which is defined by the deposition of IgA in the glomerular mesangium [1, 2]. Haematuria is the initial clinical hallmark of the disease. Early in the course of the disease episodes of visible haematuria are common and often coincide with mucosal infection (typically of the respiratory tract, and less commonly of the gastrointestinal tract). The IgA deposits may provoke little glomerular injury, but more often the consequences of IgA deposition are glomerular inflammation followed by sclerosis and progressive loss of function. This is commonly a chronic process leading inexorably to renal failure [1].

HSP, despite its name which is perpetuated for historical reasons, is a multisystem disease with a characteristic vasculitic rash. Renal involvement is common and the findings in the kidney are virtually identical with those in IgAN [3]. IgA deposition in the glomerulus and skin is a sine qua non for the diagnosis of HSP. Clinical, pathological and immunological data increasingly point to the close similarities between IgAN and HSP; IgAN is often viewed as a forme fruste of HSP [4].

Both IgAN and HSP are common in children and young adults. The implications of developing renal failure and the complexity of treatment then required justify the need for...
research into the mechanism of glomerular IgA deposition which is the initiator of injury in both IgAN and HSP.

Limitations of animal models in the study of IgAN and HSP
It is possible to provoke mesangial IgA deposition in animals by a variety of feeding and immunization protocols, although glomerular inflammation does not always follow [5]. However, there are significant species differences in the IgA immune system. No animal has IgA subclasses analogous to human IgA1 and IgA2, and mechanisms of IgA clearance also differ [6]. Although animal models are very informative about events that follow glomerular IgA deposition, they have limitations in providing information relevant to the mechanisms of deposition of human IgA1 in the human glomerulus. Nor is there any useful animal model of HSP.

As a consequence, the work reported here is restricted to human IgA1 and the human IgA immune system.

The IgA immune system in IgAN and HSP
The mesangial IgA deposits in both IgAN and HSP are largely IgA1 [4]. They are also polymeric (J-chain is present) but are not secretory IgA (secretory component is absent) [4]. Circulating polymeric IgA1 (plgA1) is modestly increased and there is considerable evidence for IgA1 immune hyper-reactivity [4,7]. There is an exaggerated plgA1 response to common antigens given by systemic immunization [8,9]. However, increases in circulating IgA (be they monoclonal or polyclonal) are not themselves enough to provoke IgAN; mesangial IgA deposits are a feature of neither IgA myeloma nor AIDS.

The presence of plgA1 in the deposits and the clinical association with mucosal infection have both promoted a view that IgAN is a consequence of an exaggerated mucosal IgA response. However, salivary IgA1 levels (unlike serum IgA1) are not increased [10]. Furthermore mucosal immunization studies show normal or diminished IgA responses in IgAN [11,12].

The studies described in this report were developed to investigate two questions which need to be addressed if there is to be progress in our understanding of glomerular IgA1 deposition in IgAN and HSP: what is the site of origin in the IgA immune system of the deposited plgA1?

What is the mechanism of mesangial plgA1 deposition?

Site of plgA production in IgAN
To investigate the site of origin of the increased circulating plgA1 in IgAN we studied J-chain synthesis which appears to be a rate-limiting step in the assembly of plgA [13].

We developed in situ hybridization for J-chain mRNA to study plgA synthesis in mucosal and non-mucosal immune systems in IgAN. Duodenal lamina propria obtained by endoscopic biopsies, bone marrow trephine biopsies and tonsils were all available from patients with IgAN and matched controls. plgA production was identified by defining J-chain mRNA expression in IgA-producing plasma cells using non-isotopic double-labelling techniques [14] (Figure 1). Unexpectedly, plgA synthesis defined in this way was consistently down-regulated in mucosal lamina propria [15] and up-regulated in bone marrow [16]. It was also up-regulated in tonsillar tissue [17]. The tonsil has previously been thought to play a key role in IgAN; there is some evidence that tonsillectomy may reduce the incidence of frank haematuria. However, tonsillar IgA production is modest compared with that of marrow or mucosal lamina propria; if tonsillar overproduction were primary, it might be expected that marrow plgA production would be unchanged or suppressed. Furthermore IgAN does occur after tonsillectomy. These points argue against a primary role for the tonsil in IgAN.

Figure 1
Expression of J-chain mRNA in IgA-producing plasma cells in IgAN and matched controls
J-chain mRNA is reduced in duodenal lamina propria, but increased in bone marrow and tonsil.

1997
It is not yet clear whether suppressed mucosal plgAl production is the primary abnormality in IgAN, perhaps allowing increased antigen penetration to stimulate an exaggerated marrow response, or is a secondary down-regulation in response to primary marrow hyper-responsiveness.

**Mechanism of mesangial IgA deposition**

The pattern of mesangial IgA deposition seen in IgAN can typically be reproduced in animal models in two ways: by infusion of preformed antigen–antibody complexes or by formation of such complexes in situ in response to fixed antigens. Such antigens could be intrinsic neoantigens or extrinsic antigens; in the human context likely antigens might have a food or viral origin. However, despite detailed study no antigen has consistently been implicated in human IgAN [13]. This has led to the concept that plgAl may be deposited by a mechanism other than classical antigen–antibody interactions. Altered glycosylation of IgA1 has been proposed as a candidate defect [18,19].

**IgA1 glycosylation**

IgA1 like all immunoglobulins is a glycoprotein. It is heavily glycosylated, containing complex N-glycans, but is unique among major serum immunoglobulins for its O-linked glycans, which are restricted to the hinge region and therefore not present in IgA2 [20]. The hinge contains 10 O-glycosylation sites linked to serine residues; the O-glycans contain GalNAc with or without terminal galactose Gal in the β1-3 position: serine-GalNAc(β1-3)Gal [21]. Each O-glycan may have variable sialylation [22].

Altered O-glycosylation could have significant structural and functional influences because of the pivotal position of the hinge region; changes in three-dimensional structure could alter interactions with the antigen-binding site as well as interactions with Fc receptors, complement and matrix proteins.

**IgA1 glycosylation in IgAN**

We have developed lectin-binding assays based on lectins specific for O-glycans [24]. In serum IgA1 from patients with IgAN we find increased binding of lectins specific for GalNAc (Figure 2), suggesting either reduced terminal galactosylation of GalNAc or increased expression of GalNAc [24]. Our initial studies relied on isolation of IgA1 by jacalin binding. Since jacalin is itself a lectin which recognizes hinge-region glycans on IgA1, there was concern that jacalin was isolating unrepresentative IgA1. However, the lectin-binding assays applied to total IgA provide the same findings (A. C. Allen, unpublished work). Preliminary studies with fluorophore-assisted carbohydrate electrophoresis, which maps O-glycans after their release from IgA1 by hydrazinolysis at 60°C, confirm a characteristic O-glycan pattern in serum IgA1 in these patients which requires further definition (A. C. Allen, unpublished work). Others have recently reported reduced sialylation of released Galβ1-3GalNAc (using HPLC after hydrazinolysis), with in vitro evidence that the altered sialylation influenced IgA1 aggregability [25]. This work provides support for the notion that altered IgA1 hinge O-glycans do indeed have functional influence. Definitive structural confirmation of the Galβ1-3GalNAc abnormality indicated by the lectin-binding studies is now required.

**IgA1 glycosylation in HSP**

Lectin-binding studies of serum IgA1 from patients with HSP show the same pattern as in IgAN (Figure 2), but only in those subjects with HSP who have renal involvement [26]. The lectin binding in HSP without nephritis is no different from matched controls. Patients with glomerulonephritis that is not associated with IgA deposition also do not show differences from controls in lectin binding of serum IgA1.
Limitations of serum IgA1-glycosylation studies

Studies of serum IgA1 are inevitably indirect; the pathogenic IgA1 molecules are by definition in the kidney and it cannot be guaranteed that circulating IgA1 molecules are representative. However, the very small amount of IgA1 that can be eluted from human renal biopsy material are not sufficient for the necessary analysis, and opportunities for study of larger samples of IgAN kidney must be awaited.

β1-3-galactosyltransferase (β1,3GT)

The glycosyltransferases are a family of highly specific enzymes [27]. Addition of Gal in the β1-3 configuration to GalNAc is catalysed by β1,3GT. If the defect implied by lectin-binding studies is indeed reduced terminal galactosylation of GalNAc, reduced β1,3GT activity would be predicted in IgA plasma cells. (An alternative could be subsequent removal of Gal, but there is no evidence for any excess glycosidase activity in these patients.) We developed a functional assay for β1,3GT in which the substrate was isolated IgA1 hinge region stripped of Gal by β-galactosidase, and the capacity of cell lysates to regalactosylate the substrate was measured by reduction in binding of a lectin (Vicia villosa) specific for GalNAc [28]. In peripheral blood B-cells from patients with IgAN there was reduced β1,3GT activity compared with matched controls (Figure 3A). Furthermore B-cell β1,3GT activity correlated significantly with the lectin-binding abnormality in serum IgA1 from the same patients (Figure 3B).

B-cells isolated from peripheral blood will only contain a minority of IgA-producing cells, and more specific studies, particularly of marrow IgA-producing cells, are required to confirm this finding. Nevertheless these studies suggest that a post-translational enzyme defect may be a key abnormality in IgAN.

Implications of altered O-glycosylation of IgA1

There are a number of putative mechanisms by which an altered hinge region could change IgA1 behaviour and promote glomerular IgA1 deposition and subsequent injury. These include: (i) reduced clearance of circulating IgA1; the hepatic asialoglycoprotein receptor, the main clearance mechanism for circulating IgA1 recognizes IgA1 through hinge-region galactose [29,30]; (ii) altered aggregability or polymerization of IgA1 [25]; (iii) modified interactions with complement (complement deposition in association with IgA is common in IgAN, yet IgA is a rather poor activator of complement [31,32]); (iv) modified interactions with glomerular matrix proteins (the IgA deposits are characteristically extracellular); (v) altered engagement of Fcγ receptors, both on circulating myeloid inflammatory cells and also on mesangial cells, where an Fcγ receptor has recently been described but not yet fully characterized [33,34]. However, it should be emphasized that as yet there is little or no evidence to support these most interesting speculations. Functional data are required before the structural abnormality of the IgA1 hinge can be confirmed as an important pathogenic element in IgAN.

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