Pathogenesis of poststreptococcal glomerulonephritis a century after Clemens von Pirquet

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Considerable insight has been gained into the etiopathogenesis of poststreptococcal glomerulonephritis since the landmark theoretical construct of Clemens von Pirquet postulated that disease-causing immune complexes were responsible for the nephritis that followed scarlet fever. Over the years, molecular mimicry between streptococcal products and renal components, autoimmune reactivity and several streptococcal antigens have been extensively studied. Recent investigations assign a critical role to both *in situ* formation and deposition of circulating immune complexes that would trigger a variety of effector mechanisms. Glomerular plasmin-binding activity of streptococcal glyceraldehyde-3-phosphate-dehydrogenase may play a role in nephritogenicity and streptococcal pyrogenic exotoxin B and its zymogen precursor may be the long-sought nephritogenic antigen.

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It has been known for a long time that scarlatina nephritis appears as a rule in the third week. None of the hypothesis thus far advanced is able to account satisfactorily for the fact that nephritis occurs just at that time.

von Pirquet C. Allergy. *Arch Int Med* **7**:259–288, 382–436, 1911.

Acute poststreptococcal glomerulonephritis (APSGN) is an ancient and well-defined renal disease. Recent decades have seen a reduction in the incidence of the disease for reasons not entirely clear, but likely associated with earlier recognition and effective treatment of streptococcal infections. Nevertheless, epidemics and clusters of cases continue to appear in several regions of the world and sporadic cases of APSGN account for 21% (4.6–51.6%) of children admitted to the hospital with acute renal failure in developing countries.[1]

In the 18th century, it was recognized that 'edematous swelling with scanty, dark and at times totally suppressed urine' was a feared complication of the convalescent period of scarlet fever,[2] but it was Wells[3] who, in 1812 published a classic paper, actually delivered 6 years before to the Society for the Improvement of Medical and Chirurgical Knowledge, defining the limits of the latent period and the characteristics of the edema, giving evidence that the urine contained the 'red matter' as well as 'the serum of the blood' and emphasized that this complication occurred more frequently in siblings than in the general population. This communication preceded by more than 10 years the landmark clinicopathological contributions of Richard Bright,[4] which established the connection between dropsy and coagulable urine, and by more than six decades the finding of 'glomerulitis' in postscarlatinal nephritis[5] and the report of Reichel[6] that gave a clear description of the glomerular lesions in a fatal cases of the disease subsequently expanded on by Osman *et al.*[7]

Despite the fact that both the clinical features and the renal pathology of the disease were well known, the reasons for the association between this clearly non-infectious ('reactive') complication of an infectious and, at that time, epidemic disease remained elusive until the seminal work of

Clemens von Pirquet.[8] In 1903, von Pirquet, then a 29-year-old pediatrics resident, purely on the basis of clinical observations, postulated the existence of antibody-driven pathogenic, rather than beneficial immune reactions that he named allergy ('altered reactivity'). Interestingly, in an unusual method of claiming scientific priority for a concept, he outlined his theory in a sealed letter sent to the Academy of Sciences in Vienna that was only to be opened at his request. This was in fact done in 1908 when the letter was read in a session of the Academy.[9]

The term poststreptococcal glomerulonephritis became usage following the demonstration that the β-hemolytic streptococcus was the cause of scarlet fever, based on the experimental work of Dick and Dick,[10] the clinical work of Dochez and Sherman[11] and particularly reports in the first half of the 20th century that identified cases of glomerulonephritis following upper respiratory and skin infections owing to streptococci. Among such reports, the work of Little *et al.*,[12] who identified bacteriologic and or serological evidence of streptococcal infections in 109 of 116 consecutive cases of acute glomerulonephritis and the association of glomerulonephritis with pyodermitis and streptococcal wound infections reported by Futcher,[13] deserve specific mention.

The next important theoretical concept was that of 'nephritogenic' streptococcal strains. This notion was advanced initially by Seegal and Earle,[14] who noted that rheumatic fever and PSGN, both nonsuppurative complications of streptococcal infection, did not coexist in the same patient, differed in geographical location, in sex incidence (2:1 male:female predominance in PSGN), and propensity to healing (PSGN) rather than to relapsing attacks (rheumatic fever). Although recognizing that host differences 'may play a definite role' in explaining these contrasting characteristics, they championed a straightforward explanation, namely the existence of hemolytic streptococcal strains that caused rheumatic fever (hence rheumatogenic strains) and other strains that caused glomerulonephritis (nephritogenic strains). Subsequent investigations[15–18] were considered to validate this concept and laid the foundations for the search for nephritogenic antigens in group A streptococcal strains isolated from patients with nephritis. As reviewed elsewhere,[19] Rebecca Lancefield's M types 1, 2, 4, 12, 18, and 25 were strains with nephritogenic potential usually recovered from the upper respiratory tract whereas M types 49, 55, 57, and 60 were usually associated with impetigo-associated nephritis.[18] More recently, the production of a lipoproteinase that makes serum opaque (opacity factor) has been used to subdivide M proteins into class I (opacity factor negative) corresponding to serotypes that cause rheumatic fever and class II (opacity factor positive) that corresponds to serotypes that cause pyoderma and acute glomerulonephritis.[19] Although these associations were the foundation of much research, accumulated evidence (see below) has demonstrated that M protein is not the decisive factor in streptococcal nephritogenicity.

EXPERIMENTAL MODELS OF PSGN

Following the demonstration of the etiologic role of the group A streptococcus in induction of acute nephritis, many attempts were made to induce experimental glomerulonephritis in animals, including rabbits, rats, mice, and monkeys. The major difficulty is clear: group A streptococci are specific human pathogens. Injections of dead streptococci, toxic extracellular products, and streptococcal vaccines, deep and superficial infections produced by inoculations with live bacteria, and implantation of diffusion chambers have been tried by many authors in attempts to reproduce the characteristics of PSGN seen in humans.[20–24] In relation to experimental infection, Reed and Matheson[25] as well as Becker and Murphy[26] were able to induce albuminuria and hematuria, and occasionally hypertension and azotemia. Unfortunately, these careful and laborious investigations did not consistently produce glomerulonephritis and did not permit evaluation of putative nephritogenic antigens.

More recently, Nordstram *et al.*[27] explored streptococcal nephritogenicity in a series of elegant studies using steel cages and osmotic pumps implanted subcutaneously in rabbits and in mice. They found clinical and histological evidence of nephritis when the cages were filled with nephritis-associated bacterial isolates and subsequently implicated streptokinase as a nephritogenic factor,[28,29] these results are discussed later.

IMMUNE COMPLEX DISEASE AND COMPLEMENT ACTIVATION

Immune complexes represent the 'toxic bodies' proposed to be responsible for the symptoms by von Pirquet.[8] Although the identity of the nephritogenic antigen remained controversial, general agreement existed in the 1960s and 1970s with respect to the nephritogenic role of circulating immune complexes because of similarities between PSGN and the acute ('one-shot') serum sickness model.[30,31]

Glomerular trapping of immune complexes is facilitated by various factors. Among these, appropriate size (300–500 000 Da), antigen:antibody relationship (combining ratios near equivalence), type of antibody (class and affinity determine half-life in plasma and ability to activate complement), and the efficiency of the reticuloendothelial system in clearing the complexes are relevant.[30–32]

It was accepted that immune complexes of appropriate size[32] (300–500 000 Da), could deposit in the glomeruli, activate the complement system and local coagulation mechanisms, and induce glomerulonephritis. In acute serum sickness, as in PSGN, there is a full recovery of the renal lesions and a transient reduction in serum complement levels.[31,33] With respect to circulating immune complexes, as many as 2/3 of PSGN patients had serum antigen–antibody complexes,[34] but these were also present in controls and in patients with uncomplicated streptococcal infections.[35] Furthermore, there was no correlation between the presence or the amount of circulating immune complexes and the clinical or pathological characteristics of the disease,[36] so these findings lacked clinical significance.

The critical role played by an *in situ* immune reaction resulting from antibody meeting free antigen deposited in the glomeruli was suspected as early as 1976.[37] This possibility was emphasized by the difficulties of inducing glomerulonephritis and the near impossibility of inducing subepithelial immune deposits (humps), which represent the prototype lesion in PSGN,[38,39] with preformed immune complexes. Large amounts of preformed immune complexes may produce glomerulonephritis but this is leukocyte-mediated, and the localization of the immune deposits is largely subendothelial. The landmark experiments of Vogt *et al.*,[40] who showed that cationic antigens could be attracted to and effortlessly penetrate the negatively charged glomerular basement membrane to induce prominent subepithelial electron-dense deposits and severe glomerulonephritis, suggested that cationic streptococcal antigens might have a role in acute PSGN. Obviously, charge alone does not govern in immune complex deposition in the glomeruli, as highlighted by the fact that patients with vasculitis and myeloperoxidase (cationic):anti-myeloperoxidase immune complexes have a pauci-immune glomerulonephritis.

Complement activation is a central feature in APSGN and the alternate pathway is preferentially activated. New insights into the activation of complement have been gained by the demonstration that immunoglobulin (Ig)-binding proteins in the streptococcal surface bind C4BP (a C4b-binding protein), thereby interfering with the classical pathway of complement activation.[41,42] Relevant for a putative nephritogenic antigen (extracellular cysteine proteinase (streptococcal pyrogenic exotoxin B, SpeB)) that will be discussed later, Wei *et al.*[43] have recently shown that complement regulatory proteins (FH and FHL-1), used for immune evasion by *Streptococcus pyogenes*, are bacterial surface proteins that may be removed by SpeB, suggesting that this protease may modulate FH and FHL-1 recruitment during infection.

Additional information on complement activation in PSGN was provided by Ohsawa *et al.*[44] who found that the lectin pathway of complement activation may be activated in PSGN, probably by the recognition of glucosamine residues in the bacterial wall by the mannan-binding lectin-starter molecule; however, recent evidence shows that individuals deficient in mannan-binding lectin may still develop glomerulonephritis[45] and the participation of this pathway of complement activation in PSGN remains a matter of speculation.

CELLULAR IMMUNE MECHANISMS IN PSGN

The presence of immunocompetent cells in biopsies of PSGN was recognized more than two decades ago. Macrophages and T helper cells were shown to infiltrate the glomeruli in early biopsies.[46] Infiltration of mononuclear cells may be promoted by chemotactic factors of the complement system, but infiltration by immune cells is not correlated with complement deposition.

Overexpression of the intercellular adhesion molecule-1 and lymphocyte function-associated antigen-1 was seen in glomeruli and tubulointerstitial regions in APSGN,[47] and Rastaldi *et al.*[48] showed that the intensity of intraglomerular and tubulointerstitial intercellular adhesion molecule-1 staining correlated with the intensity of macrophage infiltration in glomeruli and the interstitium, respectively. Furthermore, the number of intraglomerular leukocytes correlated with proteinuria.

Increased circulating levels of IL-6, IL-8, tumor necrosis factor-α, and monocyte chemotactic protein-1 have been found in APSGN[49,50] and a correlation between proteinuria and urinary monocyte chemotactic protein-1 excretion has been demonstrated.[51]

From the evidence listed above, it can be concluded that infiltrating immune cells play a role in the development and severity of the inflammatory glomerular damage in PSGN. However, the lack of a suitable animal model makes it difficult to explore the relative importance of cell-mediated immune reactivity and the good prognosis of APSGN makes it unnecessary to consider the use of immunosuppressive drugs in uncomplicated cases of the disease.

STREPTOCOCCAL NEPHRITOGENICITY

We will now consider potential pathogenetic mechanisms in PSGN, including molecular mimicry between streptococcal fractions and renal structural constituents, autoimmune reactivity (in particular anti-IgG activity), plasminogen/plasmin binding by streptococcal surface proteins, and finally, glomerular immune complex formation involving streptococcal antigenic components (Table 1).

MOLECULAR MIMICRY

Several investigators have studied structural similarities between soluble fractions of streptococci and components of the human glomerulus as a possible cause of nephritogenicity. Kefalides *et al.*[52] reported antibodies to laminin,

Table 1 | Streptococcal nephritogenicity

Molecular mimicry
Cross-reactivity of streptococcal products with laminin, collagen, GBM etc.
Anti-Ig reactivity
Streptococcal neuraminidase
Streptococcal Ig-binding receptors
Streptococcal-related glomerular plasmin-binding activity
Streptokinase
zSpeB/SpeB
Enolase
NAPlr–GAPDH
Streptococcal nephritogenic antigens
M protein
Histone-like proteins
NAPlr–GAPDH
zSpeB/SpeB

GBM, glomerular basement membrane; GAPDH, glyceraldehyde-3-phosphate-dehydrogenase; Ig, immunoglobulin; NAPLr, nephritis associated plasmin receptor; SpeB, streptococcal pyrogenic exotoxin B; zSpeB, zymogen precursor of SpeB.

collagen, and other molecules in the sera of patients with PSGN. Several authors found shared antigenic determinants between M12 streptococcal protein and glomerular basement membrane,[53–55] vimentin,[56] and mesangial proteins,[57] and Kraus and Beachey[58] found M protein epitopes of potential renal autoimmune relevance.

However, there are little, if any, differences in the cross-reactivity patterns of streptococci with rheumatogenic potential compared with those with nephritogenic potential.[59] An extensive review of the experiments reporting cross-reactivity between mammalian tissues and streptococci by Christensen *et al.*[60] concluded that most of the preparations used likely contained streptococcal Ig receptors or tissue IgG Fc receptors or, in those studies where sera were involved, anti-Igs; furthermore, they emphasized the near impossibility to induce disease with injections of cross-reactive antigens.

AUTOIMMUNE REACTIVITY IN PSGN

The existence of autoimmune mechanisms triggered by streptococci capable of causing nephritis was championed by McIntosh *et al.*[23,61,63,64] in the early 1970s. They showed that cryoglobulins had a role in experimental nephritis induced by type 12 streptococcus,[23] that streptococcal neuraminidase could eliminate the sialic acid of IgG, and that such autologous desialized IgG was capable of inducing anti-IgG reactivity and glomerular lesions.[61] IgG-rheumatoid factor was subsequently demonstrated in the serum of 32–43% of patients with PSGN and IgM-rheumatoid factor in 15% of the patients,[33,62] particularly in the first week of the disease.[63] In addition, anti-Ig deposits were demonstrated in the glomeruli of 19 of 22 biopsies[64] and IgG with anti-IgG reactivity was eluted from the kidneys of a fatal case of PSGN.[65] More evidence of anti-IgG activity was found with the skin-window technique, which showed that patients with PSGN reacted to normal human IgG with a lymphocytic infiltrate similar to the recognition reaction observed in response to antigens to which the patient had had previous exposure.[66]

Two mechanisms have been advanced to explain the development of anti-IgG activity in PSGN: neuraminidase-induced desialization of Ig and IgG-binding proteins in the streptococcal wall. Although neuraminidase production was reported in streptococci of M types 1, 4, and 12,[67] there are conflicting reports on its frequency among isolates.[68–70] likely owing to loss of neuraminidase production by bacteria after repeated subculturing.[69]

Serum neuraminidase activity and free neuraminic (sialic) acid levels were found in PSGN patients by us[71] and others.[72] Additional evidence in favor of a nephritogenic role for neuraminidase was presented in experiments that showed that desialized leukocytes have an affinity for the glomeruli[73] and by the demonstration of glomerular-binding sites for *Arachys hypogea* (peanut agglutinin), presumably identifying free galactosamine radicals exposed by the loss of sialic acid from deposited Igs[74] and desialized leukocytes.[75] The association of APSGN and thrombotic microangiopathy in a patient suggested a role of neuraminidase in the combined clinical picture.[76] Despite this evidence, it should be noted that the capacity to produce neuraminidase is not a unique characteristic of nephritogenic streptococci; in fact, rheumatogenic streptococci also produce this enzyme.[68]

Anti-Ig production could also be the result of Ig binding to receptors in the streptococcal wall. Type II receptors in groups A, C, and G streptococci have been demonstrated by several authors.[77–79] These receptors bind avidly to the Fc fragment of IgG and anti-IgG antibodies are systematically produced by the injections of group A streptococci cultured in medium containing autologous serum.[80] Anti-IgG activity induced by streptococci with Ig-binding receptors is associated with enhanced tissue deposition of IgG and immune complexes in rabbits, causing inflammatory glomerular changes.[81,82] Streptococcal receptors with affinity for IgM have also been described.[83,84]

Ig-binding capacity by streptococcal components may have additional nephritogenic relevance. Protein H, a surface protein of *S. pyogenes* interacting with the constant Fc region of IgG, is known to be released from the streptococcal surface by cysteine proteinase (SpeB) produced by the bacteria. Berge *et al.*[85] have shown that addition of protein H to human serum produces complement activation with dose-dependent cleavage of C3. Protein H–IgG complexes released from the streptococcal surface may then be relevant not only as modulators of complement activation but also as inducers of anti-IgG reactivity. The latter suggests a link between a putative nephritogenic antigen (SpeB – see later) and anti-IgG reactivity in PSGN.

In addition to anti-IgG, other autoimmune reactivity has been found in patients with PSGN. DNA–anti-DNA complexes[86] and antineutrophil-cytoplasmic antibodies have also been detected. The latter are present in as many as 2/3 of the patients in whom there is azotemia and in 70% of the patients that develop crescentic glomerulonephritis after streptococcal infection.[87]

From the evidence listed above, it may be concluded that autoimmune reactivity and, particularly, anti-IgG antibodies in serum and in glomerular deposits are frequently present in PSGN. As these autoimmune phenomena do not define a specific clinical course of the disease, it is possible that they represent epiphenomena; however, it is not unreasonable to consider that in some patients, severe autoimmune reactivity may modulate the course of PSGN.

NEPHRITOGENIC STREPTOCOCCAL ANTIGENS

The lack of a suitable animal model for PSGN made it necessary to focus attention on selected streptococcal fractions and their potential to deposit in the glomeruli and cause injury. In parallel, studies were directed to detect these putative antigens in renal biopsies of patients with APSGN and the corresponding antibody response.

M PROTEINS

Several early investigations reported M proteins in human renal biopsies,[88–90] but the results were inconsistent.[91,92] The

discrepancies were attributed to impurities of the streptococcal fractions used to produce the antisera and to variation in the timing of the biopsy, but also free antigen unmasked by antibody is more likely to be present in early biopsies.[90,93] Experimentally, it could be shown that complexes of M protein and fibrinogen could localize in the glomerulus[94,95] and mild, self-limited renal lesions were induced by repeated injections of M protein, either alone or in combination with fibrinogen.[96]

In the following years, evidence against a primary role of M protein accumulated. First is the fact that recurrence of PSGN is extremely rare, if it occurs at all, which is compatible with the notion of ubiquitous antigen(s) conferring long-lasting immunity and conflicts with the mounting number of putative nephritogenic M-proteins types that do not confer lifetime immunity. In addition, Treser *et al.*[97] showed that convalescent sera, presumably containing antibody against the specific nephritogen, could recognize free antigenic sites in early biopsies, but this staining was not prevented by preabsorbing the sera with M protein. Interestingly, attempts to evaluate the specificity of IgG eluted from the kidney in a fatal case of PSGN did not show anti-M type streptococcal reactivity.[65] Finally, in recent years, it has been shown that not only group A streptococci have nephritogenic potential, as *Streptococcus zooepidemicus* (group C), known to be the cause of equine 'strangles (fever, mucopurulent nasal discharge, lymphadenitis, and submandibular abscesses) and mastitis in cows, has been responsible for recent epidemic outbreaks and clusters of cases of PSGN in different parts of the world.[98,99]

STREPTOKINASE

In 1979, Villarreal *et al.*[100] described a protein in nephritogenic streptococci that was present in glomerular deposits in most biopsies from patients with APSGN and was recognized by the majority of sera of patients with PSGN.[101] Subsequent experiments identified this protein as streptokinase[102] and, following this lead, Nordstrand *et al.*[27] using the mouse tissue-cage model induced glomerular lesions with streptokinase-producing streptococci and showed that deletion of the gene encoding a streptokinase variant associated with nephritis resulted in loss of nephritogenicity.[29] In addition, they identified streptokinase in the glomeruli of some infected mice by the immunogold silver-staining technique.[28]

However, production of this streptokinase variant was not invariably associated with nephritis in the mouse cage model[29] and anti-streptokinase antibody titers do not offer critical information on streptococcal infections associated with nephritis.[103,104] Furthermore, streptokinase alleles that were thought to be present mainly in nephritogenic strains are just as common in non-nephritogenic streptococci.[105] In subsequent experiments, the group that had originally proposed streptokinase as a nephritigenic antigen concluded that there was no unique reactivity to streptokinase in PSGN patients and that there were no streptokinase deposits in human renal biopsy material.[106] Therefore, streptokinase has been removed from the list of candidate nephritogenic antigens in PSGN. Nevertheless, streptokinases are proteins secreted by streptococci with the capacity to convert plasminogen to plasmin[107,108] and both Poon-King *et al.*[109] and Nordstrom *et al.*[27] suggested that binding of streptokinase could convert plasminogen to active plasmin, which could cause degradation of extracellular matrix proteins, activation of matrix metalloproteinases, and local activation of the complement and coagulation pathways. Plasmin-initiated tissue injury could assist deposition of, and promote further damage by immune complexes. This notion was subsequently embraced by Yoshizawa *et al.*[110,111] in their studies on the streptococcal glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) plasmin receptor (see below). This pathogenic mechanism may also be triggered by other streptococcal antigens with the capacity to activate and bind plasmin (see later). Interestingly, mouse plasminogen is not activated by streptokinase,[112] which would imply a different mechanism for the streptokinase-induced nephritis observed in the mouse cage model.

STREPTOCOCCAL HISTONE-LIKE PROTEINS

Bacterial histone-like proteins (HlpA) of streptococci may contribute to the virulence of infections by promoting monocytes/macrophages to synthesize and release proinflammatory cytokines.[113] HlpA are highly cationic and selective binding of HlpA to proteoglycans in the rabbit glomerular basement membrane has been reported,[114] and this binding might initiate *in situ* immune complex formation.[115] The potential nephritogenicity of HlpA has been suggested because it is released into the circulation by group A streptococci *in vivo* and it elicits an antibody response.[116] To our knowledge, determination of anti-HlpA antibodies in patients with poststreptococcal sequelae and streptococcal histone localization in the glomeruli of PSGN patients has not been reported, thus its role in PSGN is speculative.

NEPHRITIS-ASSOCIATED STREPTOCOCCAL PLASMIN RECEPTOR

Identification of nephritis-associated streptococcal plasmin receptor (NAPlr) as a putative nephritogen is the culmination of a long series of studies initiated in the 1960s by Treser, Lange, Yoshizawa.[97,110] The current focus of investigations is a plasmin-binding protein on the surface of nephritogenic streptococci.[117] These studies followed the identification of a fraction obtained in the supernatant of pressure-disrupted streptococci named endostreptosin[118] or preabsorbing antigen.[119] Early biopsies of PSGN presented sites that stain positive with fluorescein isothiocyanate-labeled Ig from convalescent sera presumably identifying free antigenic sites. The preabsorbing antigen was so-named because it was shown to preabsorb the staining capacity of convalescent sera. However, it was later shown that convalescent sera had anti-IgG reactivity[33,62] that could be responsible for the positive stainings found in PSGN biopsies. Repeated injections of

preabsorbing antigen in rabbits resulted in glomerular C3 staining and mild proliferative changes with minimal or no proteinuria or hematuria.[120] Recent reports deal with an NAPlr of 43 kDa, initially identified by Winram and Lottenberg,[121] which is a glycolytic enzyme with glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) activity.[113] Streptococcal plasminogen-binding proteins may facilitate bacterial invasion since, as mentioned earlier, surface-bound plasmin activates both metalloproteinases and collagenases, which can induce local inflammation.

Studies by Yoshizawa *et al.*[104] showed that NAPlr activated the alternate complement pathway, that high antibody titers to NAPlr (as determined by Western blot in comparison with normal controls) were present in 92% of PSGN patients and 60% of uncomplicated streptococcal infections remaining elevated for at least 10 years, and that renal NAPlr deposits were found in all renal biopsies of patients with APSGN taken in the first 14 days of the disease. In a follow-up investigation,[111] the same authors demonstrated prominent glomerular plasmin-like activity in patients who had APSGN and in whom glomerular NAPlr was positive, whereas it was absent or weak in patients who had APSGN and in whom glomerular NAPlr was negative. The distribution of glomerular plasmin-like activity was identical to that of NAPlr. Importantly, the distribution of the deposits of NAPlr did not coincide with the distribution of complement or IgG deposits[111] and therefore the authors postulated that nephritogenicity of NAPlr is related to its plasmin-binding capacity, which was likely to facilitate immune complex deposition.

Several questions remain to be answered in relation to these detailed investigations. First is the possibility of cross-reactivity with human GAPDH. This needs to be considered, because the staining of certain structures, particularly infiltrating leukocytes, in the biopsies presented by the authors,[104] is a regular feature in biopsies stained with anti-human GAPDH (unpublished data from our laboratories). In addition, a recent multicentric investigation revealed different results; as evaluated by both enzyme-linked immunosorbent assay and Western blot, anti-NAPlr antibodies were a rare occurrence and glomerular deposition of streptococcal NAPlr was infrequent in PSGN.[122] One possible reason for the contrasting observations in these studies is the different genetic background of the patients in the cited studies: the patients in the study of Batsford *et al.*[122] had many different genetic backgrounds, whereas the patients in the studies by Yoshizawa *et al.*[104] were drawn from a relatively homogeneous Japanese population.

Also unanswered is the relationship between plasmin-binding activity and nephritogenicity. It is intellectually appealing to assign a pathogenic role to the capacity to bind activated plasmin to the glomeruli and this mechanism could be operating in relation to several streptococcal fractions, in addition to NAPlr–GAPDH; for instance, streptokinase, SpeB, and enolase. In fact, the latter has the strongest plasmin-binding activity and a nephritogenic potential has been suggested for α-enolase.[123] Nevertheless, plasmin-related nephritogenicity requires the participation of immune complexes and the demonstration of the colocalization of the putative antigen and the complement and Ig is a dependable characteristic of such immune reactivity. The different sites of glomerular localization for NAPlr and C3 and Ig[104] would speak against its role as the nephritogenic antigen.

SpeB/zSpeB

SpeB is a cationic cysteine proteinase that belongs to the group of exotoxins (SpeA, SpeB, SpeC, and SpeD) produced by pyogenic streptococci. One of these 'erythrogenic' toxins[10] was found by Elliot in 1945[124] to be an active proteinase of 28 kDa generated by proteolysis following reduction of an extracellular zymogen precursor of ~40 kDa produced by group A streptococci. The proteinase (SpeB) and its precursor (zSpeB) were subsequently identified by Gerlach *et al.*[125] and the crystal structure and integrin-binding properties were defined by Kagawa *et al.*[126]

SpeB is present in all *S. pyogenes* isolates and is the predominant extracellular protein, accounting for more than 90% of the total secreted protein. Patients with infections with several M types seroconvert to SpeB, indicating that the molecule is made *in vivo* in the course of streptococcal infections.[127] SpeB is a plasmin-binding receptor protein[128,129] that is capable of degrading human fibronectin, activating a 66-kDa matrix metalloproteinase and of releasing active kinins.[130,131] zSPEB and SpeB are cationic with pKs of 8.2 and 9.3, respectively.

The possibility that SpeB/zSpeB could be a nephritogenic antigen was raised after studies from Vogt *et al.*[132] showed that cationic antigens were present in the glomeruli in APSGN. These glomerular deposits were later identified as zymogen/streptococcal proteinase.[40]

Poon-King *et al.*[109] in 1993 showed that streptococcal nephritogenic strains produced a plasma-binding protein identified as zSpeB and two simultaneous and independent studies[133,134] provided evidence for a role of SpeB and zSpeB in APSGN. Cu *et al.*[134] showed that 12 of 18 renal biopsies of patients with PSGN had deposits of SpeB and high anti-SpeB antibody levels were present in patients with PSGN, but not in patients with uncomplicated streptococcal infections or in patients with acute rheumatic fever. Parra *et al.*[133] did a multicentric study of 153 patients with APSGN, 23 patients with uncomplicated streptococcal infections and 93 controls in Venezuela, Chile, and Argentina and found that anti-zSpeB titers of 1:800–1:3200 had a likelihood ratio (sensitivity/1-specificity) for the detection of streptococcal infections associated with glomerulonephritis of 2.0–44.2 and that receiver operating characteristic curves showed that anti-zSpeB titers were consistently superior to anti-streptolysin *O* titers and anti-DNAase B titers.

In a more recent study, Batsford *et al.*[122] evaluated NAPlr and zSpeB/SpeB in biopsies and sera obtained from the patients with PSGN in Venezuela, Chile, and Switzerland.

They found SpeB deposits in 12 of 17 biopsies and circulating anti-SpeB antibodies in 53 of 53 sera examined. In contrast, circulating antibodies to NAPlr were detected in five of 47 sera and unequivocal glomerular deposits of NaPlr in only one biopsy (borderline in 2). Importantly, these studies showed colocalization of SpeB and complement and IgG in the glomeruli (Figure 1b) and, in addition, they demonstrated the existence of immunogold-labeled SpeB deposits inside the electron-dense subepithelial deposits ('humps') (Figure 1e) that are the histological hallmark of APSGN. As discussed in earlier sections, other streptococcal antigens have been localized in the glomeruli and previous studies had shown intra-hump Ig;[135] however, this is the first time that streptococcal antigens have been demonstrated within this particular lesion. Not surprisingly, this was attributed to charge-facilitated penetration of SpeB, as is the case with other cationic antigens that can induce similar electron-dense subepithelial lesions experimentally.[136,137]

HOST FACTORS RESPONSIBLE FOR NEPHRITOGENICITY

Careful scrutiny of all the publications on PSGN fails to identify properties of group A streptococci that are closely correlated with the appearance of glomerulonephritis. For example, the putative nephritogenic antigens SpeB/zSpeB as well as NaPlr are found in virtually all *S. pyogenes* isolates, including those strains associated with rheumatic fever reviewed in Batsford *et al.*[138] but only a minority of patients develops nephritis. Particular properties of the bacteria are rather essential but not sufficient for induction of disease. Based on such observations, it has long been accepted that host factors must play a major and decisive role in determining who gets poststreptococcal nephritis. In fact, as early as 1812, the 'constitutional differences' among families were assumed to be responsible for a familial predisposition to PSGN.[3] Subsequent studies showed that 20[139]–38%[140] of siblings of patients with sporadic PSGN developed clinical or subclinical nephritis. Nevertheless, studies on human lymphocyte antigen antigen distribution have failed to define a specific association with PSGN.[141,142] Multiple factors are likely in play in the genetic predisposition to PSGN.

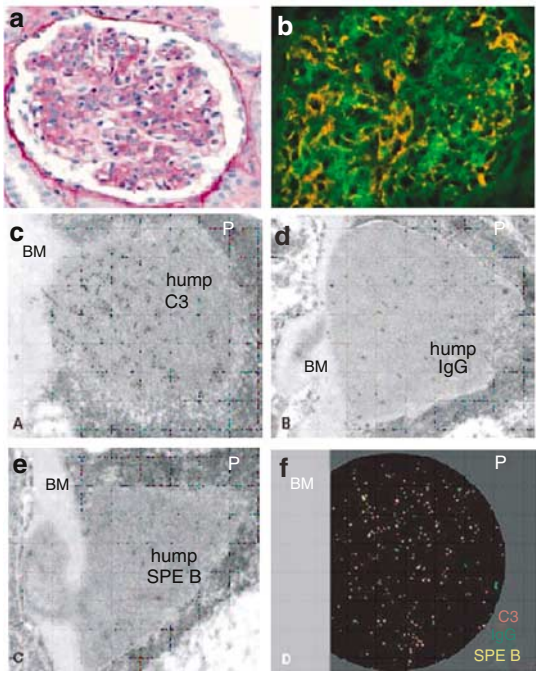

Figure 1 | SpeB is colocalized with complement and Ig and shown inside the subepithelial electron-dense deposits in APSGN. (**a**) A light microphotograph of a glomerulus of APSGN (PAS staining, original magnification ×400) and (**b**) a merge microphotograph in which zSpeB (fluorescein isothiocyanate-labeled, green) and C3 colocalization is shown in orange. The set of immune electron microphotographs show a biopsy of APSGN in which the same subepithelial electron-dense deposit ('hump') shows (**c**) C3, (**d**) IgG, and (**e**) SpeB as immunogold-positive specs inside the hump. (**f**) a composite in which the gold specs are substituted by colored spots is shown. The microphotographs and electronmicrophotographs are reproduced from Batsford *et al.*[122]

CONCLUDING REMARKS

A number of pathways by which streptococci could initiate and perpetuate glomerular injury have been delineated above. It seems unlikely that a single mechanism will be responsible in all cases, although at this time we favor the view that glomerular-immune complex formation is the critical step in the initiation of the disease that as it evolves recruits a variety of effector mechanisms. As PSGN develops in a minority of the patients infected with nephritogenic strains, it is clear that host factors are critical to determine who gets and who does not get nephritis. What these factors are is not clear at present.

It may be noted that nephritogenicity, understood as the capacity for generating renal inflammation, is not the same as nephritogenic antigen–antibody reactivity. The first may well result from plasmin-binding characteristics, whereas the later may involve colocalization of the putative antigen with complement and Ig, as has been shown for streptococcal SpeB (Figure 2). Further studies are required to elucidate the participation of these elements in the pathogenesis of poststreptococcal glomerulonephritis.

From the standpoint of diagnosis of nephritogenic streptococcal infections, rising antibody titers to SpeB/zSpeB or NaPlr represent the best evidence presently available, but they are not available in clinical practice. Yet, it is likely that they may be used to improve the etiologic diagnosis of patients with acute nephritic syndrome in the future.[133]

Clearly, in the 20th century we have gained great insight into the pathogenesis of antigen–antibody reactions and poststreptococcal nephritis, yet, to quote Wolfgang von Goethe, 'if you miss the first buttonhole you will not succeed in buttoning up your coat' (Wer das erste Knopfloch verfehlt, kommt mit dem Zuknöpfen nicht zu Rande. From 'Maximen und Reflektionen no. 900') and it is fitting to give credit to Clemens von Pirquet, a century after his sealed communication to the Vienna Academy of Sciences, for finding for us the

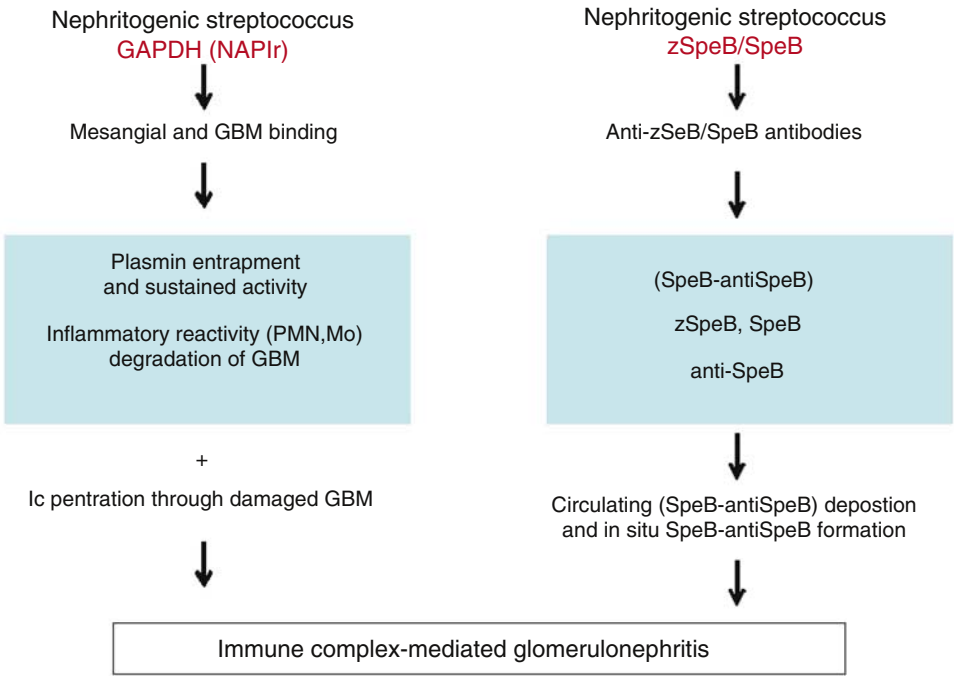

Figure 2 | Etiopathogenesis of PSGN. Nephritogenicity of streptococcal NAPIr–GAPDH (left side) may be related to its plasmin-binding activity that would induce inflammatory reactivity and glomerular basement membrane (GBM) degradation as, as demonstrated by Oda *et al.*,[111] it colocalizes in glomeruli with plasmin, but not with IgG or complement. SpeB and zSpeB (right side) may induce immune-complex-mediated glomerulonephritis as SpeB deposits colocalizes with complement and IgG and is present in the subepithelial humps that are the hallmark lesion of PSGN.[122]

elusive first buttonhole of this immune-mediated renal disease.

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