β1,4-galactosyltransferase 1 is a novel receptor for IgA in human mesangial cells

Karen Molyneux[1], David Wimbury[1], Izabella Pawluczyk[1], Masahiro Muto[1], Jasraj Bhachu[1], Peter R. Mertens[2], John Feehally[1] and Jonathan Barratt[1]

[1]Department of Infection, Immunity and Inflammation, University of Leicester, Leicester, UK; and [2]Department of Nephrology and Hypertension, Diabetes and Endocrinology, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany

IgA nephropathy is characterized by mesangial deposition of IgA, mesangial cell proliferation, and extracellular matrix production. Mesangial cells bind IgA, but the identity of all potential receptors involved remains incomplete. The transferrin receptor (CD71) acts as a mesangial cell IgA receptor and its expression is upregulated in many forms of glomerulonephritis, including IgA nephropathy. CD71 is not expressed in healthy glomeruli and blocking CD71 does not completely abrogate mesangial cell IgA binding. Previously we showed that mesangial cells express a receptor that binds the Fc portion of IgA and now report that this receptor is an isoform of β-1,4-galactosyltransferase. A human mesangial cell cDNA library was screened for IgA binding proteins and β-1,4-galactosyltransferase identified. Cell surface expression of the long isoform of β-1,4-galactosyltransferase was shown by flow cytometry and confocal microscopy and confirmed by immunoblotting. Glomerular β-1,4-galactosyltransferase expression was increased in IgA nephropathy. IgA binding and IgA-induced mesangial cell phosphorylation of spleen tyrosine kinase and IL-6 synthesis were inhibited by a panel of β-1,4-galactosyltransferase-specific antibodies, suggesting IgA binds to the catalytic domain of β-1,4-galactosyltransferase. Thus, β-1,4-galactosyltransferase is a constitutively expressed mesangial cell IgA receptor with an important role in both mesangial IgA clearance and the initial response to IgA deposition.

Kidney International (2017) **92,** 1458–1468; http://dx.doi.org/10.1016/j.kint.2017.05.002

KEYWORDS: glomerulonephritis; IgA nephropathy; mesangial cells

Correspondence: *Jonathan Barratt, Department of Infection, Immunity and Inflammation, Maurice Shock Building, University of Leicester, University Road, Leicester LE1 9HN, UK. E-mail: jb81@le.ac.uk*

Received 7 September 2016; revised 23 April 2017; accepted 11 May 2017; published online 24 July 2017

IgA nephropathy (IgAN) is characterized by mesangial IgA deposition, often accompanied by a mesangial proliferative glomerulonephritis, and is the commonest pattern of primary glomerulonephritis in all countries where renal biopsy is widely practiced.[1] IgA deposition is not necessarily an irreversible phenomenon, as it is believed that the mesangium is normally capable of clearing finite amounts of IgA and that processes distinct from IgA deposition are necessary for the development of glomerulonephritis.[2–4]

The principal pathway for mesangial IgA clearance is mesangial cell (MC) receptor–mediated endocytosis and catabolism.[5,6] In most instances IgAN is not associated with a significant glomerular cell infiltrate, suggesting that glomerular injury is mediated predominantly through IgA-induced activation of MC and local complement activation. Cross-linking of IgA receptors elicits proliferation and a proinflammatory and profibrotic phenotypic transformation in MC.[7–10] Furthermore, exposure to IgA has been shown to result in phosphorylation of a number of key intracellular kinases, including spleen tyrosine kinase (SYK).[11,12] IgA is also capable of altering MC-matrix interactions by modulating integrin expression, and this may have an important role in remodeling of the mesangium following glomerular injury.[13]

Human MCs (hMCs) express at least 1 IgA receptor, the transferrin receptor (CD71), but not the other IgA receptors thus far characterized.[14–20] CD71 is overexpressed by hMC and in glomeruli of patients with IgAN.[16,21,22] Cross-linking of CD71 by IgA, in concert with the enzyme transglutaminase 2, results in hMC proliferation and secretion of interleukin-6 (IL-6) and transforming growth factor beta.[8,23] CD71 is ubiquitously expressed by dividing cells, and it remains unclear whether CD71 is involved in the initiation of glomerular injury or rather amplifies existing hMC activation and glomerular injury in IgAN.[24,25] CD71-specific antibodies and transferrin only partially inhibit the binding of IgA to hMC, supporting the concept that hMC express at least 1 other type of IgA receptor.[12,16,21] There is preliminary evidence suggesting that this receptor could be an asialoglycoprotein receptor,[26] an Fc α/μ receptor,[27] an integrin IgA receptor,[28] and/or a Fc α receptor.[15]

Here we report that β-1,4-galactosyltransferase 1 (β-1,4-GalT1) can act as an IgA receptor, is expressed by hMC, and is found *in vivo* in the glomeruli of healthy and diseased kidneys, and that mesangial expression appears increased in IgAN.

RESULTS

hMC expression library screening

We screened 3.22×10^5 colony-forming units from an hMC expression library for their ability to increase IgA binding to HEK 293 cells. Background IgA binding to HEK 293 cells was observed and was believed to be due to CD71 expression. Clone 5S increased IgA-FITC binding to a level equivalent to that seen with the positive control, a clone containing CD89 sequence (Figure 1). Sequencing revealed that clone 5S contained 936bp of cDNA sequence coding for the catalytic region of human β-1,4-GalT1 (Figure 2).

β-1,4-GalT1 mRNA and protein expression by hMC

The 2 mRNA species of β-1,4-GalT1 were amplified from cultured hMC, and sequencing confirmed the presence of both the long (cell membrane) and short form (Golgi apparatus) of β-1,4-GalT1 (Figure 3). Immunoblotting of hMC, lysates, and cell membranes confirmed the presence of translated long and short forms of β-1,4-GalT1 (Figure 4). A 58 kDa protein corresponding to the short form of β-1,4-GalT1 was seen with total hMC lysate and 9335 (Figure 4a) but not the cell membrane fraction (blot not shown).[29] A 74 kDa protein, consistent with expression of a glycosylated cell surface variant of β-1,4-GalT1, was best seen with hMC cell membrane fractions and 9286 (Figure 4b).[30]

Mesangial cell surface expression of β1,4 GalT1

Cell surface expression of β-1,4-GalT1 was demonstrated in each of the primary hMC lines studied ($n = 5$) with each of the β-1,4-GalT1-specific antibodies: 5G4 (Figure 5), 1H11 and 1B6 (data not shown). Confocal microscopy of permeabilized hMC stained with 9335 revealed localization of β-1,4-GalT1 to both the perinuclear region, in association with the Golgi apparatus, and the cell surface of the primary processes (Figure 6a). This pattern of β-1,4-GalT1 expression is identical to that seen in other cells.[31] In nonpermeabilized cells β-1,4-GalT1 staining was limited to the cell membrane consistent with expression of the surface expression of the long form of β-1,4-GalT1 (Figure 6b).

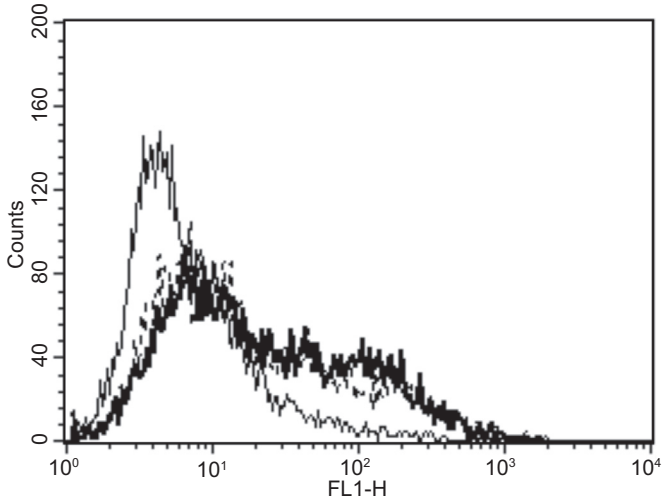

Figure 1 | IgA-FITC binding to HEK 293 cells increases after transfection with clone 5S (thick line) to a similar extent to that seen with a clone containing CD89 sequence (dotted line), and the 2 histograms overlap. IgA-FITC binding to HEK 293 cells transfected with an empty vector is also shown (thin line). Background IgA binding to HEK 293 cells was observed, and we believe it was due to expression of CD71.

Inhibition of IgA binding to hMC by β-1,4-GalT1-specific antibodies and soluble human β-1,4-GalT1

IgA binding to hMC was inhibited both by pre-incubation of IgA-FITC with soluble human β-1,4-GalT1 (Figure 7a and b) and blocking of the catalytic site of cell surface β-1,4-GalT1 (the presumed IgA binding domain of the protein) with 9335 and 1H11 (Figure 7a and c). This demonstrated that soluble β-1,4-GalT1 can compete with cell surface β-1,4-GalT1 to bind IgA and that blocking the catalytic site of β-1,4-GalT1 blocks access of IgA to its ligand binding domain. IgA binding was not completely blocked, supporting previous findings that hMCs express more than 1 IgA receptor.

Inhibition of IgA-induced hMC activation by β-1,4-GalT1 and CD71-specific antibodies

IgA1-induced IL-6 release was significantly reduced with 3191, 3192, and Sc17299 (only data for 3192 shown), which bind to different epitopes in the catalytic region of β-1,4-GalT1 but not with Sc22277, which binds to the stem region of β-1,4-GalT1 (Figure 8a). Inhibition of IL-6 synthesis was not complete, consistent with coexpression of an additional hMC IgA receptor. Expression of CD71 was confirmed, as blocking of IgA1-CD71 binding also resulted in partial inhibition of IL-6 synthesis. There was no increase in inhibition of IL-6 release when CD71/β-1,4-GalT1 dual blockade was performed, implying that at least 1 other functionally distinct IgA receptor is likely to exist and that CD71 and β-1,4-GalT1 may share intracellular signaling pathways. To investigate this latter possibility, IgA-induced SYK phosphorylation was measured. This was reduced by inhibition of both IgA1-CD71 and IgA1-β-1,4-GalT1 binding (Figure 8b).

Glomerular expression of β-1,4-GalT1 in human kidney

Low-level mesangial expression of β-1,4-GalT1 (designated in the figure by the symbol *) was seen by immunofluorescence and immunohistochemistry in kidneys without evidence of glomerular disease (Figure 9a and b), thin membrane nephropathy (Figure 9c and d), and membranous nephropathy (Figure 9e and f) consistent with the constitutive expression of β-1,4-GalT1 by mesangial cells *in vivo*. Mesangial β-1,4-GalT1 staining was increased in IgAN (Figure 9g–i). In parallel, glomeruli were isolated by laser capture microdissection (LCMD), and the presence of mRNA for the long form of β-1,4-GalT1 was confirmed by reverse-transcription polymerase chain reaction and sequencing (data not shown).

DISCUSSION

Studies examining IgA binding to hMC and IgA-induced hMC activation acknowledge that IgA receptors apart from CD71 must exist.[16,21,32] Importantly, glomerular CD71