently used immunosuppressive therapies for GN are not always uniformly effective and are frequently associated with serious side-effects. Corticosteroids are effective in several types of GN owing to their ability to inhibit the pro-inflammatory effects of cytokines known to promote glomerular inflammation. Differences between experimental and human GN complicate translation of experimental therapies into practice. More research is required to translate animal model research into a better comprehension of human GN disease. However, the complexity of GN research makes findings a challenge to replicate.

KEY WORDS: Chemokines.
Chemokine CCL2.
Glomerulonephritis.
Macrophage colony-stimulating factor.
Nephrotoxic nephritis.

Introduction

Research with animal models has shown the crucial interaction between inflammatory cells and cells intrinsic to the kidney that is both fundamental and unique to the pathogenesis of glomerulonephritis (GN).[1] The term glomerulonephritis encompasses a range of immune-mediated disorders that cause inflammation within the glomerulus and other compartments of the kidney. The mechanisms of interaction between these cells and the mediators of their coordinated response to inflammation are being elucidated. Despite these pathophysiological advances, treatments for GN remain non-specific, hazardous and only partly successful. Therefore, GN remains a common cause of chronic kidney disease (CKD) worldwide.

Animal model approaches offer effective and safer treatments for the future.[1] Evidence of complement deposition within the glomerulus is a key feature of many forms of GN, and a role of complement is implied by several observations in human disease. Indirect evidence obtained from human studies has been supported by findings in animal models.[1] The mechanisms by which macrophages mediate renal injury remains to be confirmed, but it is likely to include promotion of inflammation via release of pro-inflammatory cytokines such as tumour necrosis factor (TNF), recruitment of macrophages and other leucocytes via release of chemotactic molecules such as monocyte chemotactic protein-1 (MCP-1), cell proliferation by release of growth factors such as macrophage colony-stimulating factor (MCSF), cell death via the release of reactive oxygen species including nitric oxide, and control of fibrosis and repair via release of various enzymes.[1,2]

Epidemiology

Many cases of GN result in mild, asymptomatic illness that is not recognised by the patient, and remains undiagnosed. The incidence and prevalence of such mild episodes of GN are unknown but could be substantial. In the UK, the most common recorded renal diagnosis is GN (biopsy proven/not biopsy proven; 19%), followed by uncertain (18%). For all ages, the prevalence rate in men exceeds that in women, peaking in the 75–79 age group at 2918 per million population in males, and for females the peak is in the 65–69 age group at 1460 per million population.[2]

Population-based screening studies have shown that evidence of kidney damage by proteinuria, haematuria, low calculated glomerular filtration rate (GFR), or a combination of these features is present in 16% of adults in Australia,[1] and a similar proportion in the USA. Accurate classification requires a histological diagnosis; consequently, the reported prevalence might vary according to local indications for renal biopsy.

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A study of all renal biopsies performed in the Australian state of Victoria during 1995 and 1997 found that 21.5 people per 100,000 population per year underwent renal biopsy, yielding an annual incidence of biopsy-proven GN of 12.3 per 100 000. The most commonly diagnosed types of GN in adults were IgA nephropathy, focal and segmental glomerulosclerosis, and vasculitis; those most commonly diagnosed in children were minimal change disease, focal and segmental glomerulosclerosis, lupus nephritis and IgA nephropathy.[2]

Pathogenesis

The pathogenesis of GN is complex. Intricacy arises from factors such as autoimmunity, cancer, and structural abnormalities within the kidney. There is variability in the susceptibility to GN, which is likely to have a genetic basis; there are also complex interactions between soluble factors (including antibodies, complement, chemokines, cytokines and growth factors) and cells (both leucocytes and intrinsic kidney cells) that mediate glomerular inflammation. The contribution of non-specific factors such as hypertension and proteinuria promote continuing kidney damage, despite resolution of the initial insult.[3,4]

A constant feature of GN is the presence of inflammatory leucocytes within the glomerular and the interstitial compartments of the kidney. Macrophages and T lymphocytes are the dominant cell types present, and the intensity of cellular accumulation, particularly within the interstitium rather than the glomerulus, has repeatedly been found to correlate with the clinical and pathological severity of disease in animal models and in humans.

Within the kidney, T cells and macrophages undergo activation, which is triggered by interactions between leucocytes and intrinsic renal cells, by interactions between cells, matrix and by cytokines and other soluble factors present within the inflammatory environment. Type III hypersensitivity occurs when antigens and antibodies are present in roughly equal amounts, causing extensive crosslinking. It is characterised by soluble antigens that are not bound to cell surfaces (in the case of type II hypersensitivity); when these antigens bind antibodies, large immune complexes form that cannot be cleared. The reaction can take hours, days or even weeks to develop. Type III hypersensitivity represents the progressive forms of GN.[3,4]

Animal models

Animal model are used during the research and investigation of human diseases. The animal selected will usually meet a determined taxonomic equivalency to humans, in order to react to disease or its treatment in a way that resembles human physiology. Research relating to NTN GN can be traced back to the 1960s and late-1970s when observations were reported on the role of complement in NTN produced in the rat by either rabbit or duck nephrotoxic sera (NTS). In addition, the course of complement reactions in the glomerulus in NTN is used as a measure of the course of immunological events in GN. These observations gave the first indication of the duration of immunological reactions in the chronic form of this nephritis. Finally, the differences among the roles played by complement in NTN produced by various rabbit and duck NTS suggest the possibility of different mechanisms of mediation of injury caused by these sera.[5–13]

Matrix metalloproteinase 9 (MMP-9) is a conditionally expressed enzyme and is unregulated in GN.[14] This research showed that leucocyte-derived MMP-9 mediates the recruitment of pro-inflammatory macrophages into the kidney during experimental CRGN. In this work, the induction of NTN in wild-type mice resulted in up-regulation of MMP-9, followed by leucocyte infiltration, albuminuria and subsequent GN.[14]

Animal models of GN closely replicate features of human GN and have provided insights into the disease.[14] The reproducibility and uniformity of such animal models has also permitted a step-by-step dissection of many of the mechanisms involved. Nephrotoxic nephritis is produced experimentally by the intravenous injection of antibody (Ab) directed against glomerular basement membrane (GBM) antigens of the recipient species. Most of the accumulated inflammatory cells in many forms of human and experimental GN are macrophages.

BALB/c murine models have been helpful in understanding pathogenesis in a number of renal diseases.[14–17] In retrospective research, six-week-old female BALB/c murine models were induced with injections of 50 μg (0.2 mL) bacterial lipopolysaccharides (LPS) from *Salmonella minnesota* Re 595 twice a week for five-weeks by the intraperitoneal route. In all murine models given injections, features of polyclonal B-cell activation (PBA) and GN developed. This animal model presents features that are often encountered in human glomerulonephritis, particularly those associated with bacterial infection, as well as autoimmune diseases such as systemic lupus erythematosus (SLE).[14–17]

In contrast to commonly used animal models of immune complex-mediated NTN GN, BALB/c murine models have been highly reproducible and require only short-term injections of LPS. This BALB/c NTN murine model still holds experimental validity in the study of GN in humans. In addition, the BALB/c model can still be helpful in the investigation of the GN autoimmune phenomena. The reproducibility and uniformity of such animal models has permitted a step-by-step dissection of many of the mechanisms involved.[18,19]

The WKY rat strain is also highly susceptible to experimental models of crescentic glomerulonephritis (CRGN), including NTN and experimental autoimmune glomerulonephritis (EAG). These rats show development of NTN phenotypes, including glomerular crescents. More recently, CRGN has been induced on a normally resistant rat genetic background and identifies Lewis strain on two genetic loci (*Crgn1* and *Crgn2*) as a new, potentially valuable model for NTN GN.[20] The nephrotoxic serum nephritis (NTS) WKY rat has also been used in research.[21] Macrophages and T lymphocytes are the dominant cell types present, and the intensity of cellular accumulation, particularly within the interstitium rather than the glomerulus, has been found to correlate with the clinical and pathological severity of disease in animal models and in humans.[22]

Role of chemokines

When glomeruli are damaged by inflammation, growth factors are filtered into the urine where they activate renal tubular cells, causing them to secrete chemokines and cytokines.[23] Mutual stimulation between infiltrating leucocytes (attracted by chemokines) and fibroblasts gives rise to glomerulosclerosis, progressive tubular damage and renal fibrosis. Behind this is the activity of chemokines and their receptors. One team has described i) an initiation phase during which injury to renal tubular cells induces leucocyte infiltration, ii) an amplification phase during which infiltrating leucocytes enhance local production of cytokines and chemokines, iii) a progression phase during which macrophages in the glomeruli stimulate mesangial cells to secrete fibronectin and collagen type IV, and when there are interstitial infiltrates, iv) a terminal phase of glomerulosclerosis, extensive tubular atrophy and diffuse kidney scarring and shrinkage.[24–27]

The migration of inflammatory cells into an extravascular site requires a series of coordinated signals including the generation of a chemotactic gradient by the cells of the extravascular compartment.[3] The nature of the stimulus and the subsequent spectrum of chemotactic factors produced determine the specific leucocyte population recruited to the inflammatory site. Members of the chemokine family play a central role in this process by attracting and stimulating specific subsets of leucocytes.

Increasing our understanding of the intracellular pathway that regulates chemokine production in human mesangial cells may provide leads to the design of more effective therapies for the prevention and treatment of glomerular inflammation.[3] Animals genetically deficient in chemokines are resistant to disease induction, although results have not been uniform, with an unexpected accentuation of experimental antiglomerular basement membrane disease seen in murine models deficient in CCR1, a receptor for RANTES and macrophage inflammatory protein-1. Chemokines are thus identified as important in murine models and are clearly active in human disease.[28–30]

Interestingly, one team has generated CXCR3-deficient murine models to investigate the functional role of CXCR3 in the kidney under pathological conditions.[22] Nephrotoxic nephritis was induced to analyse the molecular mechanisms of T-cell recruitment in GN by CXCR3 and its three ligands, IP-10/CXCL10, Mig/CXCL9 and I-TAC/CXCL11, and to identify potential new targets for therapeutic interventions. This research identified that the chemokine receptor CXCR3 mediates renal T-cell recruitment and subsequent tissue injury in NTN. This study provided a rationale for considering CXCR3 blockade in Th1-mediated renal inflammation as a future therapeutic option for patients with GN and GN subsets.[22]

Treatment

Therapeutic options for human GN applicable to all cases include symptomatic treatment and strategies to delay progression; immunosuppression is appropriate for selected cases; and renal replacement therapy (RRT) with haemodialysis or renal transplantation is needed for a sizeable minority of patients. Molecular therapies designed to target key mediators of damage hold great promise and are effective in several experimental settings but have yet to achieve translation into the clinic.

Regular clinical follow-up, blood-pressure control and the use of an angiotensin-converting enzyme (ACE) inhibitor in patients with proteinuria exceeding one gram daily are advantageous. Recent findings suggest that more complete blockade of the rennin-angiotensin system by use of an ACE inhibitor and an angiotensin receptor antagonist is superior to either agent alone for prevention of progressive CKD among patients with proteinuria.[1]

Treatment of hyperlipidaemia has slowed progression in experimental models of GN, but available trial data are insufficient for lipid lowering to be recommended specifically for renal protection. Currently used immunosuppressive therapies for human GN are not always uniformly effective and are frequently associated with serious side-effects. Corticosteroids are effective in several types of GN owing to their ability to inhibit the pro-inflammatory effects of cytokines known to promote glomerular inflammation, including interleukin-1β (IL-1β) and TNFα.

Conclusions

This review provides insight into a 'suitable' model for NTN GN and relate its experimental validity in humans. Animals with experimental NTN GN are homogeneous, whereas human patients with GN are heterogeneous. Experimental disease is initiated at a time determined by the researcher and runs a predictable course, thereby permitting intervention at defined stages, including before induction, during disease establishment, or late in the disease course. Patients typically have established disease before treatment can be given.

Renal damage tends to be progressive in the longer term, despite elimination of the original source of injury. Differences between experimental and human GN complicate translation of experimental therapies into practice. Stem cell-based therapies should be explored; these could include genetic modification to produce protective molecules within the kidney or to promote regeneration of damaged intrinsic kidney cells.

There is also a need for more up-to-date research on this topic and further work is required to translate animal model research in order to achieve a better understanding of GN disease in humans. The complexity of GN research makes findings a challenge to replicate consistently. Finally, there must be continuing evidence-based assessment of immune and non-immune therapies in order to provide rational care for patients. □

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