Correlation of disease activity in proliferative glomerulonephritis with glomerular spleen tyrosine kinase expression

Stephen P. McAdoo[1], Gurjeet Bhangal[1], Theresa Page[1], H. Terence Cook[2], Charles D. Pusey[1] and Frederick W.K. Tam[1]

[1]Renal and Vascular Inflammation Section, Department of Medicine, Imperial College London, Hammersmith Hospital, London, UK and [2]Centre for Complement and Inflammation Research, Imperial College London London, UK

Spleen tyrosine kinase (SYK) is an important component of the intracellular signaling pathway for various immunoreceptors. Inhibition of SYK has shown promise in preclinical models of autoimmune and glomerular disease. However, the description of SYK expression in human renal tissue, which would be desirable ahead of clinical studies, is lacking. Here we conducted immunohistochemical analysis for total and phosphorylated SYK in biopsy specimens from >120 patients with a spectrum of renal pathologies, including thin basement membrane lesion, minimal change disease, membranous nephropathy, IgA nephropathy, lupus nephritis, ANCA-associated glomerulonephritis, antiglomerular basement membrane disease, and acute tubular necrosis. We found significant SYK expression in proliferative glomerulonephritis and that glomerular expression levels correlated with presenting serum creatinine and histological features of disease activity that predict outcome in IgA nephropathy, lupus nephritis, ANCA-associated glomerulonephritis, and antiglomerular basement membrane disease. SYK was phosphorylated within pathological lesions, such as areas of extracapillary and endocapillary proliferation, and appeared to localize to both infiltrating leucocytes and to resident renal cells within diseased glomeruli. Thus SYK is associated with the pathogenesis of proliferative glomerulonephritides, suggesting that these conditions may respond to SYK inhibitor treatment.

Kidney International advance online publication, 25 February 2015; doi:10.1038/ki.2015.29

KEYWORDS: ANCA; anti-GBM disease; glomerulonephritis; IgA nephropathy; immunohistochemistry; lupus nephritis

Correspondence: *Stephen P. McAdoo, Renal and Vascular Inflammation Section, Department of Medicine, Imperial College London, Hammersmith Hospital, London W12 0NN, UK. E-mail: s.mcadoo@imperial.ac.uk*

This work was presented in abstract form at the American Society of Nephrology Renal Week Meeting in Atlanta, GA, USA in November 2013 and at the UK Renal Association meeting in Glasgow, Scotland in May 2014.

Received 20 December 2013; revised 2 December 2014; accepted 11 December 2014

Spleen tyrosine kinase (SYK) is a non-receptor tyrosine kinase that is highly expressed in hematopoietic cells, where it has a critical function in classical immunoreceptor signaling. It has a well-characterized role in the intracellular signal transduction pathway for the B-cell surface receptor[1] and for activatory Fc receptors expressed on a variety of immune effector cells, including myeloid cells[2,3] and mast cells.[4] In addition, a role for SYK in signaling in other cell types and for other receptors, such as integrins and C-type lectins, is increasingly recognized.[5] The SYK molecule, of molecular weight 72 kDA, has a multi-domain structure,[6] characterized by two N-terminal SH2 domains, which allow it to interact with immunoreceptor-tyrosine based activation motifs on the intracellular domain (or on related adaptor proteins) of the receptors for which it mediates signaling, and a C-terminal kinase domain. Upon receptor engagement, SYK is phosphorylated on multiple tyrosine residues, leading to conformational changes that allow this C-terminal kinase domain to interact with a variety of downstream targets, including phospholipase C gamma, phosphatidylinositol 3′-kinase, and mitogen-activated protein kinases, resulting in activation of a variety of cellular responses, including proliferation, differentiation, phagocytosis, and cytokine production.

Given its important role in generating and effecting adaptive immune responses, SYK has emerged as a potential therapeutic target in autoimmune disease. Both genetic and pharmacological manipulation of SYK activity have shown efficacy in treating *in vivo* models of autoimmune disease,[7,8] and several small-molecule inhibitors have progressed to clinical investigation.[9] We have previously reported that SYK inhibition with fostamatinib, a kinase inhibitor with high selectivity for SYK that has progressed to phase III study of non-renal diseases,[10,11] is effective in treating two distinct rodent models of immune-mediated glomerulonephritis (GN).[12,13] We also reported that SYK is expressed in immunoglobulin A (IgA) nephropathy (IgAN).[14] However, a systematic analysis of SYK expression in other glomerular diseases, though desirable ahead of potential clinical studies, is lacking.

Here we have analyzed SYK expression by immunohistochemistry (IHC) on renal biopsies from patients with a

range of glomerulonephritides. We report that SYK is expressed and activated in proliferative GN and that SYK expression levels appear to correlate with disease activity, suggesting that SYK inhibition may be a rational therapeutic target warranting further clinical investigation in glomerular disease.

RESULTS

Method development and characterization of SYK staining in normal renal tissue

This was an IHC-based study for which we used commercially available primary antibodies. For total SYK (T-SYK) staining, we used an antibody directed against the N-terminus of SYK that should detect both major splice variants of the protein. For phophorylated SYK (phospho-SYK) staining, we used an antibody directed against phosphotyrosine 525/526, located within the activation loop of the catalytic domain of SYK, in order to detect functionally activated protein likely to be relevant to disease pathogenesis. We previously reported the use of these antibodies for detection of SYK in human renal mesangial cells and in rat tissue by immunoblotting and IHC, respectively.[13,14] For this study, we further confirmed their reactivity and specificity for SYK by western blotting of lysates of human peripheral blood mononuclear cells (Figure 1a). We performed positive-control IHC on human lymph node tissue and found positive staining for both T-SYK and phospho-SYK localized to lymphoid follicles, consistent with an important role for SYK in maintaining follicular B cell survival[15] and in generating adaptive immune responses (Figure 1b-e).

In order to define the pattern of T-SYK expression in normal kidney tissue, we examined tissue from nephrectomy specimens ($n=4$) and surveillance transplant biopsies with normal light micrographic findings ($n=4$). In all cases, we found positive staining for T-SYK within distal tubular epithelial cells (identified by their morphological features of cuboidal epithelium with little brush border and open tubular lumens). Minimal staining was observed within glomeruli (Figure 2a and b). For initial characterization of T-SYK staining in nephritic tissue, we examined renal biopsies from patients with postinfectious diffuse proliferative GN. We observed positive staining of distal tubular epithelial cells, comparable to that seen in normal renal tissue. In addition, there was positive staining within nephritic glomeruli that appeared to localize to proliferating cells within the tuft (Figure 2c–f).

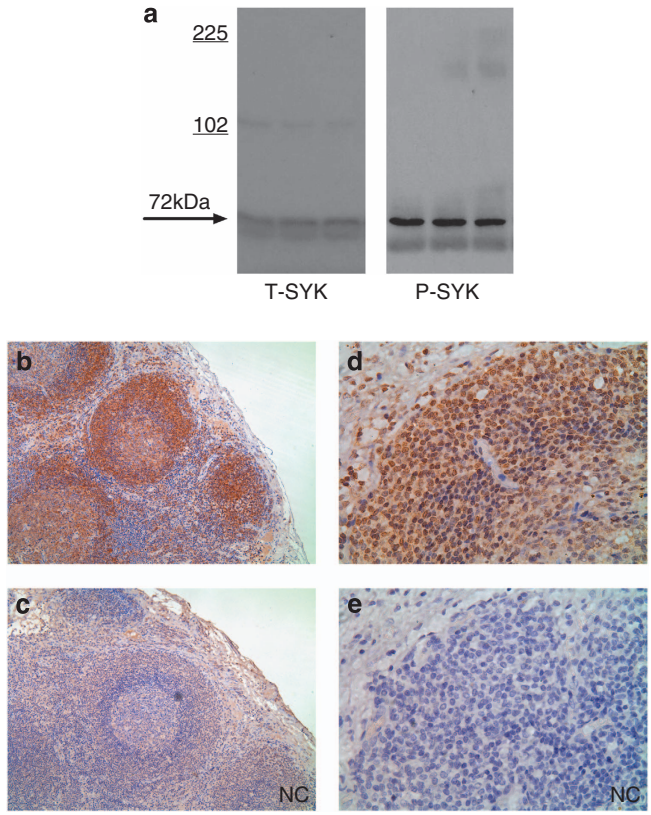

Figure 1 | Method development and control immunohistochemical stains. (**a**) Western blotting for total (T-) and phophorylated (P-) spleen tyrosine kinase (SYK) in lysates of human peripheral blood mononuclear cells, confirming detection of SYK protein at 72 kDA. (**b** and **c**) Sequential sections showing positive and negative control (NC) staining for T-SYK in human lymph node, with strong staining for SYK localized to lymphoid follicles. (**d** and **e**) Sequential sections showing positive control and NC staining for P-SYK in human lymph node, demonstrating nuclear and cytoplasmic localization, within cells in lymphoid follicles. All sections are immunoperoxidase stains with hematoxylin counterstain, ×200–400 magnification. NC stains were performed by preincubating the primary antibody with the relevant immunizing peptide.

Greatest amounts of glomerular SYK staining seen in proliferative GN

We then proceeded to perform IHC for T-SYK on renal biopsies demonstrating a range of primary glomerular lesions. In thin basement membrane lesion ($n=11$), minimal change disease ($n=11$) and idiopathic membranous nephropathy ($n=10$), there was negligible glomerular staining for T-SYK, with positive tubular staining as noted in normal tissue (Figure 3a; Figure 4). Glomerular T-SYK expression levels were the highest in anti-glomerular basement membrane (anti-GBM) disease ($n=15$), and significant expression was also seen in anti-neutrophil cytoplasm antibody (ANCA)-associated GN (AAGN; $n=18$), lupus nephritis ($n=16$), and IgAN ($n=26$) (Figure 3a). In the entire cohort ($n=107$), there was a robust association between creatinine at the time of renal biopsy and glomerular T-SYK staining ($r=0.4$; $P<0.0001$; Figure 3b). There was no association with the degree of proteinuria at the time of biopsy ($r=0.03$; $P=0.77$).

In the proliferative glomerulonephritides (anti-GBM, AAGN, lupus nephritis, IgAN), T-SYK expression localized to areas of segmental inflammation and endocapillary and extracapillary proliferation. In anti-GBM disease (Figure 5a and b) and AAGN (Figure 5d), for example, staining was observed in areas of crescent formation. No staining was