Abnormal glycosylation of IgA: is it related to the pathogenesis of IgA nephropathy?

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Introduction

IgA nephropathy (IgAN) is characterized by the mesangial deposition of IgA, which is known to be of the IgA1 subclass. Reports of recurring IgA deposits in normal kidneys transplanted into IgAN recipients provide evidence that the basic abnormality in this condition lies within the IgA immune system rather than the kidney. Numerous abnormalities and dysregulations involving IgA in IgAN have been reported. Among the best recognized are moderately raised serum IgA levels, overexpression of IgA1 plasma cells, increased in vitro IgA1 production, IgA1 overreactivity to antigen, and raised levels of IgA-containing com-

References

3. Van Den Wall Bake AWL, Daha MR, Radl J et al. Elevated production of polymeric and monomeric IgA1 by the bone
plexes in the circulation. These and other abnormalities confirm that the IgA system is flawed in these patients, but none can adequately account for the pathogenesis of IgAN.

Two key questions remain: why does IgA1 deposit in the glomerular mesangium?; and how does this lead to glomerular injury?

It is often assumed that renal immunoglobulin deposits are due to antibody complexed with a specific antigen either in situ or as circulating immune complexes. However, no convincing target antigen for mesangial IgA in IgAN has been identified. The slight autoreactivity found in serum IgA in IgAN may represent non-specific complex formation with a variety of proteins rather than specific antibody–antigen formation, and be due to some physical feature of the IgA molecule. We and others have recently described altered glycosylation of IgA1 in IgAN. The consequences of this altered glycosylation may provide answers to both the above questions.

**Protein glycosylation**

The majority of serum and membrane proteins carry carbohydrate components, which play important roles in production, maintenance, handling, and function of glycoproteins. There are two distinct forms of protein glycosylation, N-linked and O-linked. N-linked moieties, linked to asparagine residues, are by far the most common, and usually consist of complex, branched chains. O-linked sugars are less frequent, particularly in serum proteins, and are usually simpler moieties linked to serine or threonine residues. Protein glycosylation occurs during and immediately after protein synthesis, and is effected by a series of specific glycosyltransferases. Both types of moeity can carry terminal sialic acid (NeuNAc), a highly negatively charged entity that is much more labile than the core structure, and is important in determining the catabolism of many proteins.

Glycosylation defects are increasingly recognized as potentially pathogenic. In rheumatoid arthritis, IgG displays characteristic N-linked agalactosylation [1]; there are also a variety of conditions in which cells behave abnormally due to altered glycosylation of membrane proteins.

**Glycosylation of IgA1**

IgA1 is heavily glycosylated, consisting of 8% carbohydrate. In common with other immunoglobulins and many other serum proteins it has a number of N-linked glycosylation sites. It is also one of the very few serum proteins with O-linked sugars. These lie in the hinge region of the molecule, between the CH1 and CH2 domains of the heavy chain. The hinge region is made up of just 17 amino acids, of which five are O-linked glycosylation sites [2] (Figure 1). Since each IgA1 monomer consists of two α1 heavy chains, there are 10 closely adjacent O-linked sugars in this region, which form a distinctive and unusual feature of the IgA1 molecule. The IgA2 gene has a deletion in this region, so IgA2 has no hinge region and does not carry O-linked sugars. The O-linked sugars of IgA1 are believed to consist of N-acetyl galactosamine (GalNAc) O-linked to serine, usually carrying terminal D-galactose (Gal) in the β1,3 configuration (Figure 2). When it occurs in membrane proteins, this carbohydrate moiety is known as the Thomsen–Friedenrich, or T antigen, and the agalactosyl form, the Tn antigen, consisting of GalNAc only, is expressed by abnormal red cells in the rare haematological disorder, the Tn polyagglutinability syndrome [3].

**Abnormal glycosylation of IgA1 in IgAN**

We and others have described abnormal O-linked glycosylation of serum IgA1 in IgAN. In 1989, Andre et al. published the first study showing that IgA in IgAN has unusual glycosylation, demonstrated by reduced binding of IgA to the lectin jacalin [4]. Since then, studies employing carbohydrate-specific lectins and detailed carbohydrate analysis by mass spectrometry [5–7], have suggested that the abnormality is a reduction in the terminal galactosylation of the O-linked sugar moiety, leading to an increased expression of terminal GalNAc. Although recognized in membrane proteins in some diseases, this is the first time that altered O-linked glycosylation has been identified in a serum protein. In IgAN, the aberrant form of O-galactosylation seen in IgA1 is not shared by Cl inhibitor, which is another of the very few serum proteins with O-linked sugars. Therefore it appears that the abnormality is peculiar to IgA1 and arises in...
the synthesis of the molecule rather than being due to some degradative process in the circulation. N-linked glycosylation of IgA1 appears to be normal in IgAN.

Implications of altered glycosylation in IgAN

The consequences of reduced terminal galactosylation of IgA1 in IgAN are not yet established, but are potentially significant. The closely adjacent series of 10 O-linked sugar moieties in the hinge region of IgA1 is a distinctive and unusual feature of the molecule. The hinge region is devoid of charged amino acids and so the oligosaccharides, at least some of which normally carry one or more highly negatively charged sialic acids, contribute charge to the domain. The sugar moieties are large and bulky in comparison to the protein backbone, and can influence the tertiary and quaternary structure, helping to maintain the functional conformation of this area of the molecule. Lack of terminal O-linked galactose may lead to alterations in charge, since the exposed GalNAc probably does not carry sialic acid. The reduced physical size of the truncated sugar chain may possibly result in subtle conformational changes in the molecule. The carbohydrate coating of the IgA1 hinge region shields the protein chain and means that the sugars are presented to the external environment. Therefore the carbohydrate can be involved in recognition and interactions with other molecules. A change in the carbohydrate species presented to the outside of the molecule has the potential to influence such interactions.

One receptor which recognizes the hinge region sugars of IgA1 is the hepatic asialoglycoprotein receptor (ASGPR), a major clearance pathway of serum IgA [8]. The receptor binds to exposed terminal galactose sugars on glycoproteins which have lost the labile sialic acid; binding affinity depends on the number and spatial distribution of the terminal galactose residues. Asialo-IgA1, with its series of exposed galactose residues in the hinge region, is an attractive template for recognition by the ASGPR. Reduced O-galactosylation of IgA1 in IgAN may alter the template required for this high-affinity recognition, resulting in less efficient clearance from the circulation, consistent with evidence from the study of Roccatello et al. [9].

The hinge region lies in a functionally important part of the IgA1 molecule, close to the effector sites of the CH2 domain. The ligand of the leukocyte Fcγ receptor (FcγR), by which IgA mediates immune responses, lies at the very top of the CH2 domain, adjacent to the hinge region. Experiments with mutant IgA produced in vitro by site-directed mutagenesis have shown that the hinge region sugars are involved in the interaction between IgA1 and the leukocyte FcγR. Altered hinge-region glycosylation therefore has the potential to affect the affinity of this binding. The presence of a receptor for IgA on human mesangial cells in culture has recently been established [10,11], and though the ligand of this is as yet unknown, it seems reasonable to speculate that hinge region sugars may be involved.

Prolonged circulating life, altered charge or conformation, and the expression of an unusual terminal carbohydrate all have the potential to result in complex formation with other proteins. These may occur by non-immunological mechanisms, or be due to low-affinity autoantibodies to neoantigens expressed by the IgA1 molecule when it lacks terminal O-linked galactose. Circulating IgA-containing complexes are indeed described in IgAN, and such complexes may be liable to mesangial deposition. Similarly it is possible that complex formation with extracellular matrix proteins could occur in situ within the mesangium.

In summary, abnormal O-linked glycosylation of serum IgA seems established in IgAN, but its pathogenic implications are as yet only speculative. This abnormality does, however, have the potential to provide some answers to the key questions posed above.

Why does IgA deposit in the glomerular mesangium?

Altered O-linked glycosylation may:

- Reduce clearance of the various forms of IgA1 from the circulation via the ASGPR;
- Promote IgA1 binding to the mesangial cell FcγR and/or mesangial matrix proteins;
- Promote formation of nephritogenic IgA complexes.

How does deposition of IgA lead to glomerular injury?

Altered O-linked glycosylation may:

- Increase binding of deposited IgA1 to leukocyte FcγR and subsequent leukocyte activation;
- Activate mesangial cells via increased FcγR ligation, raising extracellular matrix production and initiating inflammatory cascades.

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References

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Peritoneal aspergillosis—pitfalls in the diagnosis of a rare disease

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Aspergillus spp. are filamentous fungi that are widely distributed in nature and contribute to the decay of organic debris. The most common species causing diseases in humans are Aspergillus fumigatus, A. flavus, and A. niger. Aspergillus is rarely isolated from culture specimens in the normal host, that humans are basically an insuitable environment for the growth of these fungi. Polymorphonuclear granulocytes, monocytes, and tissue macrophages effectively prevent the invasion and propagation by rapid intracellular killing of the hyphal forms.

On the other hand invasive or disseminated aspergillosis is always a very severe disease because it is difficult to treat and the outcome of manifest infections is very poor. Predisposing factors for invasive aspergillosis include almost always a seriously impaired host defence, such as prolonged neutropenia and defects in neutrophil function. Long-term administration of corticosteroids in high doses also predisposes to this infection because these drugs interfere with killing of conidia by macrophages and mobilization of neutrophils. Rare infections in normal hosts have been attributed to exposure to exceptionally high numbers of Aspergillus spores and infections following trauma to the eye, skin, bone and lung. Tissue damage by prior bacterial infection and long-term broad-spectrum antibiotic therapy has also been described as a risk factor in leukaemic patients and transplant recipients. The primary mode of acquisition of Aspergillus is the airborne route via inhalation of fungal spores. Superficial and locally invasive infections are rather uncommon and in most cases occur in immunocompromised patients.

With the exception of pulmonary fungal balls on chest X-ray, there are no characteristic signs of systemic aspergillosis. Especially in the early course of the disease, symptoms may be absent or may be masked by superimposed bacterial or viral infections. Routine microbiological cultures are of limited value and recovery of Aspergillus spp. is not indicative of infection, even if recovered repeatedly. Contamination of culture specimens may occur in the microbiology laboratory and may lead to false-positive results. Blood cultures are rarely positive even in cases of disseminated disease. Thus definitive diagnosis depends on histological examination of tissue sections obtained from endoscopic procedures or open biopsy. However, these investigations may be contraindicated in critically ill patients. Consequently invasive fungal infections often remain undiagnosed in life or are only detected at autopsy.

Immunological diagnostic procedures offer an alternative approach. Detection of antibodies directed against fungal metabolites or somatic antigens proved useful in immunocompetent patients, but have low sensitivity in immunosuppressed individuals in whom antibody response is usually poor. Another disadvantage is that seroconversion often occurs late in the course of the disease and is therefore of little value in establishing an early diagnosis. Immunodiagnostic tests based on antigen detection offer a more sensitive and specific tool in the diagnosis of systemic aspergillosis. The most reliable antigenic mycelial component of Aspergillus spp. is a group of galactomannan glycoproteins which are detectable in low concentrations during disseminated aspergillosis. However, as galactomannan antigens are rapidly removed from the circulation by the formation of immune complexes and by receptor-mediated endocytosis in the liver, antigenaemia is only a transient event. This fact results in a low overall test sensitivity, while specificity is generally close to 100%. An alternative approach is the detection of Aspergillus antigenuria which proved to be more sensitive because fungal antigens are excreted in the urine over a prolonged period in detectable concentrations.

In summary, diagnosis of invasive aspergillosis requires always a multifaceted approach including repeated conventional microbiological cultures from different body sites and combined serological tests for the detection of specific antibodies and antigens.

Against this background the case report in this issue of Tanis et al. about an Aspergillus peritonitis in a patient with peritoneal dialysis must be regarded critically. The authors describe a patient with no signs of immunosuppression, normal white-cell count, no fever, and no signs of peritonitis or exit-site infection. The only remarkable symptom was an ESR of 111 mm/h. A single dialysate culture, taken 9 days after initiation of a (successful) antibiotic therapy to treat a mixed

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