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# Study on the pathogenesis of IgA nephropathy focusing on IgA1 glycosylation and the lectin pathway of the complement system

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# Rannsókn á sykrun IgA1 sameinda og komplimentræsandi lektínum í IgA nýrnameini

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Art: as the spirit wanes the form appears.

c. bukowski

**This work is dedicated to my niece, Ingibjörg Jónsdóttir**

## ABSTRACT

IgA nephropathy (IgAN) is the most common form of primary glomerulonephritis in the world. It is characterized by IgA1 containing immune complexes that lodge in mesangial areas in the kidney glomeruli. Formation of the complexes is traced to defective glycosylation of O-linked glycans in the hinge region of the IgA1 molecule. Previous studies on IgAN and Henoch-Schönlein Purpura (HSP), which is a related disease, indicate that the complement system may play a significant role in the pathogenesis of these diseases.

The main aim of this study was to investigate whether aberrant glycosylation of IgA1 in IgAN might associate with abnormal levels or activation of the proteins involved in the lectin pathway of the complement system. Also included in the study were measurements of lectin pathway components and a complement activation product in a cohort of the HSP patients.

In the IgAN patients, two types of glycan divergence were observed in the IgA1 hinge region: a) increased N-acetylgalactosamine (GalNAc) in terminal position, and b) increased sialylation masking degalactosylated GalNAc. This is in accordance with the work of others. On the other hand, the levels of the lectins and associated proteins that can induce activation of the lectin pathway, namely MBL, L-ficolin and MASP-2 did not differ between the IgAN patients and controls. However, some striking differences were observed between HSP and IgAN patients regarding the proteins of the lectin pathway. Thus, the HSP patients had higher levels of MBL, L-ficolin and MASP-3 in the serum than both the IgAN patients and the controls. Most striking is a

marked increase in the serum levels of the MASP-3 in the HSP patients combined with relatively low levels of the C4d activation fragment. Furthermore, the HSP patients had decreased serum levels of C4 and increased prevalence of the C4B*Q0 allele, but this was not observed in the IgAN patients. Interestingly, in the IgAN patients low levels of IgA and IgA circulating immune complexes associated with increased galactosylation defects. This association was reversed regarding IgA2 deposits on biopsy sections. Patients with IgA2 in kidney deposits had significantly higher degalactosylated IgA1 when compared to other IgAN patients.

These results emphasize the divergent clinical manifestations of IgAN and HSP. The significantly increased levels of three lectin pathway components, together with low levels of C4, observed in the HSP patients, but not in IgAN, may indicate a major difference in the pathogenesis of these two related diseases. The findings do not exclude the involvement of the lectin pathway in the pathogenesis of IgAN as full investigation on functional activity of the components has not yet been conducted. The results also raise questions about the role of circulating versus locally produced complement components in the pathogenesis of IgAN and HSP. Defective galactosylation in the IgAN cohort, needs to be analyzed in the context of disease severity, activity and progression. These findings, therefore, need to be followed-up by a prospective study focusing on the relation between lectin pathway components and clinical and prognostic factors of the two diseases.

## ÁGRIP Á ÍSLENSKU

IgA nýrnamein (IgAN) er algengasta form gauklabólgu í heiminum og einkennist af IgA1 innihaldandi ónæmisfléttum sem setjast í mesangialsvæði nýrnagaukla. Myndun ónæmisfléttna er rakin til gallaðrar O-tengdrar sykrunar á hjörulið IgA1 sameinda. Fyrri rannsóknir á sjúklingum með IgAN og Henoch-Schönlein Purpura (HSP), sem er skyldur sjúkdómur, gefa til kynna að komplementkerfið eigi verulegan þátt í tilurð beggja sjúkdómanna.

Markmið þessarar rannsóknar var að kanna hvort sykrufrávik á IgA1 í IgAN tengdust óeðlilegu magni eða virkni sameinda sem þátt taka í lektínferli komplementkerfisins. Rannsóknin tók einnig til mælinga á lektínþáttum komplementkerfisins og ræsiafurð komplementþáttar C4 hjá sjúklingum með HSP.

Tvenns konar sykrufrávik greindust á IgA1 sameindum IgAN sjúklinga: a) aukin N-asetýlgalaktósamín (GalNAc) í endastöðu, og b) aukin síalsýra sem hjúpaði afgalaktósýlerað GalNAc. Þetta samrýmist niðurstöðum fyrri rannsókna. Enginn munur fannst á magni lektínþátta, það er MBL, L-fíkolín og MASP-2, milli IgAN sjúklinga og viðmiðunarhóps. Hins vegar höfðu HSP sjúklingar hærri sermisþéttni MBL, L-fíkolín og MASP-3 borið saman við bæði IgAN sjúklinga og viðmiðunarhópinn. Mest áberandi var hækkuð þéttni af MASP-3 hjá HSP sjúklingum sem einnig höfðu tiltölulega lágt magn af C4 og C4d ræsiafurðinni. Auk lítils magns af C4 höfðu HSP sjúklingarnir aukna tíðni af C4B*Q0 arfgerðinni, en þessi aukna tíðni C4B*Q0 fannst hins vegar ekki hjá IgAN sjúklingum. Athyglisvert var að lág þéttni IgA og IgA ónæmisfléttna í sermi tengdist auknu magni af IgA1 með gallaðri

galaktósýleringu. Þessu var hins vegar öfugt farið varðandi sjúklinga með IgA2 útfellingar á nýrnasneiðum; gölluð galaktósýlering hjá þeim var marktækt hærri en annarra IgAN sjúklinga.

Þessar niðurstöður endurspegla ólíkar sjúkdómsmyndir IgAN og HSP. Þannig gæti aukið magn lektínferilsþáttanna í sjúklingum með HSP, en ekki í IgAN sjúklingum, bent til mismunandi meingerðar þessara sjúkdóma. Niðurstöðurnar útiloka hins vegar ekki þátt lektínferilsins í sjúkdómsmyndun IgAN, þar sem rannsókn á virkni þessara þátta er enn ekki lokið. Niðurstöðurnar vekja ennfremur upp spurningar um hlutverk komplimentþátta sem eru staðbundnir í nýrum eða berast til þeirra með blóði í tengslum við meingerð þessara tveggja sjúkdóma. Gölluð galaktósýlering er hér staðfest í íslenskum IgAN sjúklingum, en áhrif hennar á alvarleika sjúkdómsins þarfnast frekari skoðunar með hliðsjón af klíniskri virkni og sjúkdómshorfum.

Þetta væri best gert með framskyggnri rannsókn þar sem athyglinni er beint að sambandi lektínþátta komplementkerfisins við klínisk einkenni og framþróun sjúkdómanna.

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# ABBREVIATIONS

| | |
|---|---|
| **ASGP-R** | Asialoglycoprotein Receptor |
| **Asn** | Asparagine |
| **BSA** | Bovine serum albumin |
| **C1-INH** | C1-inhibitor |
| **C4A – C4B** | Complement 4 A/B isotypes |
| **C4bp** | C4 binding protein |
| **CH1 – CH2** | Heavy chain constant regions 1-2 |
| **CIC** | Circulating immune complexes |
| **CR1** | Complement Receptor 1 |
| **C-type lectin** | Calsium dependent binding |
| **DAF** | Decay accelerating factor |
| **DM** | Diabetis mellitus |
| **EFA** | Essential fatty acid |
| **ELISA** | Enzyme-linked immunosorbent assay |
| **Fcα/μR** | Fc alpha/muReceptor |
| **FcαR1** | Fc alpha Receptor 1 |
| **FCN2** | L-ficolin encoding gene |
| **FDC** | Follicular dendritic cells |
| **FGF** | Fibroblast growth factor |
| **Gal** | Galactose |
| **GalNAc** | N-acetylgalactosamine |
| **GBM** | Glomerular basement membrane |
| **GFR** | Glomerular filtration rate |
| **GlcNAc** | N-acetylglucosamine |
| **HAA** | Helix arspesa antigen |
| **HLA** | Human leucocyte antigen |
| **HPA** | Helix pomatia antigen |
| **HR** | Homing receptor |

| | |
|---|---|
| **HSP** | Henoch-Schönlein purpura |
| **HSPN** | Henoch-Schönlein purpura nephritis |
| **ICAM-1** | Intercellular adhesion molecule-1 |
| **Ig** | Immunoglobulin |
| **IgA1 – IgA2** | IgA 1 – IgA 2 subclasses |
| **IgA1-CC** | IgA1 containing complexes |
| **IgA-ICs** | IgA immune complexes |
| **IgAN** | IgA nephropathy |
| **IL-1-6-8** | Interleukin 1-6-8 |
| **kDa** | kiloDalton |
| **mAb** | Monoclonal antibody |
| **MAC** | Membrane attack complex |
| **MASP** | MBL-associated serine protease |
| **MBL** | Mannan binding lectin |
| **MC** | Mesangial cells |
| **MCP** | Membrane cofactor protein |
| **M-CSF** | Macrophage colony stimulating factor |
| **MHC** | Major Histocompatability complex |
| **MIF** | Macrophage migration inhibitory factor |
| **NeuNAc** | N-acetylneuraminic acid |
| **PAF** | Platelet activating factor |
| **PAMP** | Pathogen-associated molecular pattern |
| **PDGF** | Platelet derived growth factor |
| **pIgR** | polymeric Immunoglobulin receptor |
| **RAA** | renin-angiotensin-aldosterone |
| **RF** | Rheumatoid factor |
| **rpm** | revolutions per minute |
| **RT** | Room temperature |
| **SLE** | Systemic lupus erythematosis |
| **SNP** | Single neucleotide polymorphism |

| | |
|---|---|
| **TCR** | T-cell receptor |
| **TfR** | Transferrin Receptor |
| **TGF- β** | Transforming growth factor-β |
| **TNF-α** | Tumor nectosing factor-α |
| **TXB2** | Thromboxane B2 |
| **UDP** | Uridin diphosphate |
| **VCAM-1** | Vascular cell adhesion molecule-1 |
| **VV** | Vicia villosa |

# INTRODUCTION

IgA nephropathy (IgAN) also known as Berger's disease, is the most common form of primary glomerulonephritis. It is characterized by immune complexes with high content of IgA1 (hence referred to as IgA1-CC) that lodge in mesangial areas in the kidney glomeruli. Since first described 40 years ago (Berger, *et al.*1968) its pathogenesis has escaped clarification. At first thought to be a benign disease, it is now known to carry life threatening consequences for a good number of the patients. The criterion for IgAN diagnosis is dominant or co-dominant IgA1 deposits in the kidney mesangium, i.e., IgA1 is the principal immunoglobulin in the deposits or co-locating with other immunoglobulins. This decisive factor has given rise to the idea that IgAN is a single entity. However, the diversity of its manifestations points to a group of diseases that have IgA deposition as a common denominator. (Barratt, *et al.*2005, Donadio, *et al.*2002, Julian, *et al.*2004).

Clinical symptoms expose the diversity of IgAN, they include; 1) a single bout of hematuria, followed by remission and, thereafter, normal renal function; 2) recurring bouts of hematuria and a gradual deterioration of the kidney function; 3) a rapid progression to kidney failure from the outset and finally, one may consider patients with Henoch-Schönlein purpura (HSP), a disease closely linked to IgAN and by many considered to be a systemic manifestation of the disease. The two diseases are indistinguishable from each other as judged by renal histopathology (Barratt, *et al.*2005, Peterman, *et al.*1991).

IgAN is considered to be a sporadic disease although a number of reports indicate that familial or regional clustering accounts for some cases, suggesting a genetic predisposition (Julian, *et al.*1988, Scolari.1999). For some time studies directed at the major histocombatability complex (MHC) region on chromosome 6 gave some promise in that direction, but HLA association has not been confirmed and, subsequently interest has moved elsewhere (Galla.2001, Julian, *et al.*1985). Familial IgAN has been linked to a locus on chromosome 6 containing 24 polymorphic markers (just distal to the MHC region) that gave a lod score of 5.6 (Gharavi, *et al.*2000) but this remains to be confirmed. In this study we did not explore the possibility of genetic association, although there were some indications for familial clustering.

The IgA1-CC sometimes observed in serum and characteristically found in the glomeruli of patients with IgAN triggered an extensive search for specific exogenous antigens. Food antigens such as gluten and microbial antigens of bacterial or viral origin have been extensively studied without success and the same can be said of potential antigens in immune complexes eluted from IgAN kidneys (Mestecky, *et al.*2008). The search for a causal agent has therefore been widened and includes now the structure and nature of the IgA molecule itself, as well as the physiological function of the kidney glomeruli (Feehally, *et al.*1999, Novak, *et al.*2001). This has led to studies on glycosylation of the immunoglobulin (Barratt, *et al.*2007, Novak, *et al.*2008) and the kidney environment, i.e., uptake of complexes by mesangial cell (MC) (Leung, *et al.*2001, Monteiro, *et al.*2002).

## 1. Structure and function of the kidney

The urinary system consists of paired bean shaped kidneys, ureter and a bladder. Of these organs the kidney is the most complex. Its chief function is to regulate the fluid and electrolyte balance of the body, i.e. maintain homeostasis by removal of waste products through blood filtration and urine production and to regulate blood pressure by production of the hormone renin.

The hilum is found at the concave medial border where nerves, blood and lymph vessels enter and exit, and the ureters exit carrying urine to the bladder. The lateral surface is convex. The interior structure of the kidney is divided into the outer cortex and the inner medulla and both serve the filtration process. Towards the hilum the renal pelvis is the receptacle where urine accumulates from the collecting ducts that run through the medullar pyramids. Distributed in the cortex are 1 – 4 million nephrons, the principal components of the filtering process. Each nephron consists of a dilated renal corpuscle - the glomerulus; the proximal convoluted tubule carrying filtrates out of the corpuscle into the loop of Henley, through to the distal convoluted tubule that empties into the collecting ducts. Only the loop of Henley and the extreme end of the ascending convoluted tubule reach into the medulla (Junqueira*, et al.*2005).

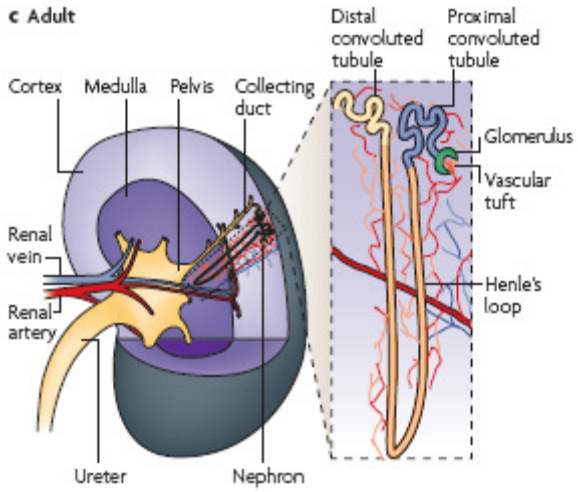


**Figure 1:** The structure of the human kindney and a nephron unit (Schedl.2007).

The kidney is densely vasculated. An intricate web of small capillaries feeds the extensive tubular system – while arterioles carry blood in and out of the glomerulus. In the renal corpuscle small particles (< 10nm in diameter or negatively charged elements of less than 70MW) are filtered over the basement membrane into the urinary space. This size-selective property makes the glomerular capillaries susceptible to damage when macromolecules get trapped in the basement membrane. Once immobilized these molecules may act as antigens for passing antibodies, resulting in obstruction and impairment of the filtering capacity of the capillaries.

After filtration the urine flows into the proximal convoluted tubule where the molecules that passed through the basement membrane are reabsorbed into the tubular epithelial cells, broken down in lysosomes and returned to the circulation. The nephrons located at the cortical-medullary junction have longer loops of Henley, which are responsible

for keeping the hypertonicity of the medullar environment, a prerequsite for production of concentrated urine.

The distal convoluted tubule touches the vascular pole of its parent nephron. This encounter between the distal tubule and the afferent arteriole brings about morphological changes both in the tubule and the arteriole. This particular segment of the tubule is called the macula densa. The cells of the macula are sensitive to the ionic content and fluid volume in the tubule, sending signals that promote secretion of the hormone renin. These signals are received by the cells of the afferent arteriole that are loaded with renin-filled granules. The renin – angiotensin – aldosterone axis (RAA) of the kidneys is responsible for the salt and water conservation which, further along controls the systemic blood pressure homeostasis.

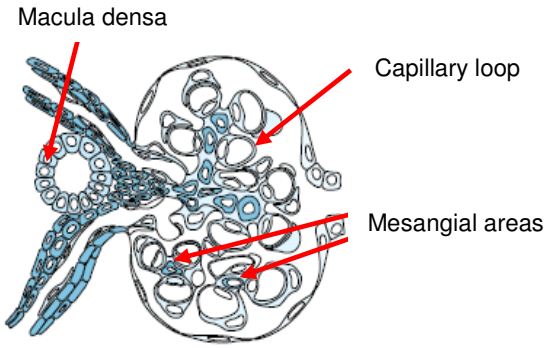


**Figure 2:** Schematic drawing of a glomerulus capillary loops, macula densa and mesangial areas (Atlas of Diseases of the Kidney, Vol. II, chapter 1. Series editor: Robert Schrier)

## 1.1. The glomerulus

The glomerulus is a vascular epithelial organ that is designed for ultrafiltration of the blood plasma. Its functional unit is enveloped by a capsule (the Bowman capsule) into which a stalk containing the arterioles

carrying blood to and from the filtrating apparatus is attached. The draining tubule is located opposite this entrance. The glomerular capillaries are lined by uniquely *fenestrated* endothelium that rests on a basement membrane. This endothelium is perforated by 100 nm pores, allowing plasma to filter onto the basement membrane. External to the glomerular basement membrane (GBM) are epithelial cells or *podocytes* that extend tentacles to the basement membrane. The effectiveness of the filtration process depends on the integrity of the basement membrane and its protective cells.

The capillary loops connect centrally and are supported by the mesangial cells. These cells are thought to be of mesenchymal origin (Junqueira*, et al.*2005).

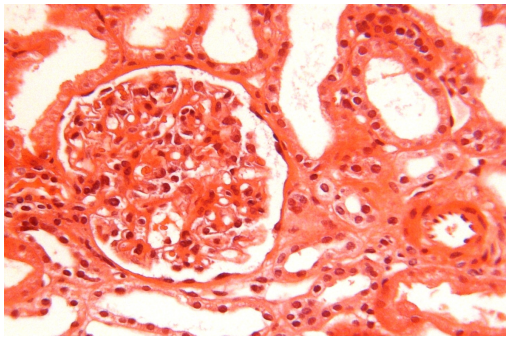

**Figure 3 :** Light microscopic appearance of an IgAN kidney biopsy. There is mesangial hypercellularity. The surrounding tubules and interstitium appears normal (HE staining) (image from S. Harðarson, 2009).

## 1.2. Mesangium

The mesangium is compiled of matrix made up of mucopolysaccharides and glycoproteins interspersed with mesangial

cells. These mesenchymal-like cells serve a variety of functions. They contain actomyosin and have smooth muscle contractile properties. They are responsible for laying down extra cellular matrix and collagen fibers, but their main task is to give structural support to the glomerular capillaries and to regulate the blood flow. This is achieved through the contractile action of micro fibrils that connect the extra cellular matrix to the basement membrane of the capillary loops. The mesangial cells have also been accredited with phagocytic capabilities.

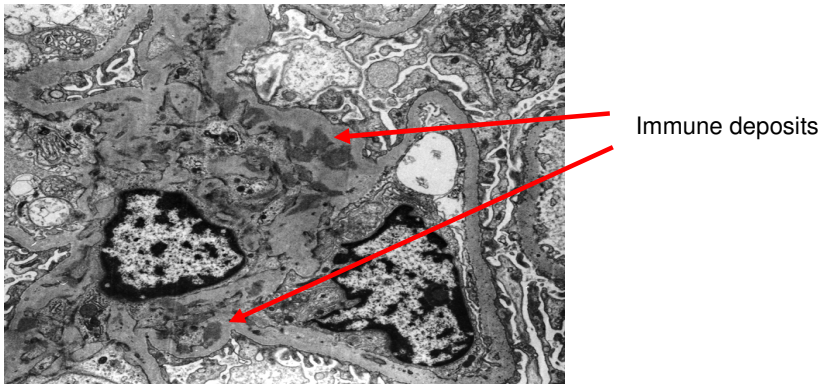


**Figure 4:** Electron microscopy of a glomerular mesangial area from an IgAN kidney containing mesangial cells and immune deposits (image from S. Harðarson, 2009).

Sternberg has reported the existence of a two cell populations in the mesangium: the smooth muscle fibrocyte and a specialized residential monocytic phagocyte derived from bone marrow (Sternberg.1992).

According to Sternberg, these macrophages make up 5-15% of the cell population in the mesangium, and the cells express Fc- and C3 receptors as well as MHC (Ia) molecules. They are responsible for the vast arsenal of chemokines and cytokines that can cause inflammation and can lead to destruction of the glomeruli. Essential fatty acids (EFA);

linoleate and arachidonate are accountable for recruitment of these macrophages into the glomerulus, assumed by the observation that dietary depletion of EFA rids the glomerulus of monocytes, while repopulation has been shown to take place after resumption of EFA (Sternberg.1992).

These residential macrophages are ascribed as active players in regulation of urine filtration through interaction with the smooth muscle fibroblasts and are also held accountable for clearance of debris from the mesangium. This function may be overstrained when the macrophages are overloaded with immune complexes and release cytokines that may cause direct mesangial damage to the mesangial environment. Distinction between these two theories is generally not clear in the literature and will therefore not be discussed further.

### 1.3. Cytokines and chemokines

The initiating factor in the pathogenesis of IgAN is considered to be deposition of immune complexes rich in IgA in the mesangium of the kidney followed by accumulation of leucocytes, leading to inflammation and, in some cases, irreversible glomerulosclerosis. Production of various inflammatory cytokines including tumour necrosing factor-alpha (TNF-$\alpha$), interleukin-1 (IL-1) and IL-6 cause increased expression of adhesion receptors such as inter-cellular-adhesion molecule-1 (ICAM-1) and vascular-cell-adhesion-molecule-1 (VCAM-1) on endothelial cells. Neutrophils, monocytes and T-cells accumulate and give rise to further release of chemotactic factors (Munro.1993, Yano*, et al.*1997). This co-operation leads to release of coagulation factors, such as platelet

activating factor (PAF), thromboxane B2 (TXB2) as well as super oxide anions, that enhance the inflammation (Chen, *et al.*1995,Schena, *et al.*1992).

The mesangial cells, as well as infiltrating macrophages, produce various mesangial growth factors such as platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), insulin-like growth factor-1 and transforming growth factor-β (TGF- β) which all participate in the inflammatory process. Proliferation of mesangial cells precedes increase in the extracellular matrix, which in turn stimulates mesangial cell to release cytokines that modulate PDGF receptor expression on mesangial cells (Haas.2005, Ruef, *et al.*1992, Tesch, *et al.*1997). Other cells in the glomerulus, including endothelial and epithelial cells, may also participate in the inflammation.

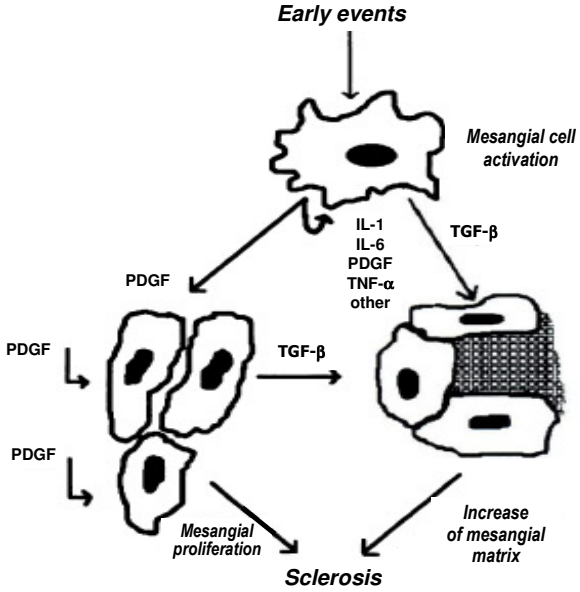


**Figure 5**: Involvement of cytokines as late pathogenic events in the progression of renal damage to sclerosis (Modified from Schena, *et al.*1992).

Mesangial cells are multifunctional. They do not only influence their own environment but are also self-regulating through production of macrophage-colony stimulating factor (M-CSF) and they can also inhibit macrophage departure from the glomerulus. Macrophage trafficking is controlled by the macrophage-migration inhibitory factor (MIF), which encourages macrophage accumulation (Leung*, et al.*2003b).

## 2. Kidney diseases

The complex nature of the kidney structure, development and function makes it susceptible to multiple malformations and defects. Many of these abnormalities arise during kidney development leading to abortion of the fetus, while others become apparent at birth or may present at a later date, even in adulthood.

Kidney diseases may be caused by several environmental factors, such as bacterial infections or toxic chemicals. They may also be caused by high blood pressure, metabolic diseases like diabetes mellitus (DM), immunological disorders including Goodpasture's syndrome and some purely heretitary conditions like Alport syndrome. Glomerular diseases may be *secondary* to systemic disorders such as systemic lupus erythematosus (SLE), amyloidosis or diabetes mellitus or *primary* disorders, where the kidney is the only or predominant organ involved.

Most glomerular diseases are immunologically mediated, caused either by circulating immune complexes (CICs) or by antibodies reacting directly within the glomerulus (Goodpasture syndrome). Antibodies may bind directly to intrinsic glomerular antigens or to immobilized antigens that have been trapped in the basement membrane. Deposition of immune

complexes can cause inflammation and lead to proliferation of the principal cells of the glomerulus. Associated clinical symptoms may be hematuria with diminished glomerular filtration rate (GFR) and hypertension (nephritic syndrome) or proteinurea followed by hypoalbuminemia (nephrotic syndrome). Manifestation of these symptoms may either present as insidious development of uremia secondary to a chronic glomerular disease or a rapid progression to acute renal failure (Kumar*, et al.*2003).

**Table 1:** Classification of kidney diseases affecting the glomerulus (modified from Robbins Basic Pathology, 2003, Saunders, Philadelphia, PA).

| |
|---|
| **Primary glomerulonephritis:** |
| Diffuse proliferating glomerulonephritis |
| Crescentic glomerulonephritis |
| Membranous glomerulonephritis |
| Lipoid nephrosis (minimal change disease) |
| Focal segmental glomerulosclerosis |
| Membranoproliferative glomerulonephritis |
| IgA nephropathy (IgAN) |
| Chronic glomerulonephritis |
| **Secondary (systemic) diseases:** |
| Systemic lupus erythematosus (SLE) |
| Diabetes mellitus |
| Amyloidosis |
| Goodpasture syndrome |
| Polyarteritis nodosa |
| Wegener granulomatosis |
| Henoch-Schölein purpura (HSP) |
| Bacterial endocarditis |
| **Hereditary disorders:** |
| Alport syndrome |
| Fabrys disease |

## 2.1 Glomerulonephritis of immunological nature

Immune mechanisms are the cause of pathological process in most patients with glomerulonephritis. This applies not only to primary glomeulonephritis but also to many of the secondary glomerular diseases. Complements, leukocytes, monocytes and platelets add to the antibody mediated damage by activating the complement system and releasing various cytokines, growth factors and proteases. Glomerulonephritis caused by circulating immune complexes is most common. Here the kidney may be considered an “innocent bystander”, as initiation of tissue damage can not be attributed to the kidney itself. CICs are formed by antibodies reacting to antigens originating within or outside the kidney. Such antigens may be: 1) cell debris as seen in many autoimmune diseases, such as SLE, 2) immunoglobulins (Ig) as in IgA nephropathy or 3) surface molecules originating from malignant tumors. CICs may also form during infections, after intake of drugs or inorganic salts.

Immune complexes are most commonly seen trapped in the mesangial area, but they may also be found along the GBM. On electron microscopy they appear as dense deposits. Immunofluorescence assays using specific antibodies will show immune complexes as granular or linear deposits of immunoglobulins and complement components.

Cell activation and release of proteases cause increased permeability of the GBM leading to nephrotic syndrome. Membranoproliferative glomerulonephritis type I and II, membranous glomerulonephritis and focal segmental glomerulonephritis present characteristically with nephrotic syndrome. Diseases presenting with nephritic syndrome include acute proliferative glomerulonephritis and

sometimes HSP and IgAN. Invasion of inflammatory cells or deposition of immune complexes into the glomerulus may affect all residential cells of the kidney. Damage is mediated by various means depending upon the mechanisms involved. Neutrophils release proteases and metabolites like nitrous oxide that affect the GFR, while T-cells and macrophages release cytokines such as IL-1 and TNF-α that augment the inflammation.

Formation of anti-GBM antibodies can cause a severe antibody mediated reaction in the kidney glomeruli (anti-GBM nephritis). Circulating or locally secreted complement factors are then activated leading to formation of the membrane attack complex (MAC: C5-C9). Residential cells react by secreting mediators that will induce increase in the extracellular matrix and thickening of the GBM follows. Direct immunofluorescence of a kidney biopsy will reveal this antigen-antibody reaction as a ribbon-like structure where the immunoglobulin deposits line up along the capillary basement membrane. GBM antibodies may cross-react with the basement membrane of lung alveoli leading to Goodpasture syndrome (Kumar, *et al.*2003).

## 2.2 IgA glomerulonephritis

Primary IgAN is the commonest glomerulonephritis in the world, accounting for up to 20% of renal biopsies in some countries (Clarkson, *et al.*1977, Kincaid-Smith.1985, Nakamoto, *et al.*1978). Peak incidence is in the second and third decades of life, although the disease may present at any age. Genetic variance has been noted and although the disease is not considered to be familial (Bisceglia, *et al.*2006, Julian, *et al.*1985, Tolkoff-Rubin, *et al.*1978) there is a racial and a geographical difference in its prevalence; Caucasians and Asians being more prone to

IgAN than Africans and South Europeans more than North Europeans. High incidence of the disease has also been reported in some American Indian tribes (Davin*, et al.*2001a, Galla*, et al.*1984, Julian*, et al.*1988).

IgAN may begin as an episode of gross hematuria, often following a nonspecific upper respiratory tract infection. The hematuria may last several days and then subside, only to recur again. IgAN was long held to be a benign disease, but it has now been shown to result in end-stage renal failure in about 1.5% of patients per year (Coppo*, et al.*2005, Galla.1995). Recurrence of IgA deposits is seen in about 60% of kidneys transplanted to IgAN patients (Floege.2004, Odum*, et al.*1994).

IgAN is an immune complex mediated disease. It is defined by deposits of immunoglobulin A1 (IgA1) in the glomerular mesangium as revealed by immunological technics. The IgA1 deposition is followed by leukocyte infiltration with subsequent increase in pro-inflammatory cytokines and chemokines; increased production of mesangial matrix, sclerosis and dysfunction of the kidney.

Despite an extensive search for a specific initiating agent none has yet been identified (Donadio*, et al.*2002). Genetic factors may be of importance but other causal mechanisms that have been suggested include defects in mucosal immunity, increased production of IgA1, defective glycosylation of IgA1 or defective immune complex clearance (Donadio*, et al.*2002). Studies have been hampered by the fact that IgA1 is only expressed in humans and humanoid primates, no small animal models are therefore available for studying IgAN (Galla.2001). Henoch-Schönlein Purpura (HSP) is by many considered to be a related disease.

## 2.3 Henoch-Schönlein purpura

Henoch-Schönlein purpura (HSP) is a systemic small vessel vasculitis that predominantly affects children. Clinical manifestations are purpuric rash found mainly on the buttocks and lower extremities. Among other symptoms is colic, arthralgia or arthritis. Macroscopic hematuria is seen in 30-50% of HSP patients (Stefansson Thors, *et al.*2005) and cronic renal disease afflicts another 20% of the patients (Davin, *et al.*2001b). Long-term renal failure ranges from 1-7% (Coppo, *et al.*1997, Koskimies, *et al.*1981, Stewart, *et al.*1988).

As serious kidney complications are much less frequent in HSP patients they are less likely to be subjected to kidney biopsies than IgAN patients, who usually do not develop systemic features and can therefore only be diagnosed by a kidney biopsy. HSP is nevertheless considered to fall within the category of IgAN associated diseases as both conditions have been known to occur consecutively in the same individual, both have been observed in pairs of identical twins, and they have identical kidney pathology (Barratt, *et al.*2005). Predicted survival after 10 years in biopsied HSP patients varies from 73% in a German cohort to 90% in an Italian cohort. A study undertaken in the United States predicted 80% survival after 20 years (Sanders, *et al.*2008).

The pathogenesis of HSP is yet not fully understood but most cases occur in the autumn or winter and disease onset is frequently related to respiratory tract infections. Numerous micro-organisms have been implicated, both viral and bacterial. Serum IgA is often elevated. Other laboratory findings are IgA containing immune complexes that lodge in vessel walls, neutrophil infiltration around the vessels and

increased cytokine levels, including IL-6, IL-8, TNF-α and TGF-β during the acute phase (Yang, *et al.*2008).

IgA1 containing immune complex deposition in the mesangium of kidney glomeruli is characteristic for both HSP nephropathy (HSPN) and IgAN. In addition, widespread deposits of IgA in vessel walls outside the kidneys are seen in HSP patients. Levels of IgA containing immune complexes have been reported in HSPN that may even exceed those reported in IgAN (Coppo, *et al.*1984). Immune complexes in HSPN patients are of special interest. Studies have shown that, like those produced in IgAN they are composed of IgA1-IgG or IgA1-IgA1 and contain incompletely glycosylated IgA1. Kidney deposits eluated from HSP patients also contain this degalactosylated IgA1 (Allen, *et al.*1998). However, numerous studies have shown that CICs from HSP patients peak at 19S or greater on sucrose gradient ultracentrifugation, while complexes in IgAN patients tend to be smaller, peaking at 7S-19S indicating a difference in complex size and composition (Davin, *et al.*2001a).

Complement activation is an important component of the immunopathogenesis of both IgAN and HSP. Hypocomplementemia has been reported but is usually transient or associated with deleted or silent C4A or C4B genes (C4A null or C4B null). Early reports showed high incidence of C4A and/or C4B null allotypes in both IgAN and HSP (Ault, *et al.*1990, McLean, *et al.*1984, Welch, *et al.*1987). In a recent Icelandic study HSP patients had a significantly increased frequency of the C4B null allele compared to healthy controls ($p=0.002$) and this was associated with a marked decrease in the serum concentration of the C4

component (Stefansson Thors, *et al.*2005). The increased frequency of C4 null alleles and the low C4 level is relevant as complement deficiency may impair complement mediated clearance of immune complexes (Saevarsdottir, *et al.*2007).

The risk for progression of HSP to HSPN is greater for adults and is associated with increasing proteinuria. The male: female ratio for HSP is approximately 1.5:1, and remission of HSP has been reported during pregnancy or sex-hormone therapy pointing to a pathogenic role of male hormones (Davin, *et al.*2001a).

## 2.4 Some other kidney diseases

Hereditary diseases of the kidney may be obvious at birth or their appearance may be delayed for some time after birth. Defects can either be X-linked or affect both sexes equally. Some diseases are caused by a combination of gene mutations or a combination of genetic and environmental factors (Kashtan.2005, Schedl.2007, Torres, *et al.*2006). Alport disease is a heriditary glomerular disease of a non immunological order. It is caused by a mutation in the collagen IV alpha-5 chain leading to irregular thickening and splitting of the glomerular basement membrane (Hudson, *et al.*1993). Alport disease leads to endstage renal failure. It is, however, a rare phenomenon. Fabrys disease is a rare X-linked lysosomal storage disorder caused by deficiency of α-galactosidase A (Zarate, *et al.*2008).

The prevalence of developmental renal abnormalities is approximately 0.2% and these account for up to a third of all prenatal

abnormalities (Schedl.2007). Of these, mechanical defects such as multiple or missing ureters are the most common. Unilateral renal agenesis occurs in about 1/5.000 births, while bilateral agenesis occurs in about 1/30.000 births. Renal hypoplasia and dysplasia are fairly common.

Hypertension or high blood pressure is a frequent cause of secondary glomerular damage. Blood pressure control is a complex phenomenon under the influence of both genetic and environmental factors. Diseases affecting the flow of blood through the vessels have detrimental effects on the kidneys function with its finely tuned mechanism. Regulation of blood pressure by the kidneys is dependent on the activation of the RAA. The RAA-axis is initiated in the juxtaglomerular region either as a response to reduced sodium content in the distal tubules or low blood pressure. Renin production leads to increased vasoconstriction through release of angiotensin I and II, causing peripheral resistance and retention of sodium and fluids. To counteract the vasoconstrictive action of angiotensin the kidneys produce vasodepressors (Kumar*, et al.*2003). Pathological thickening of arterial/arteriolar vessel-walls raises renin production as seen in malignant hypertention. Diabetes mellitus is commonly associated with hypertension. Patients with advanced glomerular disease usually do have hypertension.

Diseases that primarily affect kidney tubules and interstitium are generally caused by drug allergies, toxins or bacterial invasion. Infections of the urinary tract are most often limited to the bladder (cystitis). Pyelonephritis, i.e. suppurative inflammation of the kidney, is characterized by invasion of bacteria into the interstitium causing inflammation of the kidney parenchyma. Pyelonephritis is usually caused

by ascending bacteria from the bladder, however on occasion bacteria may enter the kidney through the blood stream. In its chronic form, pyelonephritis will leave scarring, kidney deformities and even chronic renal failure (Kumar*, et al.*2003).

Urolithiasis (kidney stones) causes obstruction of urine outflow. Stone formation may occur at any level within the urinary collecting system, but they arise most frequently within the kidney. About 75% of stones are composed of calcium oxalate or calcium phosphate (Kumar*, et al.*2003).

### 3. Immunoglobulin A

Immunoglobulin A (IgA) is the main immunoglobulin protecting mucosal surfaces of the body against microbial invasion of potential pathogens. IgA is, like other immunoglobulins a tetramer consisting of two heavy chains and two light chains. The light chains, containing the variable binding sites, sit on the forked arms of the heavy chains (Fab) that then come together to form a single stem (Fc). IgA antibodies are of two subclasses, IgA1 and IgA2. Plasma cells associated with the gastrointestinal- or the respiratory tract produce both subclasses, while serum IgA is mainly synthesized in the bone marrow and tonsils. IgA is the most abundant immunoglobulin in body secretions but the serum concentration of IgA is only about one-fifth of that of IgG. However, the turnover of IgA is more rapid and it is estimated that twice as much IgA than IgG is produced in the body each day, i.e. approximately 70 mg IgA/kg of body weight (Kerr.1990, Novak*, et al.*2001, Yoo*, et al.*2005).

Distribution of the two IgA subclasses differs in various body fluids (fig. 6). IgA1 is most abundant in serum and nasal fluid, accounting for approximately 90% of the total IgA in serum. However, relatively more IgA2 is produced in the gut, especially the colon. There it gives protection against bacteria and alimentary antigens that might otherwise bind to intestinal epithelial cells causing disruption of the defence barrier.

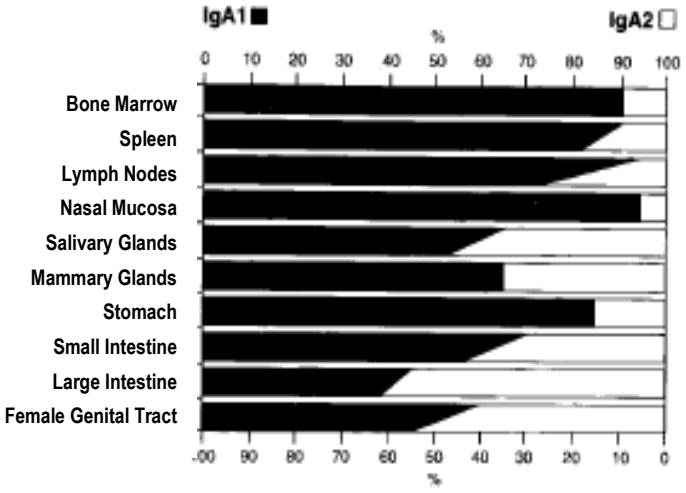


**Figure 6:** The proportions of IgA1 and IgA2 producing cells in different human tissues (as percentage of all IgA producing cells in each of the tissues) (Novak, *et al.*2001).

IgA in the gut is secreted by submucosal B-cells of the lamina propria and lymphoid nodules. It travels through epithelial cells into the luminal space as a dimer, i.e. two IgA molecules linked by a single J-chain (fig. 7). This dimer is chaperoned across the epithelial cell aided by a poly-Ig receptor (pIgR). On exit, at the apical surface of the the cell, this pIgR is enzymatically cleaved leaving only a portion of the receptor

attached to the IgA dimer. This appendix is called secretory component. Both the J-chain and the secretory component add N-linked glycans to the dimer (Roitt*, et al.*2006).

Increased levels of polymeric IgA (pIgA) have been noted in the serum of IgAN patients. pIgA together with its J-chain is synthesized by B-cells stemming from the bone marrow and tonsils (Bene*, et al.*1993, Harper*, et al.*1995, Tomana*, et al.*1999).

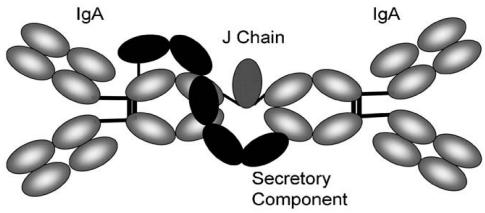


**Figure 7:** Dimeric IgA with a J-chain and secretory component (Arnold*, et al.*2006).

Like other immunoglobulins the IgA molecule is a glycoprotein and the glycosylation affects both its structure and its function. Both isotypes carry N-linked carbohydrates. Two conserved sites are present in the Fc-region on both isotypes: one at Aspargine 263 (Asn) and another at Asn-459. IgA2 has additional N-linked glycans, one in constant region 2 ($C_H2$) and a variable number of glycan sites on the Fab arms (Arnold*, et al.*2006). This variability in glycosylation of the two subclasses may give a clue to differences in their physical distribution and effectiveness in the fight against microbes. High number of N-linked glycans, such as terminal mannose and N-acetylglucosamine (GlcNAc), increase the binding properties of IgA2 to lectin-like molecules, that are found on various bacteria in the gut, thus facilitating their removal

(Arnold, *et al.*2006). IgA1, lacking the additional N-linked glycans is more dependant on antigen recognition by the variable binding sites on the Fab arms.

The main structural difference of the two subclasses is the O-linked glycans found in the hinge region of IgA1 situated between the $C_H1$ and $C_H2$ on the antibodies heavy chain. The flexibility of the hinge region in IgA1 allows for greater mobility of the Fab parts compared to the stationary Fab region of IgA2. The O-linked glycans make IgA1 practically unique for a serum protein as O-linked glycans rarely occur in other circulating proteins (Allen, *et al.*1995, Smith, *et al.*2006a).

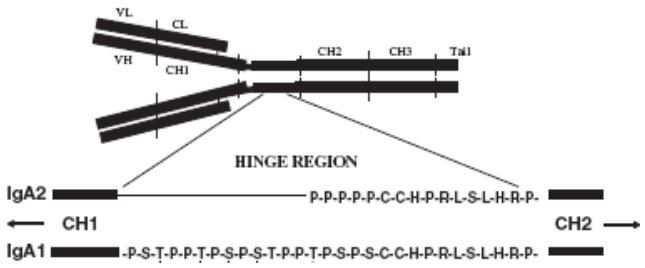


**Figure 8:** The hinge region of human IgA1 and IgA2 including the potential structures of O-linked glycan (Mestecky, et al.2008) .

Defects in the galactosylation of the O-linked glycans of IgA are believed to be of major importance in IgAN (Barratt, *et al.*2007, Gharavi, *et al.*2008). Immune complexes contain these defective glycans are consistently found in glomerular deposits of IgAN patients (Allen, *et al.*2001). These IgA containing immune complexes (IgA-IC) are believed to be important to disease development in IgAN. More important than increased serum levels of IgA, as raised levels of IgA are often found in

diseases like colitis ulcerosa, Crohn´s disease, some liver diseases and HIV, without leading to IgAN (Donadio*, et al.*2002) .

## 3.1 Immune complexes

Antibody production is of primary importance in the adaptive immune response. Binding of antibodies to antigens through the hypervariable domain in the Fab-region is part of the natural clearing process of foreign antigens. This process may give rise to immune complex formation in an interwoven network of antigens and antibodies, Immune complexes mediate effector functions, such as complement activation and opsonization through the Fc-region of the antibodies. Clearance of circulating immune complexes is facilitated by complement receptors on red blood cells that help transporting the complexes to the liver and spleen. IgA-ICs are cleared by means of asialoglycoprotein receptors (ASGP-R) on liver cells (Kupffer cells) or through interaction with localized phagocytes bearing Fc-alphaReceptor1 (FcαR1, CD89). Once complexes are taken up by phagocytes they are broken down and recycled (Monteiro*, et al.*1990, Stockert.1995).

IgA rich immune complexes may develop by at least three distinct mechanisms: 1) interaction of IgA with circulating peptides, 2) self-aggregation through altered glycosylation of IgA and 3) formation of complexes with soluble FcαR1. Mechanisms 1 and 2 will be addressed here, while the third is discussed under the heading of *Receptors*. It has been suggested that the hepatic clearance of IgA1 containing immune complexes in IgAN might be reduced, while mesangial cell uptake of

polymeric IgA1 molecules is increased (Feehally, *et al.*1999, Ivan, *et al.*2008).

Search for an external antigen has for years been the focus of IgAN research. This work has been hampered by the fact that IgA-ICs also occur in healthy individuals complicating identification of IgAN specific antigens. Interest in microbe and food antigens as a causal agent in IgAN has declined in recent years (Jackson, *et al.*1992, van Es, *et al.*1988), shifting the attention increasingly to the structure of the IgA molecule itself (Allen, *et al.*1995, Tomana, *et al.*1999). There is now a general consensus that defects or alterations of the O-linked glycans in the hinge region of IgA may be considered the primary cause of IgA-ICs in IgAN (Kokubo, *et al.*2000, Tomana, *et al.*1999).

Serum IgA1 from IgAN patients has been shown to contain an increased proportion of degalactosylated N-acetylgalactosamin (GalNAc) when compared to healthy individuals (Moldoveanu, *et al.*2007). Anti-GalNAc antibodies are found in serum of all IgAN patients and to a lesser degree in the blood of healthy individuals, cord blood and intravenous gammaglobulin preparations (Jackson, *et al.*1987a, Jackson, *et al.*1987b, Tomana, *et al.*1999). Cells secreting such antibodies are more numerous in IgAN patients than in healthy population. The origin of these antibody is uncertain, but production might be initiated by any number of micro-organisms expressing terminal GalNAc that infect the human body. It has been suggested that these antibodies might then cross-react with O-linked glycans on human IgA1 and thereby become the main source of immune complexes in IgAN (Mestecky, *et al.*2008, Tomana, *et al.*1999). Suzuki suggests that these anti-GalNAc antibodies, especially of the IgG type, might represent a disease specific marker as

elevated levels of these antibodies are seen to correlate with proteinuria (Suzuki, *et al.*2009).

Immune complexes in serum from IgAN patients may also contain IgA, IgG or IgM antibodies (Donadio, *et al.*2002) against diverse antigens, including fibronectin (Cederholm, *et al.*1988), IgA secretory component (Oortwijn, *et al.*2006) and rheumatoid factor (RF) (Czerkinsky, *et al.*1986). These antibodies may impede the immune complex clearance but, otherwise their participation in the pathogenesis is unclear. Self-aggregation of IgA1 is thought to contribute directly to the pathogenesis of the disease. Aggregation of IgA1 may partly be due to exposed degalactosylated GalNAc, but aberrant sialylation of negatively charged molecules is thought more likely to be the culprit. Several studies show that pIgA (J-chain containing degalactosylated dimeric IgA1) has a greater tendency to aggregate than monomeric IgA and mesangial cells show substantially greater affinity for this aggregated IgA1 (Almogren, *et al.*2008, Kokubo, *et al.*1998, Moura, *et al.*2004b).

IgA1–IgG containing immune complexes in the urine are shown to be of medium size, i.e. about 650-850 kDa and their levels in the urine of IgAN patients are significantly higher than similar complex in other proteinuric patients. (Matousovic, *et al.*2006, Mestecky, *et al.*2008). IgA1 complexes eluated from IgAN and HSP kidneys were shown to be more heavily degalactosylated than serum IgA complexes from the same patients (Allen, *et al.*2001) .

Complement mediated clearance of IgA containing immune complexes was, until recently, not seriously considered. Older studies had shown that the classical pathway was not activated by IgA. This left the alternative pathway as the sole complement activation pathway in

IgAN. Discovery of the lectin pathway cast a new light on complement activation and according to one report IgA may be capable of activating complement through mannan binding lectin (MBL) (Roos, *et al.*2001) but Thiel and Jensenius could not demonstrate MBL binding to IgA (personal communication). However, although Terai could not demonstrate complement activation by natural IgA he showed that MBL could bind denatured IgA and degalactosylated IgA1, making the connection to IgAN possible (Terai, *et al.*2006). MBL has been demonstrated in mesangial deposits in biopsies from some IgAN patients (Roos, *et al.*2006), and this echoes reports from a Japanese group that found MBL and other lectin pathway proteins in mesangial kidney deposits in up to half of IgAN and HSPN patients. This group maintains however, that the presence of these components is always co-located with IgA2 (Hisano, *et al.*2001).

## 3.2 Receptors

Deposition of IgA1–CC in the mesangium of the kidney is the diagnostic criteria for IgAN, but it has proven elusive to pinpoint the exact nature of the immune complex uptake and the receptor involved. It should be noted that none of the commonly known IgA receptors, i.e. pIgR, FcαR1 (CD89) or ASGP-R are expressed on mesangial cells (Leung, *et al.*2000, Monteiro, *et al.*2002, Moura, *et al.*2005).

Of late, attention has been turned to a non-classical IgA receptor detected on mesangial cells. This is the transferrin receptor (TfR, CD71) whose main role is to transfer iron from the circulation into cells, but immune complex uptake may be their secondary function as they have

been shown to be highly expressed on mesangial cells from IgAN patient. TfR bind avidly to the polymeric form of IgA1 (pIgA1) and are seen to co-locate with IgA1 deposits in the mesangium. They do not, however bind to IgA2. How IgA2 antibodies come to lodge in the mesangium is yet another unanswered question (Moura*, et al.*2004b, Moura*, et al.*2001, Julian*, et al.*1999, Moura*, et al.*2005, van der Boog*, et al.*2002). Other receptors may also be involved in IgA uptake. One such might be the Fcα/μR, a transmembrane glycoprotein receptor capable of binding to the Fc portion of IgA and IgM. McDonald reported the Fcα/μR to be expressed on mesangial cells, but whether it participates in the pathogenesis of IgAN has yet to be established (McDonald*, et al.*2002).

A French group led by Monteiro has proposed that immune complex uptake in IgAN is brought about by the TfR receptor and suggests a model in which two receptors participate (Monteiro*, et al.*2002). Their work on human mesangial cells shows that pIgA1 binds avidly to FcαR1 on phagocytes, causing instability in the receptor leading to its shedding. This is followed by recognition and uptake of the pIgA1-FcαR1 complexes by TfR on mesangial cells (Fig. 9). The complex binding can be blocked in two ways: 1) by circulating soluble TfR1 and TfR2 ectodomain fractions and 2) by application of a monoclonal antibody (mAb) against the TfR. TfR binding to IgA-ICs is increased when hypoglycosylated IgA is present. Both O-linked and N-linked glycans participate in the uptake (Launay*, et al.*2000, Moura*, et al.*2008).

In this model it is assumed that when FcαR1 binds degalactosylated IgA1 it induces shedding of the ectodomain portion of the receptor, increasing the presence of a 50-70 kDa soluble fraction of

FcαR1 in the circulation. According to Monteiro not only does degalactosylation destabilize the cell-bound FcαR1; it also plays a part in immune complex uptake. Degalactosylated IgA1 amplifies human mesangial cell uptake of immune complexes 3-4 fold by inducing upregulation of the TfR receptors on the cells. Proliferation of the mesangial cells is dependent on this TfR engagement. These results have been confirmed by experiments on transgenic mice that serve as models for spontaneous development of IgAN (Monteiro, *et al.*2002).

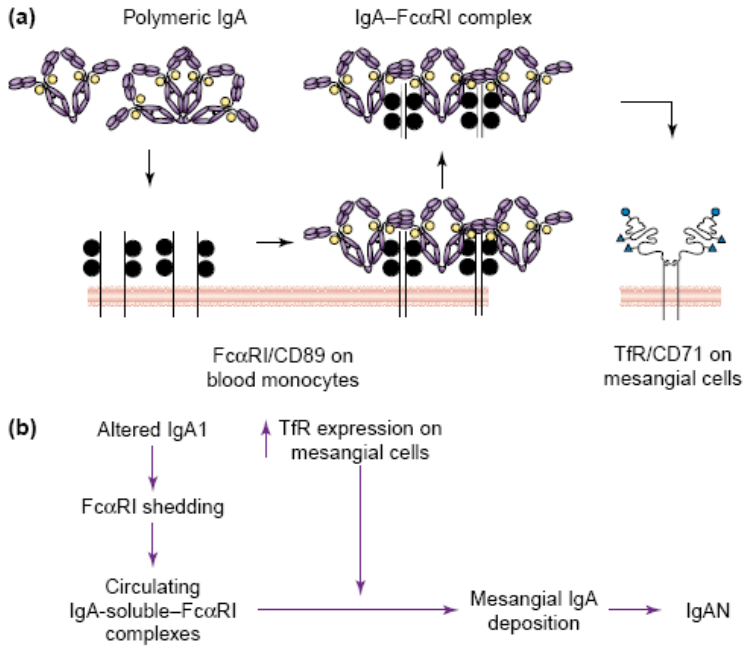


**Figure 9:** (a) Shedding of IgA1 immune complexes from FcαRI receptrors, forming IgA1–FcαRI complexes. IgA1–FcαRI complexes may be deposited in the kidneys via TfR/CD71 expressed on mesangial cells. (b) Proposed sequence of events leading to IgA deposition and IgA nephropathy (Monteiro, *et al.*2002).

## 3.3. The role of T-cells in IgAN

Class switching to IgA and increased serum pIgA1 in IgAN may be caused by abnormal function or regulation of T lymphocytes. Numerous studies have shown exaggerated and prolonged pIgA1 systemic response to both systemic and mucosal challenge in IgAN, at the same time pIgA response at mucosal sites is often decreased. Initially, circulating pIgA in IgAN was believed to be of mucosal origin, but was later traced to bone marrow B-cells. That is, a systemic response driven by mucosally encountered antigens (Batra*, et al.*2007). This has lead the search to the usual suspect; the T-cell.

T-cells are regulators of mucosal IgA production and oral tolerance wielding their effect through cytokine production or cell-cell interaction. T-cells recognize antigens through a set of receptors known as T-cell receptor (TCR); variable region heterodimers αβ and γδ. Antigen recognition appears different between the two sets of T-cells. There still is much to be discovered about the role of γδ cell, but some collaboration between the two cell types may exist. The γδ T-cells may operate closer to the innate response, as their activation does not always require antigen presentation by MHC class I and class II molecules. Steering away from the lymphoid pathway the γδ T-cells take up residence in non lymphoid organs like lungs and intestine where they seem to monitor epithelial cells for infection and damage. Diverse effector functions are attributed to the γδ T-cell population. They are seen to be increased in circulation after systemic antigen challenge but their main function is believed to be as contributors to mucosal B-cell

response. Unlike CD4+ αβ T-cells, γδ T-cells tend to be biased towards Th1 and away from Th2 response (Hayday, *et al.*2000). Irregularities of γδ T-cells have been noted in IgAN. Enhance IgA class switching in naïve IgD+ B-cells is seen to correlate with an increase in a subset of γδ T-cells (Toyabe, *et al.*2001). Moreover, a subset of γδ T-cells expressing the Vγ3 and Vδ3 families is reported reduced in the duodenum of the patients (Olive, *et al.*1997). Yet another study shows this reduction to be evident in the bone marrow (Buck, *et al.*2002). Diminished number of γδ T-cells in these tissues may aid and abet the abnormal production of IgA in IgAN patients.

pIgA producing B-cells have been reported to be reduced in the duodenum in IgAN patients (Harper, *et al.*1994), but increased in the bone marrow (Harper, *et al.*1996). It has been suggested that altered homing of lymphocytes may account for this deviation. A study on T-cell homing receptors in IgAN patients shows reduced L-selectin on CD3 and CD4 T-cells coinciding with an increased expression of the systemic homing receptor α4β1. Difference in expression of the mucosal homing receptor α4β7 between patients and controls was not observed (Batra, *et al.*2007). Down regulation of L-selectin and increased expression of the regulatory molecule α4β1-integrin indicate increased activation of the CD4 subset in IgAN and increased T-cell trafficking into systemic effector sites.

Increased traffic of effector T-cells primed by specific antigens at mucosal sites into the bone marrow may be the underlying cause of aberrant systemic pIgA1 production in IgAN patients (Smith, *et al.*2006b). This may be supported by diminished regulation of γδ cells in

the gut, the hypotheses being that mucosally activated effector memory CD4 T-cells (Tem) directed to the bone marrow by surface bound homing receptors, may encounter a secondary signal needed for clonal expansion and, thus, feed the continuous loop of abnormal pIgA production (Batra*, et al.*2007). The secondary signal may not need to be of the same microbial origin initially encountered; it need only carry similar antigenic properties. This $T_{em}$ - antigen encounter might also take place within the circulatory pathway that T-cells are known to migrate, i.e. on their route between bone marrow and non-lymphoid organs (Di Rosa.2009).

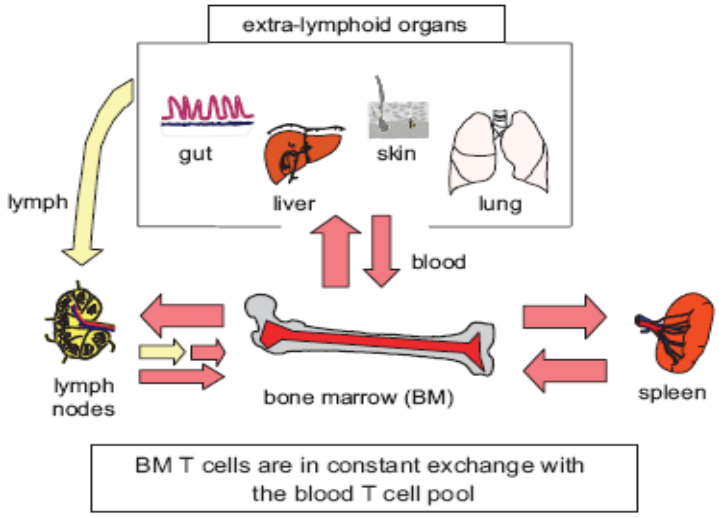


**Figure 10:** Schematic presenatation of the recirculatory pathways for bone marrow T-cells. Mature T-cells get in and out of the bone marrow via the blood (Di Rosa.2009)**.**

## 4. Glycosylation

Glycosylation takes place in the Golgi system concurrently with and immediately after protein synthesis. It is a process where saccharides are enzymatically linked to proteins in a step-wise manner to form glycoproteins. Glycans are found on cell surfaces, were they may be linked to proteins or they may be secreted in free form. Their structure as well as their presence or absence on proteins may strongly influence the effector function and binding specificities of the proteins.

Immunoglobulin A is a glycoprotein that carries both N-linked and O-linked glycans. N-linked glycans are attached to asparagine (Asn) residues found in the Fc region of both IgA isotypes; one near the terminal C-end of $C_H2$ at Asn residue 263, while a second one is situated at the tail-end at position 459.

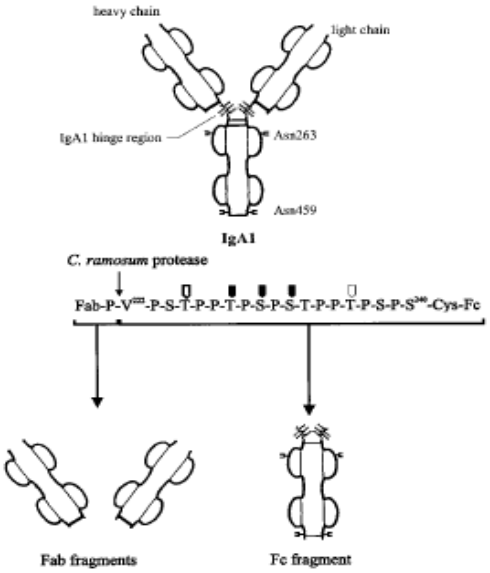


**Figure 11:** Structure of the IgA1 molecule. The glycosylation sites present on the hinge region (proline rich amino acid string and O-linkage sites) and the Fc fragment (Asn263 and Asn459) fragment are shown (Mattu*, et al.*1998).

Of the two isotypes IgA2 is more heavily glycosylated. IgA2 carries an additional N- linked glycan on the $C_H2$ segment as well as a variable number of N-linked glycans on each Fab arm. The number of N-linked glycans in the IgA2 Fab portion define its allotype (Yoo, *et al.*2005). Absence of N-linked glycans on the Fab arms of IgA1 is reported by majority of investigators, however, one study describes N-linked glycans on the Fab arms in approximately 30% of IgA1 molecules (Mattu, *et al.*1998).

N-linked glycans on the IgA molecule comprise several binding sites for lectins. Biantennary complex carbohydrates are found at residue 263, while the tail-end glycans are mainly triantennary. N-linked glycans attach at the Asn position as a core consisting of one mannose and two N-acetylglucosamine (GlcNAc) moieties. Fucose and GlcNAc may or may not bisect the core, while the biantennary branching takes off from the mannose. Mannose, fucose and GlcNAc may be found in terminal position, but analysis of pooled monomeric IgA1 show N-linked glycans to be approximately 80% sialylated. Furthermore, the J-chain connecting two IgA molecules, forming the dimer, carries one N-linked glycan and the secretory piece carries an additional seven (Mattu, *et al.*1998).

O-linked glycans are generally associated with cell membranes or membrane-bound proteins, but rarely on free circulating proteins. Of the three known serum proteins carrying O-linked glycoforms (IgA1, IgD and C1-inhibitor (C1-INH)) IgA1 is the most abundant. O-linked glycans on IgA1 are found in the hinge region, where a proline rich string of 17 amino acids connects the $C_H1$ to $C_H2$. There GalNAc is found in O-linkage to serine (Ser) or threonine (Thr) residues. Nine potential binding sites exist, but in the IgA1 hinge region O-linkage occurs only at Thr

228, Ser 230 and Ser 232. To make up the basic unit the enzyme UDP-N-acetylgalactosyltransferase-2 catalyzes GalNAc to the hydrogen oxygen side chains of the protein backbone. Galactose (Gal) may then occupy the GalNAc site or the GalNAc may take on sialic acid (NeuNAc). Sialic acid may attach to one or both glycans. Different sialyltransferases participate in each event. The commonest O-linked glycan structure on IgA1 is; GalNAc-β1,3GT-Gal-α2,3-sialyltransferase (α2,3-ST), while α2,6- ST arrangement links sialic acid directly to GalNAc (Yoo*, et al.*2005).

Many pathogenic bacteria have developed means to evade elimination by producing proteases that affect immunoglobulin structure and function. Absence of N-glycans, especially at the $C_H2$ - $C_H3$ junction, has been shown to increase susceptibility to cleavage by some bacteria (Chintalacharuvu*, et al.*2003). IgA1 proteases from various species of *Streptococcus* cleave the IgA1 hinge region, separating the Fab parts from the Fc region, thereby rendering the antibody ineffective. Cleavage takes place where proline residues sit adjacent to Ser or Thr residues. (Batten*, et al.*2003, Yoo*, et al.*2005).

## 4.1. Defective glycosylation of IgA1

As stated earlier, the search for a causal agent in IgAN has mainly been directed towards microbial or food born antigens. However, this search for exogenous antigens has not been rewarding.

Currently, the general consensus is that the epitopes provoking immune complex formation in IgAN are located in the hinge region of the IgA1 molecule, i.e. where the O-linked glycans are positioned. This

conclusion was originally reached on the premise that serum IgA1 from some IgAN patients showed reduced reactivity to jacalin. Jacalin is a lectin that binds selectively to the β1,3 glycosidic bond linking Gal-GalNAc and has been used for IgA isolation (Andre, *et al.*1990). The observation brought the focus onto the carbohydrate moieties in the hinge region and has since been followed up with extensive glycoresearch. Several reports on defective glycosylation of IgA1 in IgAN patients have now emerged. Two defects have been identified; the degalactosylation that is seen to participate in immune complex formation and the over-sialylation that is believed to play a critical role in mesangial cell uptake of the complexes (Allen, *et al.*2001, Coppo, *et al.*2004, Leung, *et al.*1999, Tomana, *et al.*1999). The glycosylation defect is restricted to O-linked glycans on IgA1 secreted by bone marrow B-cells. O-linked glycans on other circulating proteins (IgD and C1-INH) and N-linked glycans are not affected. The synonyms defective galactosylation or degalactosylation of IgA1 refer either to exposed GalNAc; a pattern seen to resemble that on various microbial surfaces or it may apply to the blocking of the galactose site on GalNAc by sialic acid. B-cell diversity accounts for each variant of the glycans and only a small proportion of B-cells is responsible for the galactose deficient IgA1 pool (Smith, *et al.*2006b). Degalactosylation is not entirely an aberrant phenomenon as it may also occur in healthy individuals, albeit at a much lower level.

J-chains are consistently detected in the mesangial deposits of patients with IgAN, where they co-locate with under-galactosylated IgA1. The J-chains involved are produced by the same B-cells that produce the degalactosylated IgA1 (Bene, *et al.*1982, Harper, *et al.*1994). Degalactosylated dimeric IgA1 has an increased tendency to self-

aggregate, whereby augmenting the size of the immune complexes (Iwase*, et al.*1999, Yan*, et al.*2006).

O-linked glycans form at least five distinctive patterns (fig. 12, table 2): I) the basic unit N-acetylgalactosamine (GalNAc) O-linked to Ser/Thr: II) sialic acid linked by α2,6 sialyltransferase (ST) to GalNAc: III) galactose taking terminal position on GalNAc: IV) α2,3-ST linked sialic acid in terminal position on GalNAc-Gal complex and V) a glycan complex with α2,3ST and α2,6ST sialylation (over-sialylation) (Tomana*, et al.*1999). Pattern I and II are considered defective galactosylation while patterns III to V are normally galactosylated IgA1 and patterns II and V show abnormal sialylation or over-sialylation.

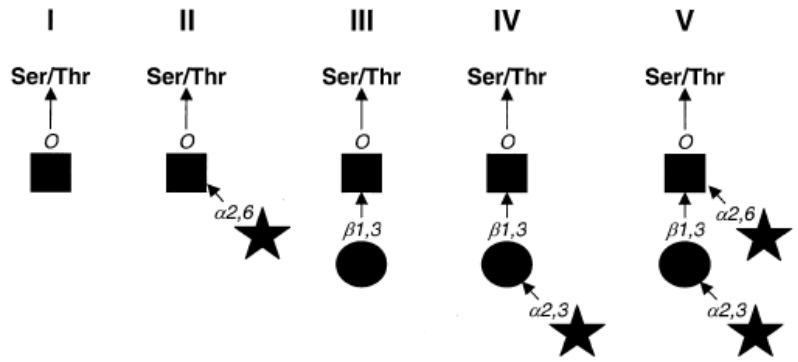


**Figure 12:** Different possibilities for O-linked glycosylation of the IgA1 hinge region. Black cubes represent N-acetylgalactosamine (GalNAc), black circles represent galactose and the black stars are sialic acid (Smith*, et al.*2003).

**Table 2:** Main characteristics of IgA1 hinge region galactosylation (see fig. 12).

| Pattern | Galactosylation | Characteristics |
|---|---|---|
| I | Defective | No galactose |
| II | Defective | No galactose, sialic acid in position α2,6 |
| III | Normal | Galactose in position β1,3, no sialic acid |
| IV | Normal | Galactose in position β1,3, sialic acid in position α2,3 |
| V | Normal | Galactose in position β1,3, sialic acid in positions α2,3 and α2,6 (over-sialylation) |

Sialic acid is found on all human cells, where it participates in cell protection against complement activation. Sialylation of O-linked glycans on IgA1 molecules results from activation of two sialyltransferases; the α2,3-ST-Gal and α2,6-ST-GalNAc. These transferases link sialic acid to their respective glycan. Sialylation also alters the tertiary structure of proteins and their electrostatic charge. It is responsible for the negative charge of IgA and increased sialylation of IgA1 may be a factor in diminished immune complex clearance through the asialoglycoprotein receptors (ASGP-R) of the liver (Leung, *et al.*1999). Not all of the IgA in circulation is negatively charged; the greater part is cationic, but only the anionic fraction is considered to be pathogenic in IgAN. This has been demonstrated in several studies where cultured human mesangial cells show high affinity binding to anionic polymeric IgA1 (pIgA1), while monomeric IgA1 (mIgA1) from the same patients is not taken up (Leung, *et al.*1999, Leung, *et al.*2001, Monteiro, *et al.*1985) Leung also found that the larger the complexes the stronger

is the affinity (Leung, *et al.*2003a, Moura, *et al.*2005, Moura, *et al.*2004a, Novak, *et al.*2002). Furthermore, it seems that large immune complexes (>800 kDa) of galactose deficient IgA1 are not only more appealing to the mesangial cells they are also more stimulating for their proliferation. Thus, immune complexes collected during bouts of macroscopic hematuria stimulate mesangial proliferation more than complexes collected during quiescent periods (Novak, *et al.*2005a, Novak, *et al.*2002).

A number of techniques have been used to assess the glycosylation status of IgA1. These include mass spectroscopy (Hiki, *et al.*1998, Yasuda, *et al.*2004), chromatography (Allen.1999, Allen, *et al.*1999) and lectin binding (Allen, *et al.*1995, Coppo, *et al.*1993, Moldoveanu, *et al.*2007, Moore, *et al.*2007). Although these methods are not fully comparable, they all agree on reduced galactosylation of IgA1 in IgAN patients, while conflicting results have been reported for sialylation; depending on the method sialylation has been shown to be either increased or decreased. Altered sialylation, as well as degalactosylation, are now considered central to IgAN pathogenesis. (Allen, *et al.*1999, Leung, *et al.*1999, Matsushita, *et al.*1996, Odani, *et al.*2000).

The mechanism behind the IgA1 glycosylation defect still needs clarifying, but the isolation of the defect to a B-cell subset suggests that it takes place after class switching (Smith, *et al.*2006a, Smith, *et al.*2006b). Isolated B-cells from IgAN patients have been shown to express reduced β1,3-galactosyltransferase and its chaperon protein *cosmc* while at the same time the α2,6-sialyltransferase expression is increased (Suzuki, *et*

*al.*2008). Imbalance between mucosal and systemic production of pIgA1 has advanced the idea that the glycosylation defect may be of mucosal origin or under mucosal influence; determined at the site of antigen encounter. It has therefore been suggested that specific antigens may prompt a T-cell response that alters cell surface homing receptors. Cells, otherwise destined for the systemic compartment, might then be shunted into the bone marrow where production of the altered antibody takes place (Barratt*, et al.*2007, Smith*, et al.*2006b).

This theory of site specific antigen challenge leading to aberrations in glycosylation has been put to a test. IgAN patients and healthy controls with confirmed *Helicobacter pylori* (HP) infections made up the mucosal challenge group and comparable cohorts immunized with subcutaneous injection of tetanus toxoid (TT) made up the systemic challenge group. Antibody response for each antigen did not differ between patients and controls. However, exposure to *Helicobacter pylori* revealed a significant elevation of undergalactosylated IgA1 antibodies; both in the patients and the controls. These results are taken to indicate that antigens of different nature can bring about distinctly different glycosylation profiles on IgA1 antibodies (Smith*, et al.*2006b).

Genetic predisposition for defective glycosylation in IgAN has not been ruled out (Gharavi*, et al.*2008), but evidence pointing to microbial and environmental factors are increasingly gaining weight in the discussion.

## 4.2 Lectins

Many biological phenomena depend on recognition in cell-cell interactions. Diverse events such as fertilization, cell migration, organ formation, immune system activation and microbial infections depend on recognition, and lectins play a big role in this activity. Lectins are proteins derived from plants or animals that bind specifically and with great affinity to carbodydrates. Lectins can distinguish between different monosaccharides, bind to oligosaccharides and detect subtle differences in complex carbohydrate structures. They may be found as soluble proteins in the circulation or cell-bound. As such, lectins may be involved in binding, uptake and killing of cells. Thus, red blood cells and desialized glycoproteins are taken up and disposed off by lectins on Kupffer cells in the liver. Lectins on phagocytes may recognize glycan motifs on bacteria and thus facilitate their removal, while other microbes use lectins to their own advantage. Such is the case of the influenza virus that uses lectins against surface glycoproteins in order to enter and infect cell. Haemagglutinating properties of many lectins are used in a number of laboratory techniques; for example blood typing where cell bound glycoproteins need to be identified (Sharon*, et al.*1989)**.** An array of lectins is found on all cells of the human body. The importance of lectin-ligand interactions is demonstrated by the fact that the dendritic cell carries more than 15 surface bound C-type lectins (calcium dependent binding) for the purpose of recognizing and ingesting antigens (van Kooyk*, et al.*2004).

Lectin assays have been extensively used to study glycosylation of IgA1 in IgAN and HSP (Allen*, et al.*1995, Moldoveanu*, et al.*2007,

Saulsbury.1997). Various lectins have been used, among them Jacalin for the Gal-GalNAc bond, Peanut agglutinin for detection of desialylated Gal, Vicia villosa and Helix aspersa for terminal GalNAc and Sambucus nigra for identification of sialic acid to name only a few. As suggested by Tomana, and discussed in a previous chapter there are at least five glycan motifs found in the IgA1 hinge region (Tomana, *et al.*1999). The presence and proportions of each of these glycans can be demonstrated with the right selection of lectins. For detection of pathological glycans in IgAN and HSPN patients, identification of Gal deficient GalNAc and sialic acid on IgA1 is of great importance as these glycoforms characterize the defective IgA1 (Allen, *et al.*2001, Hiki, *et al.*1995, Julian, *et al.*2004).

The most commonly used lectins for detection of GalNAc are snail lectins from *Helix aspersa* (HAA) and *Helix pomatia* (HPA) and the plant lectin *Vicia villosa* (VV). Plant and animal lectins may be obtained from different manufacturers and their specificity may vary depending on purification methods, environmental conditions and geographical origin. The assay used for detection may also affect the results.

**Table 3:** Binding characteristics of the lectins used in the present study (see materials and methods). The Roman numerals refer to the five different possibilites for O-linked glycosylation described in figure 12 and table 2.

| | **Possibilities for O-linked glycosylation** | | | | |
|---|---|---|---|---|---|
| | **I** | **II** | **III** | **IV** | **V** |
| **Helix aspersa (HAA)** | + | | | | |
| **Desial-Helix aspersa (desial-HAA)**[*)] | + | + | | | |
| **Helix pomatia (HPA)** | + | | | | |
| **Sambuca nigra (SN)** | | + | | + | + |

[*)] Samples treated with neuraminidase before Helix aspersa lectin binding assay.

## 5. The complement system

The complement system plays a critical role in host defense. It is an important component of the innate immune system that consists of no fewer than 35 proteins co-operating in an intricate manner to eliminate microbial infections (Thiel.2007). Complement activation sets into motion a cascade like process where anchoring proteins are acted upon by specific enzymes. Distinct cell-surface receptors on phagocytic cells recognize fragments of complement components and aid in waste-product removal. The system is controled by various regulatory proteins

that may be either soluble or membrane bound. The regulatory proteins prevent spontaneous complement activation that otherwise might be harmful to host cells (Meri.2007, Muller-Eberhard.1988).

The three main functions of the complement system are: 1) Host defence against infection involving opsonization, chemotaxis and bacterial lysis, 2) Interface between innate and adaptive immunity, i.e. augmentation of antibody response and enhancement of immunological memory, 3) Disposal of waste, i.e. clearance of micro-organisms, immune complexes and apoptotic cells (Walport.2001).

The system was originally set forth in a numerical string according to its assumed activation, i.e. C1 – C9. This was later revised, and more to the point, as additional proteins were discovered and the complexity of the system was recognized new proteins were referred to as factors. Most recent additions to the system are now named by their function or genetic affiliation.

Three distinct activation pathways are known. These are: 1) the classical, 2) the alternative and 3) the lectin pathways (fig. 13). All three merge in complement factor C3, the pivotal protein of the whole complement system. Activation of C3 may lead to interaction with phagocytic elements through opsonization or it may trigger activation of the lytic pathway, i.e. the membrane attack complex (MAC or C5b-C9).

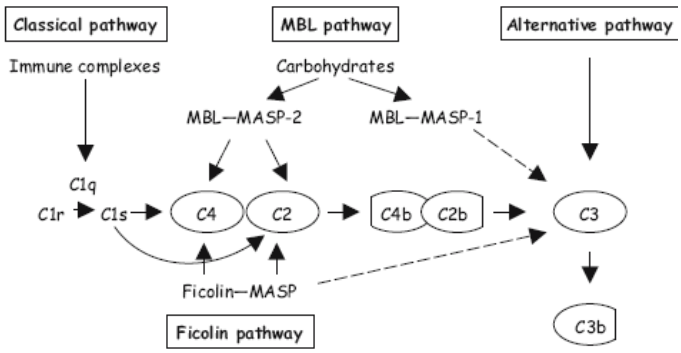


**Figure 13:** Overview of the complement system with focus on the MBL pathway (Sorensen, et al.2005)**.**

MAC is activated when surface bound C3b in association with C4bC2a or factor B forms the enzymatic complex C4bC2aC3b or C3bBbC3b. This is known as the C5 convertase capable of cleaving C5. Cleaving of C5 produces two fragments; the larger one C5b participates in the MAC cascade, while the smaller C5a has chemotactic activity. Cleavage of C5 allows the assembly of the "killer unit" to take place, forming a supramolecular structure consisting of one C5b, C6 and C7 that bind to the cell membrane, forming a receptor for C8. The C8 α-chain is then able to penetrate the lipid bilayer of the cell wall and, in turn, mediate the binding of several C9 molecules. The final event of MAC assembly is the unfolding and polymerization of C9 within the target membrane; weakening the membrane structure and forming transmembrane channels (Muller-Eberhard.1988). C9 is not essential for lysis of erythrocytes but is required for the lysis of bacteria (MacKay*, et al.*1990).

The small fragments, C3a and C5a produced by enzymatic cleavage of C3 and C5 are powerful chemoattractants. They form a gradient along which leucocytes migrate to the site of injury. Mast cells and macrophages that are capable of provoking an inflammatory response enter the arena to secrete a number of pro-inflammatory molecules, such as vasoactive amines, chemokines and cytokines that augment the injury inflicted (Roitt*, et al.*2006). These chemoattractants in conjunction with resident macrophages are the initiators of mesangial damage in IgAN.

Activation of the complement system in IgAN and HSPN is well known. Complement components, most consistenly C3 and MAC, are found in mesangial deposits in both diseases. It has generally been assumed that the inability of IgA to activate the complement system through C1q rules out the participation of the classical pathway. The blame for activation and deposition of C3 has therefore been put on the alternative pathway. This theory is supported by studies showing intraglomerular expression of C3 mRNA in mesangial and epithelial cells (Abe*, et al.*2001, Miyazaki*, et al.*1996). However, the significance of local synthesis of complements is still unclear and neither has the relatively high content of IgG and/or IgM in the IgAN complexes been ruled out as activators of the classical pathway.

The C4 component has, on occasion, also been found in kidney deposits of IgAN and HSP patients (Endo*, et al.*2000, Roos*, et al.*2006)**.** As C4 was previously known only to be activated through the classical pathway, its presence in the deposits has been somewhat puzzling. The discovery that C4 activation also occurs through the lectin pathway has

put a new light on complement participation in IgAN; reviving a previous discussion of a potential role of C4 in IgAN and HSP.

## 5.1 The classical pathway

The classical pathway is activated through membrane bound IgM and IgG antibodies or immune complexes of the same type. Certain micro-organisms can also directly activate this pathway and so do polyanions such as DNA, cardiolipin and dextran sulphate (Roitt*, et al.*2006). The initiating component of the classical pathway is C1. It is a complex of three types of molecules: a recognition unit, C1q and two molecules each of C1r and C1s. C1q is made up of six subunits of three polypeptide chains each, that come together in a collagen-like triple helix towards the N-terminal. The binding domain for the Fc regions of immunoglobulins is in the globular heads at the C-terminal. A tetrameric chain of Cs-Cr-Cr-Cs is inserted into the collagenous part of the subunits (Colomb*, et al.*1984, Muller-Eberhard.1988, Weiss*, et al.*1986). Activation of the complex needs a minimum of 2 globular heads to be occupied for the conformational change required for C1r to cleave C1s. These changes allow C1s to extend outside the C1q stalk and consequently cleave C4 and C2 to form the C3 convertase, C4bC2a.

C4 is coded for by two tangently positioned genes within the MHC region on chromosome six, yielding two isotypes of the protein: C4A and C4B. Cleavage of the α-chain of C4 by C1s enables the internal thioester to form amide or ester bonds with respective groups on the target surface. C4A shows preference to amide groups like those found on immune complexes, while C4B binds more readily to hydroxyl groups such as found on erythrocytes. Absence of either isotype may account for

diminished clearance of immune complexes (Christiansen, *et al.*1983, Muller-Eberhard.1988, Roos, *et al.*1982). Like C4, the C2 and Factor B are also coded for within the MHC region. Once activated C2 becomes an enzym aiding C4 cleavage of C3.

The classical pathway is also a powerful activator of the alternative pathway, which functions as an amplification loop for the classical pathway, generating more deposited C3.

## 5.2 The alternative pathway

Activation of the alternative pathway is a true innate response as it does not involve specific recognition molecules. It provides antibody independent complement activation on bacterial surfaces by covalent binding of C3b to hydroxyl groups on micro-organisms. Beside C3, five other proteins participate in the process. Four of thes are factors B, D, H and I, and the fifth is a stabilizing protein called properdin (P) (Walport.2001).

Activation of the alternative pathway has its origin in spontaneous low rate hydrolysis of the thioester bond within the C3 molecule. This produces C3(H2O) the equivalent of C3b. A slow tick-over of C3 hydrolyses is believed to occur continuously. Hydrolyzed C3 may then be deposited onto a target cell surface. In the presence of Mg2+ hydrolyzed C3 will bind to factor B and, in turn, be activated by factor D to produce C3bBb (Pangburn, *et al.*1980, Roitt, *et al.*2006). C3bBb is not a stable complex, but it is capable of splitting more C3. In order to effectively split a functional number of C3 molecules the C3 convertase needs stablilization by properdin to extend its half-life activity. As such it

can split sufficient amounts of C3 to provide phagocytic opsonization or activation of MAC. (Daha*, et al.*1976).

## 5.3 The lectin pathway

Phylogenetic studies date the lectin pathway of the innate immune system back some 600 million years; its earliest components tracing back to a primitive marine invertebrate, the sea urchin. Investigations show that lectin pathway components were already in place long before adaptive immunity evolved; adaptive immunity first appearing in cartilaginous fish. Similarities between components of the activation pathways indicate that complements are a link between true innate immunity and the adaptive immunity. Innate immunity is the first line of defence against micro-organisms; becoming active within seconds after the initiating event. Previously thought to be entirely a non-specific response resulting in phagocytosis, but now recognised to have considerable specificity to discriminate between self and non-self.

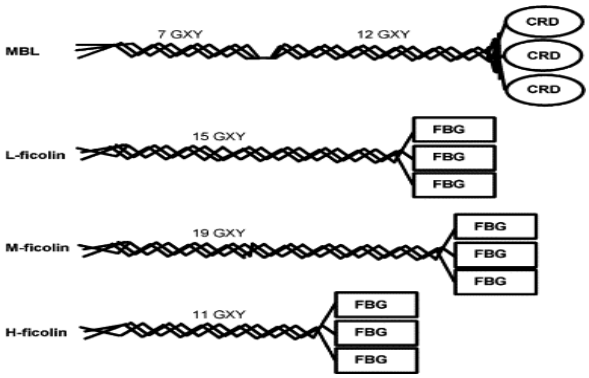


**Figure 14:** The structural subunits of MBL and the ficolins. Three identical polypeptide chains form a collagen-like strand terminating in three recognition domains in the C-terminal end (Thiel.2007).

Lectins are proteins in animals or plants that have specificity for certain glycans; monosaccharides or oligosaccharides. Their role in the innate response is to recognize conserved carbohydrate patterns on pathogens, so called pathogen associated molecular patterns (PAMPs). In humans this is mediated through recognition domains of two types of soluble lectins, a true lectin, the MBL and lectin-like proteins named ficolins, i.e. L- and H- ficolin (fig. 14). Additionally, there is a cell bound lectin-like protein, M-ficolin with high homology to L-ficolin. Lectins work in conjunction with a number of serine proteases that cleave complement components, thus activating the complement system (Fujita, *et al.*2004).

### *5.3.1. Mannan-binding lectin*

MBL is the best defined human lectin in circulation. It belongs to the collectin family of proteins that carry a collagen-like domain, a neck region and a carbohydrate recognition domain (CRD). Through the CRD it recognizes sugar patterns on microbial surfaces, recognizing primarily oligosaccharides terminating in monosaccharides with 3´ and 4´OH groups in the equatorial plane, such as mannose and glucose derivatives (Dommett, *et al.*2006). MBL is an oligomer of structural subunits that are composed of 3 identical polypeptide chains. Each polypeptide chain contains a cysteine rich N-terminal region. This region is involved in the covalent interaction between the three polypeptide chains as well as the oligomerization of the subunits (Endo, *et al.*2006, Thiel.2007, Turner.2003).

MBL is a C-type lectin, meaning that the binding between lectins and MBL is calcium dependent. Each CRD binds a calcium ion that in

turn can establish a co-ordinating bond with the 3-and 4-hydroxyl groups in the pyranose ring of the appropriate carbohydrate. Ligands for MBL CRDs are preferably mannose, fucose, N-acetyl-D-glucosamine and N-acetyl-mannosamine, while carbohydrates that do not fit the steric requirement, such as galactose and sialic acid commonly found on mammalian cells go undetected (Fujita*, et al.*2004). CRDs mediate binding to ligands in a terminal position with a constant distance of 45Å. The dissociation constant of one CRD binding to a ligand is weak, only about $10^{-3}$ M. This demands that several CRDs on the same MBL molecule bind at any point of time in order to form effective attachment (Iobst*, et al.*1994). For sufficient binding strength this calls for a minimum concentration of the carbohydrates involved as well as the right spatial arrangement (Gadjeva*, et al.*2004, Thiel.2007).

Human MBL genes are situated on chromosome 10 q21-24 (Hansen*, et al.*1998). Of the two genes found there, one is a pseudogene *MBL1,* while *MBL2* encodes for the protein. MBL is mainly produced in the liver. Functional oligomers are made up of subunits that vary in numbers, ranging from dimers to hexamers. Disruption of the assembled subunits that leads to diminished levels of functional protein molecules is traced to three single nucleotide polymorphisms (SNP). These SNPs are all positioned in exon 1 at codons 52, 54 and 57 in (D, B, C while A is the wild type) (Turner.2003). Variant B is most commonly found in the Caucasian population (Lipscombe*, et al.*1996). Three polymorphisms, H/L, X/Y and P/Q occuring in the promoter region of the gene can also affect MBL levels. The three loci are linked, making up 4 promoter haplotypes (LXP, LYP, LYQ and HYP) of which HYP is associated with normal or high levels of MBL, while LXP is frequently associated with

low levels (Madsen, *et al.*1998). This is most obvious when it associates with A allele in individuals who also carry B, C or D variant allele on the other chromosome (Saevarsdottir, *et al.*2006). Although MBL levels are relatively constant for each individual they may vary up to 1000 fold between individuals, i.e. ranging from a few ng/mL up to 5 – 10 μg/mL. It is generally held, that 500 ng/mL is sufficient for protection while 100 ng/mL are considered a state of deficiency (Valdimarsson, *et al.*2004). An estimated 10-12% of Caucasians fall into this category, some of whom may never show any symptoms due to MBL deficiency (Kilpatrick.2002, Thiel.2007, Turner.2003).

MBL is nevertheless a major component of the innate immune system. Its main functions are: 1) activation of complement, 2) promotion of complement-independent opsono-phagocytosis, 3) modulation of inflammation and 4) promotion of apoptosis. Of these functions complement activation is best defined (Turner.2003).

Activation of the lectin pathway is attained through participation of proteolytic enzymes, the MBL-associated serine proteases or MASPs. The MASPs form homodimeric complexes with the lectin molecules. Binding of MBL to its preferred carbohydrates brings on conformational changes allowing activation of the MASP. Activated MASP-2 cleaves C4 and MASP-1 cleaves C2 and possibly also C3 (Moller-Kristensen, *et al.*2007). Complement independent action of MBL is believed to take place through a putative collectin receptor on phagocytes, while lectin binding to apoptotic cells has been shown to be mediated through the recognition domain in the collagen region. This binding may compete with C1q for the cC1qR/calreticulin receptor in association with the α2-

macroglobulin receptor on phagocytes (Turner.2003). MBL is reported to enhance the inflammatory response of phagocytes to *N. meningitides* by inducing macrophages to secrete pro-inflammatory cytokines, such as TNF-α, IL-1β and IL-6 (Jack*, et al.*2001a, Jack*, et al.*2001b).

The role of MBL in disease pathogenesis has not yet been fully elucidated. MBL deficiency was first recognised as an opsonic defect in children presenting with frequent infections. It has since been shown that MBL deficiency is associated with infections caused by extracellular pathogens, particularly respiratory infections in early childhood (Koch*, et al.*2001). Mutant MBL alleles or low levels of the protein play a role in susceptibility to some autoimmune diseases, as low levels of MBL will affect clearance of apoptotic cells. This has been confirmed in numerous studies on SLE patients (Davies*, et al.*1995, Lau*, et al.*1996, Saevarsdottir*, et al.*2006, Sullivan*, et al.*1996). On the other side of the coin, low MBL levels may provide protection against intracellular organisms, such as *Leishmania* and *M. leprae,* as a result of diminished opsonization. Abundant MBL binding and C3 opsonization facilitates the entry of these organisms into the phagocytes, while diminished recognition of the microbes impedes their entrance (Dornelles*, et al.*2006, Santos*, et al.*2001).

The involvement of MBL in disease pathogenesis has mainly focused on low levels of the protein. More recently attention has been directed on the possibility that high levels might aggravate tissue damage through acute phase mechanisms, for instance in reperfused ischemic tissue. There MBL has been shown to participate in complement activation at the site of injury (Collard*, et al.*2000, Jordan*, et al.*2001).

Inappropriate activation of the lectin pathway in glomerular tissue has been demonstrated in a number of studies on patients with IgAN and HSP (Endo*, et al.*1998a, Endo*, et al.*2000, Roos*, et al.*2006). However, other studies have not shown MBL activation to be a special feature of IgAN (Lhotta*, et al.*1999, Ohsawa*, et al.*1999).

### *5.3.2. Ficolins*

Ficolins have certain structural similarities with the MBL molecule, both carrying collagen regions as well as recognition domains. Three types of ficolins are found in humans: L-ficolin, H-ficolin and M-ficolin. L-and H-ficolins are soluble proteins, while M-ficolin is membrane bound on phagocytic cells.

L-ficolin is a tetramer made up of 35 kDa trimeric subunits of identical polypeptide chains; consisting of cysteine rich NH3-terminal region, collagen-like sequence, a short neck region and a fibrinogen-like recognition domain (Matsushita*, et al.*1996). The gene coding for L-ficolin (*FCN2*) is located on chromosome 9 q34 and protein synthesis takes place in the liver.

L-ficolin levels are influenced by three polymorphisms found in the promoter region of the FCN2 gene (-986, -602, -4), while their binding ability is determined by a SNP on exon 8 (Hummelshoj*, et al.*2005, Thiel.2007).

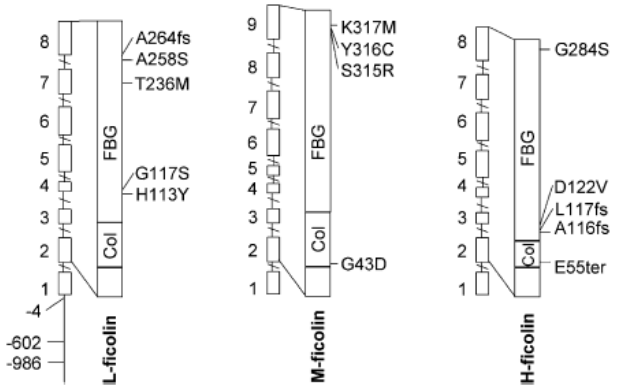


**Figure 15:** The genes and polypeptide chains of human ficolins. SNPs or mutations are indicated (fs, frame shift; ter, termination). Polymorphisms in L-ficolin promoter region are shown (Thiel.2007).

Ficolins are not true lectins, their recognition domain is fibrinogen-like and their preference of binding is not mono-saccharides but acetylated groups like GlcNAc and GalNAc on bacteria. Recent studies have shown that, unlike MBL, L-ficolin binding depends on structural conformation of glycan motifs containing a string of trisaccharide sequences with terminal GlcNAc and an early positon of an α-bond in the linkage is important (Krarup*, et al.*2008). Binding specificities of different ficolins may vary while some overlapping of binding also takes place between MBL and ficolins. L-ficolin also binds to lipoteichoic acids on Gram-positive bacteria, C-reactive protein and altered structures on bacteria, including some strains of *S. pneumoniae*. Some strains of *S. aureus* are commonly bound by MBL and L-ficolin, while others escape recognition or are recognized only by one but not the other. This is due to different binding specificities of the two proteins. L-ficolin binds to GlcNAc next to galactose and it does not bind to

mannose or fucose (Fujita, *et al.*2004). L-and H-ficolin participate in the clearance of apoptotic cells, by binding to cC1qR/calreticulin or C3R and thus promote opsonization and phagocytosis (Kuraya, *et al.*2005). All of the ficolins have been shown to activate complement by associating with MASPs (Endo, *et al.*2006, Thiel.2007).

Overlapping binding specificities of the different lectins increase the defence coverage by the lectin pathway. Deficiency of L-ficolin has not been reported, but low L-ficolin levels have been associated with recurrent respiratory tract infections in children (Atkinson, *et al.*2004)

M-ficolin is encoded by the *FCN1* gene on chromosome 9 and is shown to have 79% homology with L-ficolin at the amino acid level. Its intron-exon structure is similar to that of L-ficolin. It has been suggested that these proteins are the consequence of gene duplication and that the gene for M-ficolin may have evolved first. M-ficolin is found on the surface of circulating blood leucocytes and type II alveolar epithelial cells as well as in their secretory granules. The mRNA of M-ficolin is expressed in monocytes, lungs and spleen and may be secreted on demand.

The binding properties of M-ficolin have not been fully defined but, to some extent, it shows affinity for GlcNAc. Antibodies against the recombinant protein inhibit phagocytosis of *E. coli* by U937 cells (Teh, *et al.*2000). Unlike the other two ficolins M -ficolin may also bind to sialic acid residues (Liu, *et al.*2005).

H-ficolin has been shown by electron microscopy to have a hexameric structure, made up of 34 kDa identical polypeptide chains. Initially, H-ficolin was identified as a soluble antigen recognized by autoantibodies found in the serum of SLE patients (Hakata antigen).

Thorough screening of 200,000 Japanese blood donors did not expose other H-ficolin deficiencies than those associated with SLE (Fujita, *et al.*2004, Thiel.2007).

H-ficolin differs from the other two ficolins at the amino acid level and its homology to L- and M-ficolin is only 45%. H-ficolin is encoded for by a gene on chromosome 1 p35.3 and is mainly secreted by liver cells, but also by various epithelial cells, e.g. bile duct, bronchial epithelia and type II alveolar epithelial cells (Akaiwa, *et al.*1999). As a functional protein H-ficolin is only expressed in primates (Endo, *et al.*2004).

Aside from the fact that H-ficolin binds GlcNAc its ligand specificity is not yet fully defined. H-ficolin will agglutinate human erythrocytes coated with lipopolysaccharides from *S. typhimurium*, *S. minnesota* and *E. coli*. This binding was inhibited by GlcNAc and GalNAc and was calcium independent. H-ficolin does not recognize mannose or lactose (Fujita, *et al.*2004). *A. viridans* is the only bacteria H-ficolin has been shown to bind, but the ligand remains elusive as the binding, so far, can not be inhibited by any of the carbohydrates tested (Krarup, *et al.*2005, Tsujimura, *et al.*2002).

### *5.3.3 MBL-associated serine protease (MASP)*

MASPs are proteolytic enzymes that activate the lectin pathway in association with MBL and ficolins. Three MASPs have so far been identified. They are referred to as MASP-1, MASP-2 and MASP-3. MASP1 and MASP-3 are encoded for by genes on chromosome 3 q27-28 while the gene for MASP-2 and Map19 is on chromosome 1 q36.2-3. MASP-3 and Map19 are generated by alternative splicing (Thiel.2007).

Alternative splicing allows for multiple mRNA products from a given gene; often encoding for proteins with distinct or opposing functions and thus acting as regulators for over-expression of the alternative mRNA product and thereby increasing diversity.

The basic structure of genes encoding for the MASPs consists of 10 exons that code for a CUB1 domain (C1r/C1s/Uegf/Bone morphogenic protein-1), one EGF domain (epidermal growth factor), a second CUB2 domain and two CCP domains (complement control proteins). A single exon codes for the serine protease domain on MASP-2 and MASP-3, while 6 exons code for the protease domain of MASP-1 (Thiel.2007). From evolutionary perspective MASP-1 dates with MASPs from ascidians. The active serine site on MASP-1 carries a histidine-loop in the split exon. This loop is absent in MASP-2 and MASP-3 that seem to have evolved at about the same time as that of the lamprey MASP (Fujita*, et al.*2004).

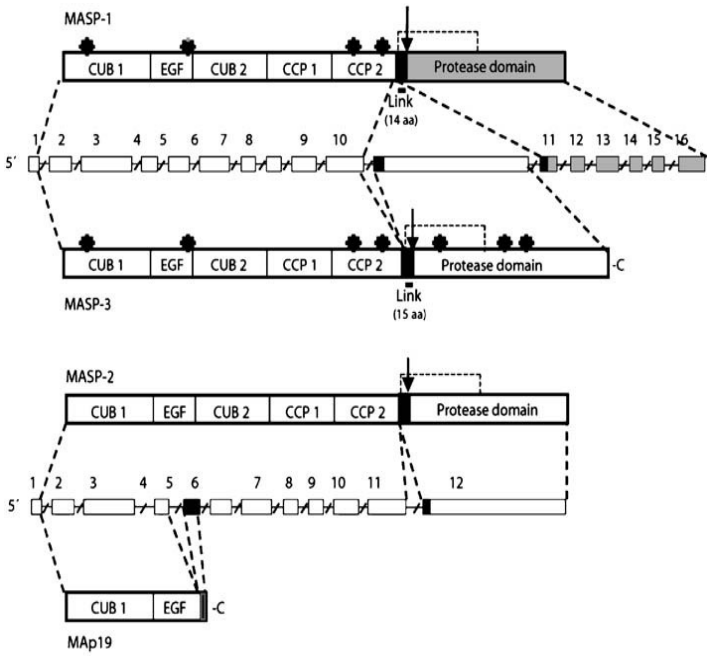


**Figure 16:** Genomic organization and protein structure of MASPs and MAp19 (truncated form of MASP-2). At the top the exon–intron structure of the MASP1/3 gene is shown. A single exon encodes the MASP-3 B-chain, whereas this region in MASP-1 is encoded by six exons. The A-chain of MASP-1 and MASP-3 is encoded by 10 shared exons. MASP-2/Map19 exon-intron structure shown at the bottom (Sorensen*, et al.*2005).

Organ distribution of mRNA of the MASPs and MBL has now been described (Seyfarth*, et al.*2006). The liver is the main producer of MASP-1 and MASP-2, although small amounts were also found in the small intestine and the testis. MASP-3 mRNA, on the other hand was ubiquitously dispersed in all organs tested except in peripheral leukocytes. Overwhelming production of MASP-3 was found in the colon and the liver and lesser amounts in extra-hepatic organs. Relevant to IgAN and HSP may be the copious presence of MASP-3 mRNA in, the kidneys.

All of the MASPs form homodimers that associate with MBL and ficolins. Activation of the lectin pathway occurs when the MBL (or ficolin) recognition domain binds with sufficient strength for conformational changes to take place. This allows MASPs complexed with MBL or ficolin to cleave C4 and C2 and, thereby produce the C3 convertase (Sorensen, *et al.*2005).

Although, the substrate specificity of MASPs has not been fully elucidated the present understanding is that MASP-2 is the workhorse when it comes to activation of C4. MASP-2 is capable of self-activation but is more effective when working together with MBL or ficolins. It is a robust cleaver of C4 and may, on molar basis, even be up to 20 times more effective than C1s (Rossi, *et al.*2001). MASP-2 also cleaves C2 but with much lower proficiency (Matsushita, *et al.*2000a, Matsushita, *et al.*2000b). MASP-1 cleaves C2 and it seems that the combined effort of MASP-1 and MASP-2 is needed for efficient activation of the lectin pathway (Moller-Kristensen, *et al.*2007). MASP-1 may also be capable of direct cleavage of C3. So far no substrate has been identified for MASP-3 but one report shows that the recombinant serine protease domain of MASP-3 is capable of cleaving insulin-like growth factor binding protein (Cortesio, *et al.*2006). Distribution of MASPs varies between MBL oligomers of different size. MASP-1 associates with smaller oligomeres, while MASP-2 and MASP-3 are found with larger oligomers (Dahl, *et al.*2001, Moller-Kristensen, *et al.*2007).

In order to control the activity of the complement system a number of inhibitors have evolved. The MASP-proteases are controlled by C1-inhibitor (C1-INH), also known for its inhibition of C1s activation of C4. Inhibition of MASP-1 and MASP-2 is brought about by C1-INH

forming complexes with the activated proteases. MASP-1 has been shown to cleave Factor XIII and fibrinogen, while cleavage of C4 by MASP-1 is inhibited by anti-thrombin III in presence of heparin. This may reflect the involvement of coagulation-based defence mechanisms in the early evolution of innate immunity. (Petersen*, et al.*2001, Presanis*, et al.*2004). Importantly, MASP-3 may also have a role in controling complement activation. Thus, MASP-3 has been shown to inhibit complement activation when added to MBL/MASP-1/MASP-2 complexes (Dahl*, et al.*2001, Iwaki*, et al.*2006, Moller-Kristensen*, et al.*2007), This may take place through competition with MASP-2 for a position on MBL whereby C4 cleavage is inhibited.

## 5.4 Complement regulatory proteins

A number of regulators have evolved whose sole purpose is to prevent autoactivation of the complement system. Most of these regulators control classical pathway activation. This begins with C1 inhibitor (C1-INH), which blocks the activation of the C1 complex and thereby prevents the autoactivation of C4 and C2. C1-INH bound to C1r inhibits the cleavage of C1s that in turn obstructs the initiation of C4 activation (Muller-Eberhard.1988). The regulatory proteins of the complement system may either be soluble proteins like C1-INH, factor H (fH) and C4 binding protein (C4bp) or they may be cell-membrane anchored like decay accelerating factor (DAF, CD55), complement receptor 1 (CR1) and membrane cofactor protein (MCP). C4bp, fH, CR1 and MCP act as cofactors to the serine protease, factor I (fI) that inactivates C3b/C4b by cleavage. Inactivation of the C3 convertase by DAF is achieved by dissociating C2a/Bb from their corresponding C3

convertases, C4bC2a and C3bBb (Medof, *et al.*1984). Regulation of MAC is provided by the S-protein that binds to C5b67 in fluid phase and prevents its attachment to cell surfaces. CD59 is a membrane protein that binds to cell bound C5b678 and blocks the addition of C9 to the complex (Muller-Eberhard.1988, Podack, *et al.*1984, Roitt, *et al.*2006, Schonermark, *et al.*1986).

Factor H and factor I serve as inhibitors of both classical and alternative pathway activation. Factor H will compete with C2b and Bb for binding successfully to C3b and thereby inhibit complement activation on host cells. This is aided by the presence of sialic acid. C3b can bind unhindered to bacterial surface. Lack of sialic acid makes bacterial surfaces natural depositories for C3bBb(Mg2+) whereas human cells are protected by sialic acid that allows recognition by factor H (Isenman, *et al.*1981).

## 5.5 Complement receptors

Many types of cells express receptors for numerous complement fragments, mediating the effect of complement activation throughout the immune system. Best characterized are reseptors for various fragments of C3, like the complement receptor 1 (CR1) that binds C3b/C4b and is crucial for clearance of immune complexes. CR1 is found on phagocytes, follicular dendritic cells (FDC) and red blood cells. CR2 is another important receptor that binds to C3d and inactivated C3b (iC3b). It is found on B-cells, FDC, some T-cells, basophils and endothelial cells. Both these receptors play a role in humoral immune responses, lowering the threshold for B-cell activation. They help to maintain B-cell tolerance and promote development of optimal B-cell memory (Molina, *et al.*1996,

Nielsen, *et al.*2000, Pepys.1974, Roitt, *et al.*2006). Antigen presenting cells (APC) carry full repertoire of complement reseptors including CR1, CR2, CR3, CR4, as well as C3aR, C5aR and C1qR. Exposure to ligands modulates their maturation status and cytokine and chemokine expression. This, in turn, influences the T-cell response (Kemper, *et al.*2007).

CR3 and CR4 belong to the integrin family and bind iC3b. Receptors for the small fragments C3a and C5a are widely distributed on blood and tissue cells through which they mediate chemotaxis and cell activation. There are two receptors for C1q; one for the collagen tail cC1qR, another for the globular heads, C1qRp. Although most C1q is bound in the C1 complex and as such does not interact with these receptors, they are believed to aid in phagocytosis of free C1q during complement activation (Steinberger, *et al.*2002).

## 5.6 C4 allotypes

Complement component 4 is encoded by two closely linked genes located within the MHC region on chromosome 6. Products of these genes encompass two functionally distinct isotypes, C4A and C4B. Activated C4A binds to amino-rich surfaces, while C4B forms ester bonds with carbohydrate residues. The difference in the C4 isotypes is due to a histidine residue in C4B that is missing in the C4A isotype. This minor variation results in a slight alteration in the mobility of the gene product on agarose electrophoresis, giving the more anionic C4A an advanced position. It is thought that C4A has a role in the solubilization of immune complexes and their clearance, while C4B may be more important for the activation of the membrane attack complex (Rupert, *et*

*al.*2002). Alper et al first demonstrated a genetic polymorphism in complement factor 4, which was later shown to be one of the most polymorphic of the human proteins (Awdeh*, et al.*1979, Truedsson*, et al.*1989) . More than 40 allotypic variants have now been recognized.

Most individuals carry two alleles for each isotype, but studies show that approximately 2% of healthy Caucasians lack the C4B isotype and the same applies for C4A (Welch*, et al.*1987). However, absence of one allele from either of the C4 isotypes is quite common in the healthy population, ranging from 11-15%. Complete C4A deficiency has been shown to predispose to autoimmune diseases, especially SLE (Welch*, et al.*1989). Increased frequency of homozygous C4 null genotypes in patients with IgAN and HSP (17.8% of patients vs 3.9% of controls) was first reported in 1984 (McLean*, et al.*1984). In that study HSP carried the heavier burden, 26.3% against 14.6% homozygous C4B null for IgAN patients. In a recent Icelandic study the frequency of the C4B null allele was significantly increased in patients with HSP compared to controls (allele frequency 0.25 versus 0.11), while homozygous C4B deficiency was observed in 7.1% of the patients versus only 2% in the controls. In contrast, no difference was observed regarding C4A deficiency between patients and controls in this study (Stefansson Thors*, et al.*2005).

## 6. Histopathology

Evaluation of tissue damage in IgAN is routinely assessed by conventional histopathology. Light microscopy showing excessive mesangial proliferation (cells and matrix), degree of glomerular sclerosis and extent of interstitial fibrosis is an indication of disease severity. Immune deposits are usually not apparent by light microscopy, but on

ultrasections dense mesangial deposits are observed at the interphase between mesangium and the capillary lumen. More rarely deposits can be located at the union of mesangium and the glomerular basement membrane and these may even extend into the membrane.

Dominant or co-dominant deposits of IgA in kidney mesangium are determined by immunofluorescent technique based on antibody labelled with fluorescein, as described in 1950 and later used by Berger and Hinglais (Coons, *et al.*1950). All IgAN patients have IgA1 deposits in the kidney. In a study of 239 IgAN patients Sinniah found that 45.7% had mesangial deposits containing only IgA, while 50.1% had IgG and 21.4% had IgM blended with the IgA (Sinniah, *et al.*1981). Depending on the study these figures vary due to different criteria for selecting patients for biopsy. Complement component C3 in the deposits has been reported in 80-100% of cased. Ikezumi found C3c deposits significantly greater in children than in adults. (Ikezumi, *et al.*2006). Properdin and MAC (C5b-9) are present in up to 95% of cases, more rarely C4.

Deposition of IgA in the glomerulus is associated with complement activation via C3 and the alternative pathway. Until recently this was considered to be the sole cause of complement activation in IgAN; classical pathway activation having been ruled out due to absence of C1q in the deposits. Complement activation in the IgAN kidney has been readdressed after the discovery of the lectin pathway. In the late 1990s Endo and co-workers showed MBL and MASP-1 to be present in 24.4% of IgAN renal biopsies (Endo, *et al.*1998b). Taking this study further they found that MBL, MASP-1 and C4 were invariably co-located with IgA2. This combination was only observed in IgAN and found to be present in over 50% of these patients. About 30% of SLE patients, used

as controls, had MBL and MASP-1 but never in combination with IgA2 (Hisano*, et al.*2001). No difference in laboratory parameters or clinical symptoms between the IgA2 positive and IgA2 negative IgAN patients was seen. In a later study the group reported HSP patients with kidney deposition of both IgA1 and IgA2 to have more severe renal disease than those with deposition of IgA1 alone (Hisano*, et al.*2005). In a study on 60 IgAN patients Roos and colleagues reported that MBL, MASP-1/3, L-ficolin and C4d were found in 25% of IgAN glomerular deposits. They did not find IgA2 in kidney biopsies from this group of patients. Serum levels for MBL, L-ficolin or IgA did not differ in IgAN patients with or without lectin pathway proteins, but patients with glomerular deposits of lectin pathway components had significantly more proteinuria indicating more severe renal damage (Roos*, et al.*2006).

## 7. Previous study of patients with Henoch-Schönlein Purpura

As previously mentioned HSP patients have markedly increased frequency of the C4B null allele, and this was associated with strikingly low levels of serum C4 (Stefansson Thors*, et al.*2005). This led us to look for a similar pattern in IgAN patients; the hypothesis being that; 1) low or dysfunctional C4 could be associated with diminished clearance of immune complexes of the IgA1 isotype, or 2) that IgAN could be traced to irregularities in the lectin pathway proteins that activate C4 or, 3) that complement dysfunction could be associated with defects in IgA1 glycosylation. To the best of my knowledge no attempt has previously been made to analyze defective IgA1 glycososylation in IgAN patients in the context of the components of the lectin pathway of the complement system.

# AIM OF THIS STUDY

Previous studies on IgA nephropathy and Henoch-Schönlein Purpura (HSP) have indicated that the complement system may play a significant role in the pathology of these diseases. The discovery of the lectin pathway for complement activation has opened a new frontier in complement research and the role this pathway may play in the pathogenesis of certain diseases. The main aim of the present study was therefore to investigate whether aberrant glycosylation of IgA1 in IgA nephropathy might be associated with abnormal levels or activation of the proteins involved in the lectin pathway of complement activation. Components of the lectin pathway and complement activation were also investegated in a cohort of HSP patients.

**Specific aims:**

- To analyze the IgA1 glycosylation status in patients with IgA nephropathy and compare this to healthy controls.

- To evaluate whether aberration[s] in the lectin pathway of complement activation might be involved in the pathogenesis of IgA nephropathy.

- To compare components of the lectin pathway of complement activation in patients with IgA nephropathy and HSP.

# MATERIAL AND METHODS

## 1. Study cohort

A total of 49 patients with biopsy documented IgAN were enrolled for the study, 21 females and 28 males. Mean age was 44.8 years (SD 17.2 years). Patient samples, both serum and EDTA plasma, were collected either at the Landspítali outpatient clinic or private clinics. Patients were recruited into the study exclusively on the grounds of histopathological findings; no selection in relation to disease progress or activity was applied. Five patients in the study (10%) carried the diagnosis "IgAN compatible with HSP". Corresponding control samples were collected from 46 healthy individuals. All samples were aliquoted and kept frozen at -70°C until measurements were performed. No personal information regarding disease status or treatment other than age, sex and disease duration were recorded. Additionally, at a later stage, HSP patients from a previous study were enrolled and their serum samples used for evaluation of the lectin pathway proteins. This was a retrospective study as patients had received diagnosis over a period ranging from 1 year upto 17 years. The participants were symptom free at the time of sampling (Stefansson Thors*, et al.*2005). The study protocol was approved by the Data Protection Authority (Persónuvernd) and the Ethical committee (Siðanefnd) at Landspitalinn and all study participants signed a standard informed consent.

As routine procedures for evaluating renal biopsies in the department of Pathology at Landspítalinn do not include staining for IgA1, IgA2, C4 or other complement components of the lectin pathway a special permission was obtained to include detection of some of these

proteins for this study. Included here are results from staining of biopsies for IgA1 and IgA2.

The IgAN patients were compared to a group of HSP patients that had participated in an earlier study at Landspitalinn (Stefansson Thors*, et al.*2005). The samples from the HSP patients were tested for L-ficolin, H-ficolin, MASP-2 and MASP-3, but data for MBL and C4d levels from the original study were used.

**2. Serum IgA levels**

Serum IgA was measured by radial diffusion as described by (Mancini*, et al.*1965) and modified by Fahey and McKelvey the same year. Rabbit anti-IgA (A0092; Dako, Glostrup, Denmark) was incorporated into 1.2% agarose (Merck, Damstadt, Germany) in phosphate buffered saline (PBS, pH 7.4) containing 3% polyethyleneglycol (PEG) 6000 (Merck) and cast on plates. Serum dilutions and serial dilutions of a normal serum pool that had been standardized by nephelomertry were applied to wells punched into the gel. After incubation the plates were dried and stained with 0.1% Coomassie Brilliant Blue protein stain (B1131; Sigma Aldrich, St.Louis, MO, USA) in a solution of acetic acid-methanol-distilled water (10%-45%-45%). The diameter of the resulting precipitate of complexes was then measured and calculated against the standard curve. Upper reference limit was 5.16 g/L (mean: 2.76±2SD).

IgA levels measured in the previous study on HSP patients were not applicable for comparison with IgAN patients due to age related differences.

### 3. IgA immune complexes

Circulating immune complexes (CIC) were precipitated by PEG 6000 as described by Imai (Imai, *et al.*1987). In short, the sera were mixed with equal volumes of 14% PEG 6000 in borate buffered saline at pH 8.0. After centrifugation at 1800g for 20 minutes, the precipitate was washed with the appropriate PEG-buffer (7%), spun again and re-suspended in 900 µL $H_2O$ to dissolve the pellet. Finally, 100 µL 0.9 M TRIS-borate (pH 8.0) was added.

IgA in the immune complexes was measured by enzyme linked immunosorbent assay (ELISA). Microtiter plates (Maxisorp; Nunc AS, Roskilde, Denmark) were coated with 100 µL/well of rabbit anti-IgA $F(ab)_2$ (A0408; Dako, Glostrup, Denmark) at a concentration of 2 µg/mL in PBS without Tween at pH 7.4. After incubation overnight at 4°C plates were washed with PBS (containing 0.05% Tween) and blocked with 1% BSA (A7030; Sigma Aldrich, St.Louis, MO, USA) for 2 hour. After washing, 100 µL of suspended precipitate was added to the plates in duplicates. Double dilutions of a known IgA concentration as determined by nephelometry (Beckman Coulter Array 360 System, Fullerton, CA, USA) were carried along in duplicates as a standard. Wells with dilution buffer alone served as blank. Alkaline phosphatase (AP) conjugated mouse anti-IgA1/IgA2 (Cat#555885, BD Pharmingen, Franklin Lakes, NJ, USA) was applied and the reaction visualized with p-nitrophenyl phosphate disodium salt hexahydrate (p-NPP) (S0942; Sigma-Aldrich) in diethanolamine-HCl buffer (Merck, Damstadt, Germany). Absorbance was measured at 405 nm (Titertek Multiskan, Plus MK II; ICN Flow Laboratories, Irvin, UK) and the concentration of

IgA calculated from the standard curve using an in-house computer-program (Titri) developed by G. Viðarsson (no reference). Upper reference value was set at 19.5 µg/mL (mean: 10.98±2SD).

Common to all ELISAs used in this study (excluding the lectin pathway assays) was development of colour with pNPP, absorbance reading on Titertek Multiskan followed by Titri calculations. 100 µL volumes were applied to wells and test serum and standards were applied in duplicates unless otherwise stated. Plates were washed three times after each step with PBS containing 0.05% Tween-20.

### 4. IgA-rheumatoid factor

Serum samples were screened for rheumatoid factor (RF) by an in-house ELISA (Jonsson*, et al.*1986). Immulon 2HB plates (Thermo, Milford, MA, USA) were coated with 100 µL, of 20 µg/mL of rabbit IgG (I5006; Sigma-Aldrich, St.Louis, MO, USA), in a coating buffer (carbonate-bicarbonate 0.1 M pH 9.6). After binding overnight at 4°C plates were washed, emptied and kept cold until used.

Control sera were diluted to high-medium-low concentrations with PBS buffer containing 0.5 M NaCl and 0.5% Tween-20. Serum dilutions in duplicates along with twofold dilutions of the standard pool were applied to plates. All incubation times were 2 hours. After washing in PBS-Tween, monoclonal mouse anti-human kappa light chain (K4377; Sigma-Aldrich) was applied, followed by AP-conjugated anti mouse antibody (D0314; Dako, Glostrup, Denmark). After development with pNPP substrate, absorbance was read at 405 nm (Titertek Multiskan). Calculations were made from a standard curve using Titri-program and

expressed in Units. All sera giving a reading ≥ 3 Units were tested for RF-isotypes of IgG and IgM and all patients sera was tested for IgA-RF regardless of screening results.

RF-isotypes were measured by ELISA in plates as used for the RF-screening. Test serum, standard and control were diluted in serum dilution buffer and applied to plates in duplicates. After incubation 100 µL of appropriate AP-conjugated monoclonal anti-IgG, IgM or IgA (anti-IgG, #A2064, Sigma-Aldrich, anti-IgM, #A2189, Sigma-Aldrich and IgA1-IgA2, Cat#555885, from BD Pharmigen, Franklin Lakes, NJ, USA) was added and allowed to react, washed and reacted with pNPP in substrate buffer. Readings were taken at 405 nm on Titertek Multiskan and calculations taken off a standard curve (Titri).

**5. Complement C3d and C4d**

EDTA plasma was precipitated in equal volumes of 22% PEG 6000 in cold Borat buffer, 0.2 M. After standing on ice for 1 hour the tubes were centrifuged at 1500g and the supernatant kept cold until used. Maxisorp ELISA plates (Nunc AS, Roskilde, Denmark) were coated with rabbit anti-human C3d (A063; Dako, Glostrup, Denmark) in a coating buffer (carbonate-bicarbonate pH 9.6) overnight at 4°C. The supernatants were diluted 1/100 with PBS with 0.01% BSA and applied to the plates in triplicates along with a plasma pool control and twofold dilutions of a Zymosan (Z4250; Sigma-Aldrich, St.Louis, MO, USA) activated serum that served as standard. (This standard was prepared by incubating 50 mg zymosan with 5 mL serum-pool for 60 minutes at 37°Cfollowed by centrifugation at 43.000g. Results were calculated as arbitrary units (AU) where 1/200 dilution of the standard was taken to be 500 Aus).

The plates were incubated at room temperature (RT) for 4 hours. AP-conjugated anti-C3d (A063; Dako, Glostrup, Denmark) was applied and allowed to stand overnight at RT to be followed by colour development with pNPP. Upper reference value was 35 AU (mean: 22.55±2SD).

C4d levels were also measured by ELISA as described (Rebmann*, et al.*1992). In short, Maxisorp plates (Nunc) were coated with goat anti-C4 (DiaSorin, Stillwater, MA, USA) in a coating buffer (carbonate-bicarbonate 0.1 M pH 9.6) and allowed to stand overnight at 4°C. Serum diluted in PBS-Tween 0.05% was applied along with a reference standard, the concentration of which had previously been determined twice by rocket immunoelectrophoresis. After incubation for 2 hours at 37°C, mouse anti human C4d (Cat.#213; Quidel, San Diego, CA, USA) was applied for 2 hours at RT, followed by AP conjugated anti-mouse antibody (D314; Dako, Glostrup, Denmark) for 1 hour and reaction visualized by pNPP. Reference values used were mean values for control ±2SD (mean: 0.195±2SD)

## 6. C4 allotypes

C4 allotypes were determined by high voltage agarose electrophoresis after treatment with carboxypeptidase B (Cat.# 103 233; Boehringer Mannheim, Germany) and neuraminidase type VIII (N5631; Sigma-Aldrich, St.Louis, MO, USA) in barbitone buffer (pH 8.8) containing glycene (G7403; Sigma-Aldrich) and Tris (T87602; Sigma). For electrophoresis 0.5% agarose SeaKem LE (Cambrex Bio Science, Wokingham, UK) in 1:3 diluted barbitone buffer, was cast on Gelbond

film (Amersham Bioscience, Uppsala, Sweden). Treated samples were laid in 5mm slits in the gel and 100 mA current applied. Samples were run until haemoglobin indicator had travelled desired distance across the film (6-8 cm). The agarose was then treated with 1:2 diluted anti-C4 (DiaSorin, Stillwater, MA, USA) for 2 hours for immunofixation to take place. Afterwards the plates were washed in physiological saline, dried and stained with Coomassie Brilliant Blue (B1131; Sigma-Aldrich) and Amido Schwarts (Merck, Damstadt, Germany). C4 allotypes were then visually scored by comparison with reference control sera and null alleles were determined by relative intensity.

The electrophoresis appatatus with a cooling system was purchased from Amersham Bioscience (Multiphor II) and so was the power-pack used (Power supply – EPS 1001).

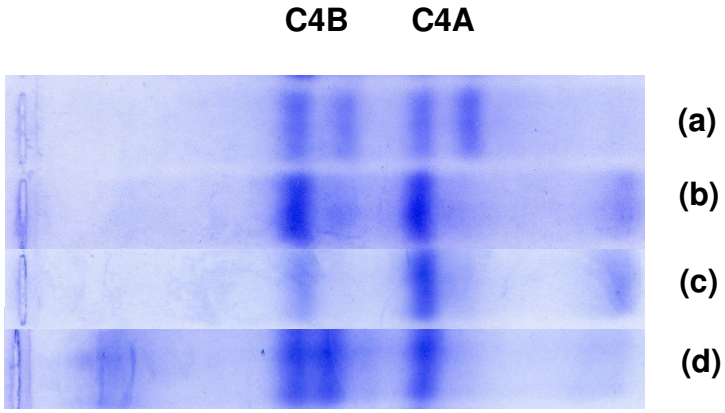


**Figure 17:** Electrophoresis gel showing different C4A and C4B haplotypes. A single strong band indicates homozygosity for either C4A or C4B. (a) C4 haplotype 6-3-3-1 with two C4A bands and two C4B bands, (b) C4 haplotype 3-3-1-1 i.e. homozygosity for both C4A and C4B, (c) C4 haplotype 3-3-1-0, i.e. only one C4B band can be seen, (d) C4 haplotype 0-4-2-1, i.e. only one C4A band can be seen.

**7. MBL**

MBL levels were determined by an ELISA. Plates were coated with 4 μg/mL anti-human MBL (HYB 131-1; Statens Serum Institute, Copenhagen, Denmark) in PBS pH 7.2 (without azide). Standard and test sera were diluted in PBS containing 0.37 M NaCl/ 0.05% Tween-20/ 1% BSA. Serum was diluted 1/25 and 1/200 and standard serum pool (standardized against purified MBL obtained from Statens Serum Institute) was diluted 1/35- 1/360, incubated for 1 hour at RT on a shaking table. Biotin labelled HYB 131-1 diluted 1/6000 was applied for 1 hr on shaking table followed by horseradish peroxidase-conjugated streptavidin (S5512; Sigma-Aldrich, St.Louis, MO, USA) on shaker for 1 hour. Visualization was accomplished with 100 μL/well of tetramethylbenzidine peroxidase substrate (TMB; KPL, Gaithersburg, MD, USA), incubated at RT until satisfactory colour had developed. The reaction was stopped using 150 μL of 0.18 M sulphuric acid (H2SO4; Merck, Damstadt, Germany). Optical density reading was taken at 450 nm on the Titertek Multiscan reader.

**8. L-ficolin and H-ficolin**

L- and H-ficolins were measured at the Microbiology and immunology laboratory at Aarhus University in Denmark.by a time-resolved immunofluorometric assay (TRIFMA). Fluoro Nunc plates (Nunc AS, Roskilde, Denmark) were coated with 1 μg/mL of mouse anti-L-ficollin antibody (GN5; HyCult, Uden, The Netherlands) in PBS (pH 7.4) overnight at 4°C. The plates were blocked with 1 μg/mL human serum albumin (HSA) in 10mM Tris buffer (pH 7.4) without Tween. The

same Tris buffer, but containing 0.05% Tween and 5mM $CaCl_2$, was used for diluting serum and a standard pool containing 5 µg/mL L-ficolin. Tris/Tween/$CaCl_2$ buffer was also used for washing. Test sera were incubated overnight at 4°C. After washing 1 µg/mL biotinylated anti-L-ficolin (GN5; HyCult) was applied to the wells and after incubation for 2 hours at RT, washed and EU-labelled steptavedin (PerkinElmer, Zaventem, Belgium) was applied for 1 hour, followed by washing and application of DELFIA EU-enhancement solution (PerkinElmer). Counts by time-resolved fluorometry were obtained by a DELFIA-Victor3 reader (PerkinElmer).

TRIFMA plates were coated with 1 µg/mL mouse anti-H-ficolin antibody (4H5; HyCult) in PBS (pH 7.4). The procedure was as for L-ficolin with the exception that serum was diluted 1/1000 and MASP-3 serum dilution buffer was used. Biotinylated anti-H-ficolin (4H5; HyCult) was diluted in regular Tris-Tween- $CaCl_2$ as for L-ficolin.

## 9. MASP-2 and MASP-3

TRIFMA plates were coated with 5 µg/mL of mouse anti-MASP-2 antibody (8B5; HyCult, Uden, The Netherlands) in a carbonate-bicarbonate coating buffer, 0.1 M (pH 9.6). The procedure was similar to that for the L-ficolin with the exception that samples were diluted in Tris buffer with high salt content (1 M NaCl), 10 mM EDTA and 100 µg/mL heat aggregated human IgG. Regular Tris buffer with $CaCl_2$, 1% ox serum and heat aggregated IgG was used to dilute the biotinylated antibody (6G12; HyCult).

TRIFMA plates were coated with 2 µg/mL mouse anti-MASP-3 antibody (1E2; HyCult) in PBS (pH 7.4). The procedure was the same as that for L-ficolin except the serum dilution buffer contained 20 mM Tris, 1 M NaCl, 0.05% TritonX-100, 10 mM $CaCl_2$ with 1 mg/mL HSA. Regular Tris with 5 mM $CaCl_2$ and 1% ox serum was used for diluting biotinylated anti MASP-3 antibody 38:12-3 (in-house product).

## 10. Isolation of IgA

The isolation of IgA has long been a cumbersome affair. Numerous studies describe the use of the lectin jacalin for isolation of IgA1, followed by various purification steps. As jacalin binds selectively to the β1,3-GT bond connecting Gal to GalNAc, this method fails to catch IgA1 from serum of IgAN patients lacking this bond, yielding decreased harvest of IgA1 (Andre, *et al.*1990). Sandin and co-workers applied a single step affinity chromatography to isolate IgA from body-fluids using Streptococcal IgA-binding protein (Sap). This method catches all IgA, as Sap interacts with the antibody at the Fc level (Sandin, *et al.*2002). The method was therefore employed with satisfactory result.

Serum IgA was isolated by affinity chromatography on N-hydroxy-succinimide (NHS) activated 1 mL HiTrap columns (GE Healthcare, Uppsala, Sweden). Streptococcal IgA-binding peptide (Sap) (Peptron, Daejeon, South Korea) was used to catch IgA from serum. Sap was immobilized on a NHS column (Sandin, *et al.*2002). Five (5) mg of Sap was coupled to the gel following the manufacturer´s instructions. Preparation of serum samples included centrifugation at 2500 revolutions per minute (rpm) prior followed by filtering through 0.45 µm pore size

filter (Spin-X Costar; Corning Incorporated, Corning, NY, USA) at 3000g for 20 minutes. Filtered samples were diluted to 0.5-1 mg/mL in 0.05 M PBS before running through the column. Bound protein was eluted with 0.1 M sodium acetate (pH 4.0) and fractions were collected into equal volume of 1 M Tris (pH 8.3). The fractions were then pooled and centrifuged in an iCON concentrator (Pierce, Rockford, IL, USA) with a molecular weight cut-off at 20 kDa. The concentrate was adjusted to 500 μL with PBSx2. The protein content was then measured at A280 on a NanoDrop 1000 Spectrophotometer (ThermoFisherScientific, Waltham, MA, USA) supplemented with 5 μL 10% BSA and 15 mmol/l of NaN3 (S8032; Sigma-Aldrich, St.Louis, MO, USA) and kept at -70°C until used.

## 11. Evaluation of IgA1 glycosylation

An in-house ELISA assay was developed to measure the O-linked carbohydrate content of the harvested protein. This was necessary in order to give lectins a direct access to the glycans and eliminate unspecific binding to coating antibodies, a problem especially prominent with regard to measurements of sialic acid.

Maxisorp plates (Nunc, Roskilde, Denmark) were coated with 7.5 μg/mL of the isolated IgA in filtered PBS (pH 7.4) without Tween. After overnight incubation at 4°C, the plates were blocked with 1% BSA (A7030; Sigma-Aldrich, St.Louis, MO, USA) /PBS/Tween for 1 hour at 37°C. Each carbohydrate was tested in duplicates and so was the IgA1 content of each sample. A blank was carried with each sample.

All lectins used were biotinylated: they were Helix aspersa (L8764; Sigma) binding GalNAc, Helix pomatia (L6516; Sigma) also binding GalNAc, Sambucus nigra (sialic acid) and Jacalin (β1,3-GT) (Vector laboratories, Peterborough, UK). Vicia villosa (GalNAc) and Peanut agglutinin (Gal) were also tried but did not give satisfactory binding (Vector laboratories). The lectins used in the assays were diluted 1/500 in BSA/PBS/Tween. Unlabelled mouse anti-human IgA1 clone NI 69-11 (A89-036; Nordic Immunology, Tilburg, The Netherlands), was diluted 1/1500 in BSA/PBS/Tween. AP-conjugated Avedin (D0365; Dako, Glostrup, Denmark) was applied to the lectin containing wells and AP-conjugated rabbit anti-mouse antibody (D0314; Dako) to the IgA1 wells. Reading of absorbance was taken after pNPP development and lectin absorbance adjusted by the absorbance of IgA1.

For neuraminidase treatment of IgA1 glycans: 100 µl of 10 U/mL of neuraminidase (P0720S; BioLabs, Ipswich, MA, USA) in 0.1 M sodium acetate buffer (pH 5) was applied to coated plates, allowed to react for 3 hours at 37°C before proceeding with the lectin assay as described above.

## 12. Immunopathology

Unfixed renal tissue was orientated and embedded in O.C.T compound (Sakura Finetek, Zoeterwoude, The Netherlands) on cold zink blocks and frozen at -70°C. Subsequently, 4 µm sections were placed on slides and stored at -70°C until immunostaining was performed. For direct immunofluoresce-staining sections were dried, washed in Tris buffer (pH7.6) and stained with FITC-labelled primary antibody IgA1,

clone NI69-11 (A89-036; Nordic Immunology, Tilburg, The Netherlands) or FITC-labelled IgA2, clone NI512 (A89-038; Nordic Immunology) for 30 minutes. Both antibodies were diluted 1/20 in Tris buffer. The sections were washed 3 times in Tris buffer and mounted in Tris-Glycene (Glycerolum 85%; Bufa, Uitgeest, The Netherlands) diluted 1:9. As this gave poor results for the IgA2 antibody detection an extra step was added: after the first wash the sections were exposed to and allowed to react with FITC-labelled F(ab)2 rabbit anti-mouse IgG (Star9B; Serotec, Oxford, UK) for 30 minutes before they were washed and mounted in Tris-Glycene.

Evaluation of stained sections was performed in a blinded manner. Sections were given new numbers by an outsider and the staining was graded as negative or positive (1 to 3+) according to the intensity of the staining.

The same Tris buffer (0.05 M Tris (hydroxymethyl)-aminomethane (Merk, Damstadt, Germany) and 0.15 M NaCl, pH 7.6) was used throughout for diluting antibodies and washing between steps.

## 13. Statistical analysis

SigmaStat software (version 3.11; Jandel Scientific, Erkrath, Germany) and Microsoft® Office Exel (version 11.0; Microsoft Corporation, Redmond, WA, USA) were used for all statistical evaluations. Comparison between groups was made by the Mann-Whitney Rank Sum Test. All tests were two sided and the level of significance was set at $p<0.05$. Correlations were calculated by

Spearman's rank correlation and contingency table calculations by GraphPad Software using Fishers exact test (GraphPad Software, San Diego, CA, USA). Graphs were made with GraphPad Prism software (GraphPad Software) and bars are set at median values.

# RESULTS

As both serum and EDTA plasma samples were available from the controls and the IgAN patients all tests were performed on these subjects. However, HSP samples were retrieved from a previous study and did not include EDTA plasma. Therefore C3d levels could not be estimated in the HSP cohort. As IgA levels are age dependent and the HSP patients were much younger than the IgAN patients it was not relevant to compare their IgA levels.

## 1.1. IgA nephropathy patients and controls

All patients entering the study had biopsy proven IgAN. When the present study was performed the disease duration ranged from less than one year to 21 years, with a mean duration of 6.1 years. The patient´s age ranged from 16 to 70 years, with a mean age of 44.9 years. The control group, 46 healthy adult laboratory workers, turned out to be somewhat younger than the IgAN patients, with a mean of 36.8 years. There was also a bias towards women in the control group (table 4).

## 1.2. Serum IgA levels

The IgAN patients had significantly higher levels of IgA in serum than the controls. The mean serum level for IgA in the IgAN patients was 3.51 g/L, compared with 2.75 g/L in the controls (fig. 18, p=0.003).

**Table 4:** Characteristics of the IgAN patients and controls studied.

| | **IgAN patients** (n=49) | **Controls** (n=46) |
|---|---|---|
| **Sex ratio** (M/F) | 28 / 21 | 14 / 32 |
| **Age** (years): | | |
| <30 y | 12 | 18 |
| 31-50 y | 16 | 20 |
| >50 y | 21 | 8 |
| Mean (±SD) | 44.9 (17.4) | 36.8 (12.6) |
| **Disease duration** (years): | | |
| < 5 y | 20 | – |
| 5-10 y | 19 | – |
| >10 y | 10 | – |
| Mean (±SD) | 6.1 (4.4) | – |

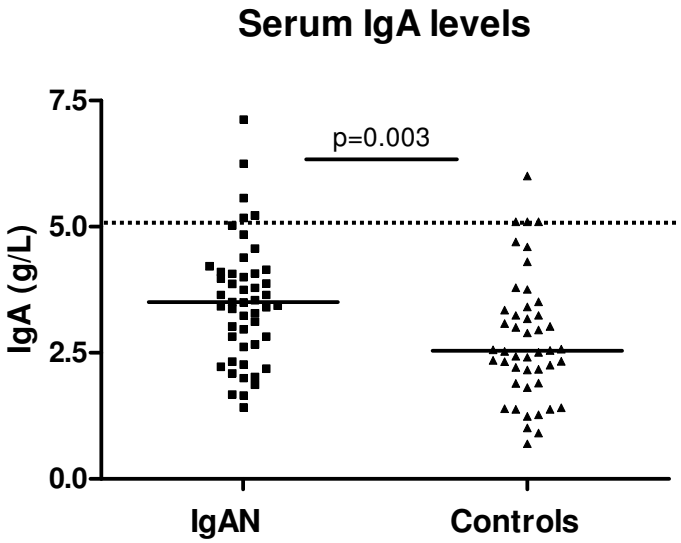


**Figure 18:** Distribution of serum IgA levels in IgAN patients (n=49) and controls (n=46). The dotted line indicates the upper limit of normal for serum IgA and the short horizontal lines indicate the median values for each group.

The 5 patients with abnormal IgA glycosylation (HAA OD >0.289) had relatively low levels of serum IgA, ranging from only 2.00 to 2.33 g/L, which was below the median value for the control group (2.54 g/L), and significantly lower than in the remaining IgAN patients (p=0.006).

### 1.3. IgA-containing complexes (IgA-ICs)

The upper limit of normal for serum IgA-ICs was defined as the mean value + 2 SD for the control group or 19.5 μg/mL. The IgAN patients had increased levels of IgA-ICs in the blood compared to the control group (fig. 19, p=0.004). Of the IgAN patients 25.6% (10/39) had IgA-IC levels above the upper reference value (p=0.0075). No association was found between disease duration and IgA-IC levels. Furthermore, IgA-IC levels did not correlate with other serological parameters measured in this study, other than total serum IgA (r=0.397, p=0.012).

### 1.4. IgA rheumatoid factor

All participants were tested for RF. Those with RF values above 3 U/mL in the RF screening test (Jonsson*, et al.*1986) were further tested for IgA RF, IgG RF and IgM RF. Furthermore, all samples from the IgAN patients were tested for IgA RF regardless of the RF screening test results.

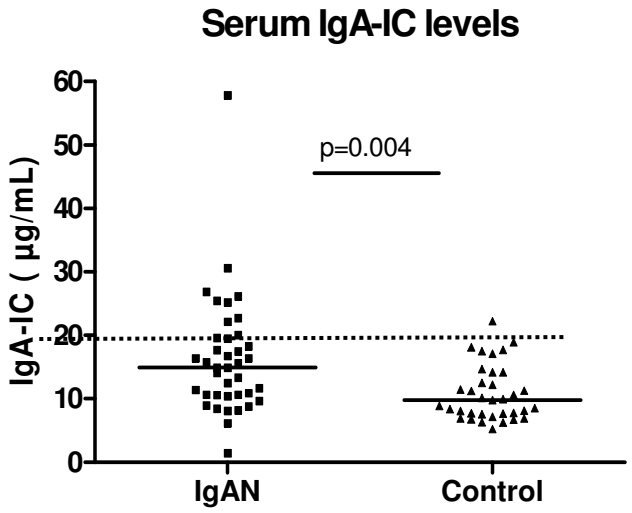


**Figure 19:** IgA-IC levels in IgAN patients (n=39) and controls (n=35). The dotted line indicates the upper limit of normal for serum IgA-ICs and the short horizontal lines indicate the median values for each group.

Only one IgAN patient (2.0%) had raised IgA RF, two (4.1%) had raised IgM RF and six (12.2%) had raised IgG RF. The IgAN patient with raised IgA RF also had raised IgG RF and IgM RF. None of the individuals in the control group had elevated IgA RF, IgM RF or IgG RF.

### 1.5 C4 allotypes

Table 5 shows the prevalence of different C4A and C4B haplotypes in the IgAN patients and controls. No significant differences were found in the prevalence of different C4 haplotypes between the IgAN patients and the control group, including the C4B null alleles (24.5% in the IgAN patients vs. 17.4% in the control group). None of the participants were homozygous for the C4B null alleles.

**Table 5:** C4A and C4B haplotypes in the IgAN patients and the control group.

| | IgAN | HSP | Controls |
|---|---|---|---|
| **C4A haplotypes:** | | | |
| **33 xx** | 29 (59.2%) | 27 (48.2%) | 23 (50.0%) |
| **C4A null** | 8 (16.3%) | 15 (26.8%) | 13 (28.3%) |
| **Other combinations** | 12 (24.5%) | 14 (25.0%) | 10 (21.7%) |
| **C4B haplotypes:** | | | |
| **xx 11** | 20 (40.8%) | 22 (39.3%) | 26 (56.5%) |
| **C4B null** | 12 (24.5%) | 24 (42.9%)* | 8 (17.4%) |
| **Other combinations** | 17 (34.7%) | 10 (17.9%) | 12 (26.1%) |

xx: any C4B combination (above) or C4A combination (below)
* : $p<0.01$ compared to the control group

**Table 6:** MBL, C3d, C4d and IgA containing immune complexes in IgAN patients in relation to C4B haplotypes.

| C4B haplotypes: | MBL [#] (μg/mL) | C3d [#] (AU) | C4d [#] (g/L) | Elevated IgA-ICs |
|---|---|---|---|---|
| **xx11** | 495 * | 30.5 | 0.190 | 37.5%** |
| **C4B null** | 896 | 34.0 | 0.180 | 18.2% |
| **Other combinations** | 1426 | 35.5 | 0.215 | 16.7% |

[#] Medians for the groups are shown
* P=0.019 compared to other combinations
**P=0.062 compared to other combinations

### 1.6. Complement C3d and C4d

The upper limit of normal for the complement activation product C3d was defined as the mean value + 2SD for the control group or 33 AU. Eighteen (36.7%) of the IgAN patients had C3d above this level compared to only two (4.3%) of the controls (fig. 20, $p<0.0001$). Elevated C3d was not related to disease duration or any parameter measured in the blood samples.

Another complement activation product, the C4d fragment, was also measured but no difference was found between the IgAN patients and the controls for the C4d values (fig. 21). In contrast, patients with HSP had significantly lower C4d levels compared both to the controls ($p=0.01$) and the IgAN patients ($p=0.003$).

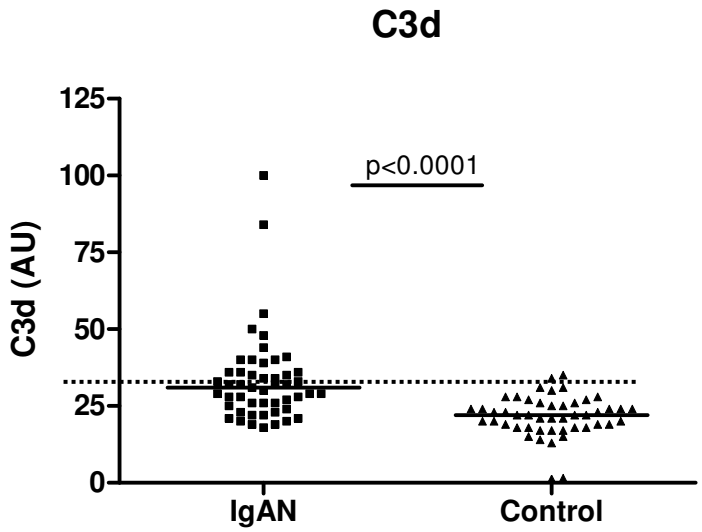


**Figure 20:** C3d levels in IgAN patients (n=49) and controls (n=46). The dotted line indicates the upper limit of normal for C3d and the short horizontal lines indicate the median values for each group.

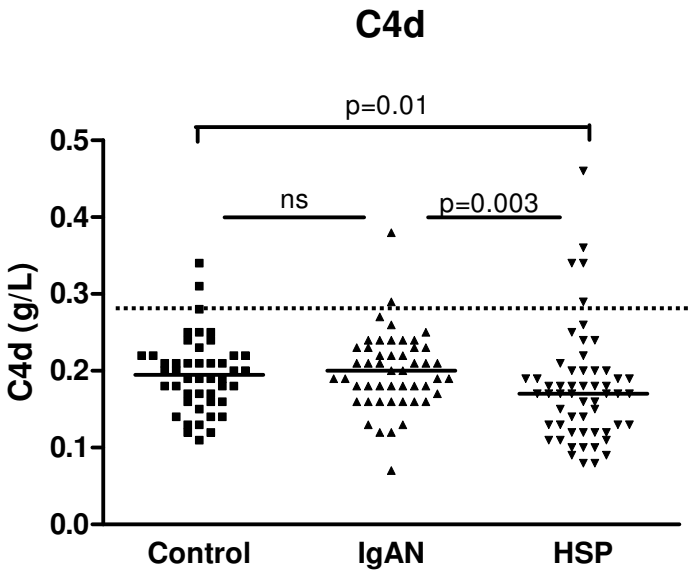


**Figure 21:** C4d levels in IgAN patients (n=49), HSP patients (n=56) and controls (n=46). The dotted line indicates the upper limit of normal for serum C4d and the short horizontal lines indicate the median values for each group.

In a previous study on HSP patients C4d levels were found to be significantly reduced compared to controls (Stefansson Thors, *et al.*2005). Further analyses revealed that the low C4d levels in the HSP patients were primarily associated with C4BQ*0. A similar analysis of the IgAN patients in this study showed that patients with C4BQ*0 had lower C4d levels than other patients (fig. 22).

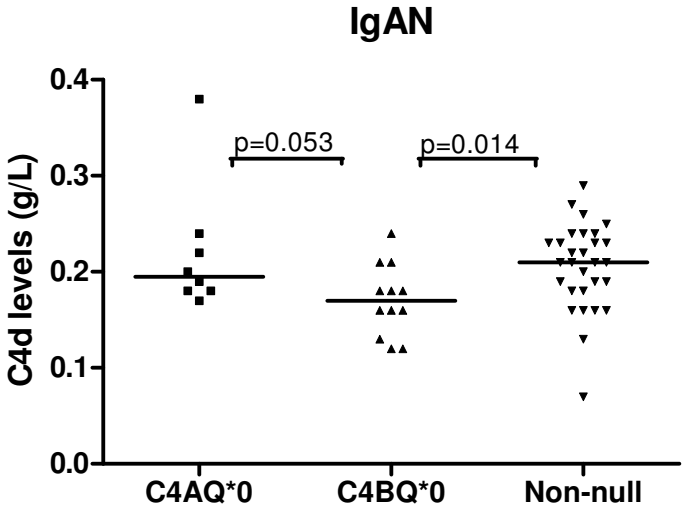


**Figure 22:** C4d levels in IgAN patients in relation to C4 null allotypic variants.

### 1.7. MBL

MBL levels did not differ significantly between IgAN patients and controls (fig. 23). In contrast, the patients with HSP had higher MBL levels compared to the controls (p=0.0004) and the IgAN patients (p=0.002).

Of the 49 IgAN patients 39 were measured for IgA-ICs and assessed for C4 haplotypes. Of these 16 had the C4B 1-1 haplotype and table 7 shows correlations between MBL, C4d, C3d, serum IgA and IgA-ICs in this group of patients.

**Table 7:** Correlations between MBL, C4d, C3d, serum IgA and IgA-ICs in the IgAN patients with the C4B 1-1 haplotype (n=16).

| | **C4d** | **C3d** | **IgA-ICs** | **serum IgA** |
|---|---|---|---|---|
| **MBL** | r= 0.564<br>p=0.022 | r= 0.664<br>p=0.005 | r= 0.711<br>p=0.002 | r= 0.534<br>p=0.031 |
| **C4d** | | r= 0.596<br>p=0.014 | r= 0.275<br>ns | r= –0.010<br>ns |
| **C3d** | | | r= 0.768<br>p<0.001 | r= 0.268<br>ns |

In the IgAN patients a weak negative association was found between MBL levels and defective IgA glycosylation (HAA OD >0.289; r= –0.335, p=0.019). No significant associations were observed between MBL and other parameters that were measured.

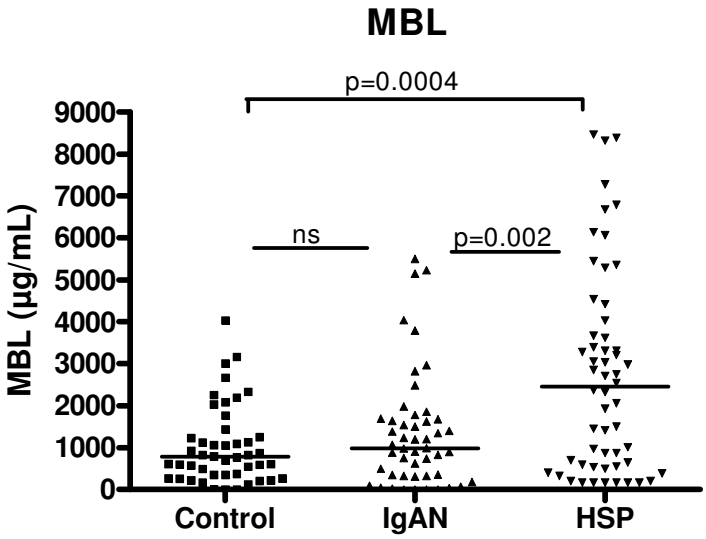


**Figure 23:** MBL levels in IgAN patients (n=49), HSP patients (n=56) and controls (n=46). Horizontal lines indicate the median values for each group.

## 1.8 L-ficolin and H-ficolin

No significant difference was observed in L-ficolin levels between the IgAN patients and the control group (fig. 24). However, the HSP patients turned out to have significantly higher L-ficolin levels than both the control group ($p<0.0001$) and the IgAN patients ($p=0.0005$).

Patients with IgAN had slightly higher levels of H-ficolin compared to the control group (fig. 25, $p=0.041$). However, no differences in H-ficolin levels were observed between the controls and HSP patients or between the IgAN and HSP patients.

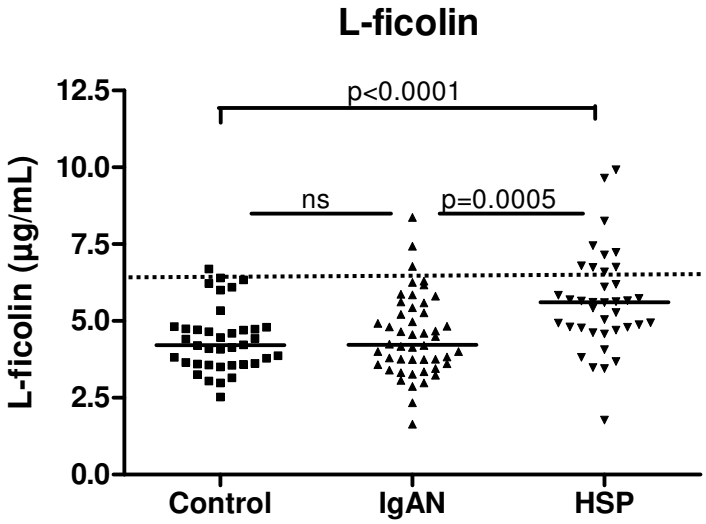


**Figure 24:** L-ficolin levels in IgAN patients (n=48), HSP patients (n=38) and controls (n=38). The dotted line indicates the upper limit of normal for L-ficolin and the short horizontal lines indicate the median values for each group.

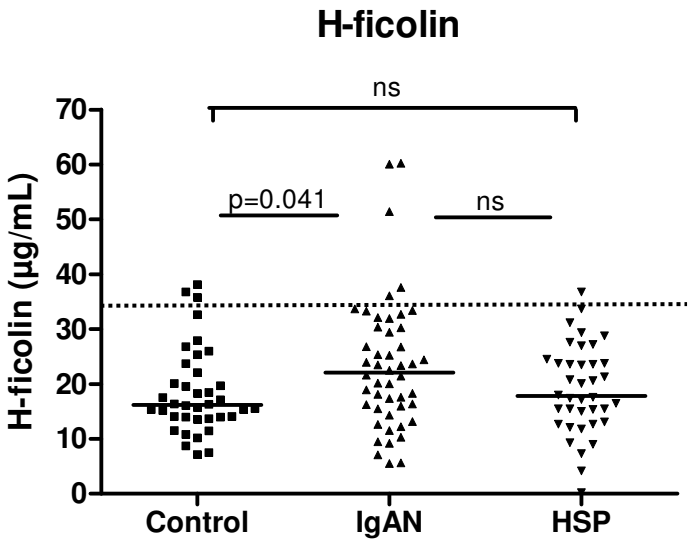


**Figure 25:** H-ficolin levels in IgAN patients (n=48), HSP patients (n=38) and controls (n=38). The dotted line indicates the upper limit of normal for H-ficolin and the short horizontal lines indicate the median values for each group.

### 1. 9 MASP-2 and MASP-3

No significant differences were found between the IgAN patients, HSP patients and the control group for MASP-2 levels (fig. 26).

No difference was observed in MASP-3 levels between the IgAN patients and the control group (fig. 27). However, the HSP patients had markedly higher MASP-3 levels than both the control group ($p<0.0001$) and the IgAN patients ($p<0.0001$).

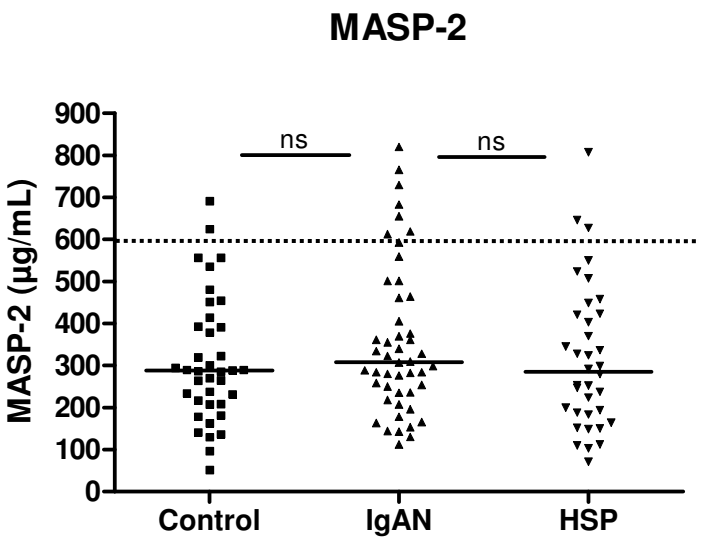


**Figure 26:** MASP-2 levels in IgAN patients (n=48), HSP patients (n=36) and controls (n=38). The dotted line indicates the upper limit of normal for MASP-2 and the short horizontal lines indicate the median values for each group.

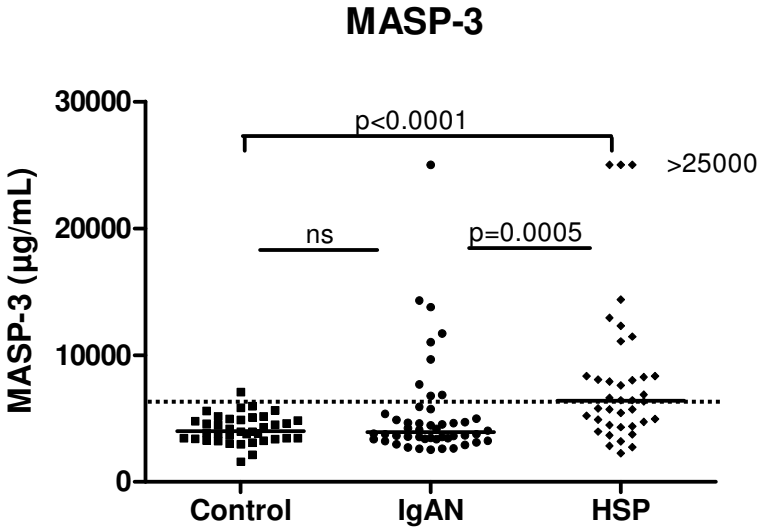


**Figure 27:** MASP-3 levels in IgAN patients (n=48), HSP patients (n=38) and controls (n=38). The dotted line indicates the upper limit of normal for MASP-3 and the short horizontal lines indicate the median values for each group.

## 1.10 Defective IgA1 glycosylation

Using Helix aspersa (HAA) to test IgA1 glycosylation an increased binding was observed for the IgAN patients compared to the control group (fig. 28, p=0.041), and this was mainly due to 5 patients who had abnormally high binding, indicating increased defective glycosylation of IgA1 (HAA OD >0.289). When sialic acid was removed from the sugar residues on IgA1 by neuraminidase treatment (desial-HAA), the differences between the IgAN patients and the controls became even more apparent (fig. 29, p=0.0004). Supporting these observations a significantly increased binding was found between Helix pomatia (HPA) and IgA1 in the IgAN patients (fig. 30, p=0.037).

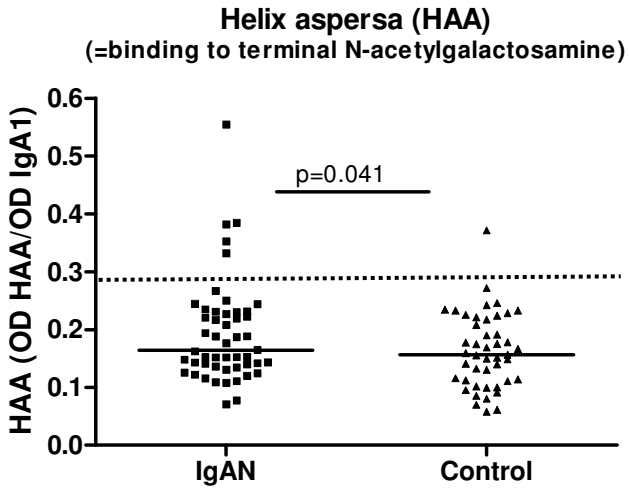


**Figure 28:** Helix aspersa (HAA) binding to terminal N-acetyl galactosamine (GalNAc) on IgA1 in IgAN patients (n=49) and controls (n=44). The short horizontal lines indicate median values for each group.

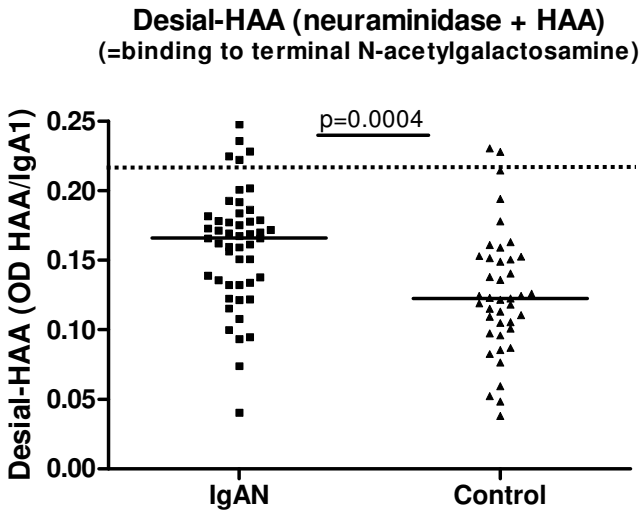


**Figure 29:** Helix aspersa (HAA) binding to terminal N-acetyl galactosamine (GalNAc) on IgA1 after treatment with neuraminidase in IgAN patients (n=49) and controls (n=41). The short horizontal lines indicate median values for each group.

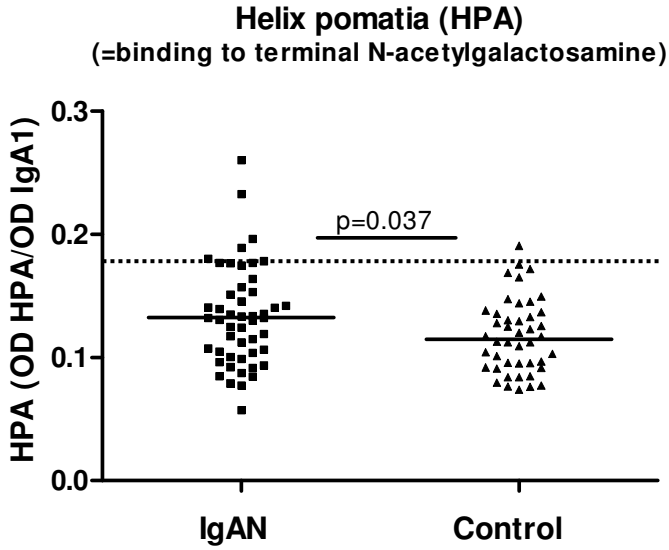


**Figure 30:** Helix pomatia (HPA) binding to terminal N-acetyl galactosamine (GalNAc) on IgA1 in IgAN patients (n=49) and contols (n=44). The short horizontal lines indicate mean values for each group.

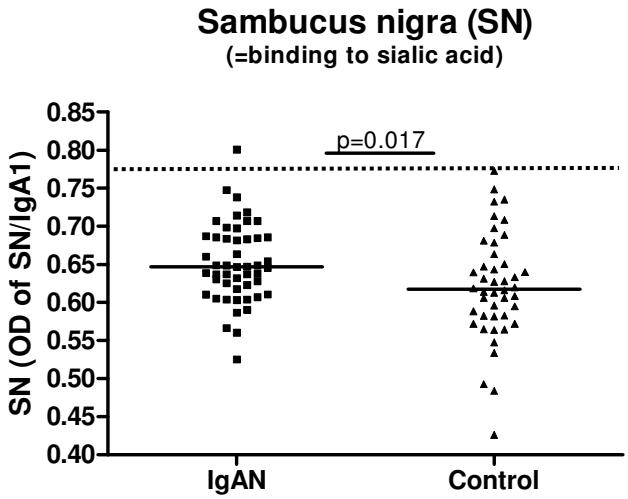


**Figure 31:** Measurement of sialic acid on IgA1 with the lectin Sambucus nigra in IgAN patients (n=49) and controls (n=44). The short horizontal lines indicate the mean values for each group.

Using Sambucus nigra (SN), a sialic acid specific lectin, increased binding to IgA1 was found in the IgAN patients compared to the controls (fig. 31, p=0.017), further confirming increased defective IgA1 glycosylation in the IgAN patients.

The 5 IgAN patients with the highest levels of defective IgA1 glycosylation (by HAA and desial-HAA) all had low levels of MASP-3. It should also be noted that these patients all had relatively low serum IgA levels (less than 2.75 g/L).

## 1.11 Associations between defective IgA glycosylation and complement components

Table 8 summarizes the associations between different complement components and defective IgA1 glycosylation in the IgAN

patients. A negative association was observed between MASP-3 and defective IgA glycosylation, as measured both by HAA (r= −0.381, p=0.007) and desial-HAA (r= −0.474, p<0.001). Weaker negative associations were found between MBL levels and defective IgA glycosylation as measured by HAA (r= −0.335, p=0.019) and between C4d and defective IgA glycosylation as measured by HPA (r= −0.293, p=0.041).

**Table 8:** Correlations between IgA glycosylation (HAA, desial-HAA, HPA and SN) and different complement components in the IgAN patients.

| | **HAA** | **desial-HAA** | **HPA** | **SN** |
|---|---|---|---|---|
| **MBL** | r= −0.335<br>p= 0.019 | r= −0.261<br>ns | r= −0.208<br>ns | r=−0.078<br>ns |
| **C3d** | r= 0.039<br>ns | r= −0.149<br>ns | r= 0.018<br>ns | r=−0.136<br>ns |
| **C4d** | r= −0.029<br>ns | r= 0.053<br>ns | r= −0.293<br>p= 0.041 | r=0.274<br>ns |
| **L-ficolin** | r= −0.077<br>ns | r= −0.233<br>ns | r= 0.068<br>ns | r=−0.275<br>ns |
| **H-ficolin** | r= −0.063<br>ns | r= −0.116<br>ns | r= −0.085<br>ns | r=−0.107<br>ns |
| **MASP-2** | r= 0.068<br>ns | r= 0.106<br>ns | r= 0.074<br>ns | r=−0.035<br>ns |
| **MASP-3** | r= −0.381<br>p= 0.007 | r= −0.474<br>p <0.001 | r= −0.129<br>ns | r=−0.022<br>ns |

## 1.12 Immunopathology

Twenty six biopsies from the IgAN patients were available for this study. Of the 25 biopsies stained, 18 (69.2%) stained positive for IgA1 only while 8 (30.7%) stained positive for both IgA1 and IgA2. All "HSP compatible" biopsies available (3/3) stained positive for both IgA subclasses.

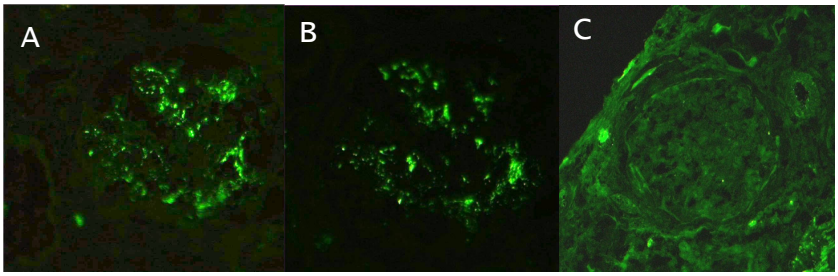


**Figure 32:** Immunofluorescence staining of kidney biopsies (bx). A glomerulus staining positive for $IgA_1$ (A), glomerulus staining positive for $IgA_2$ (B) and a negative control staining (C).

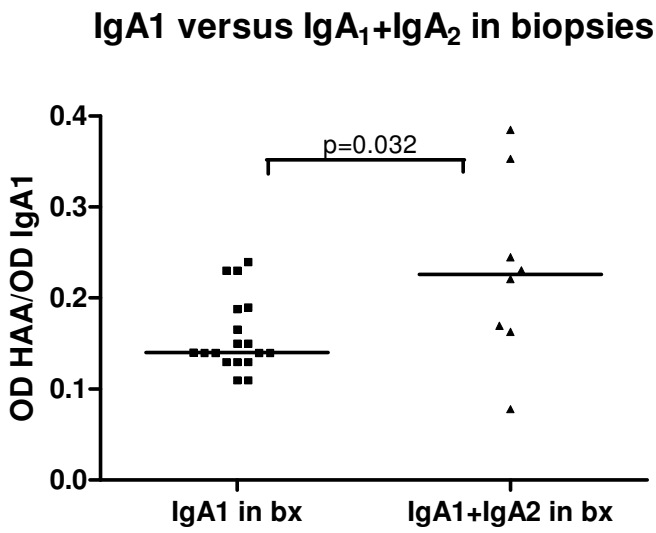


**Figure 33:** Helix aspersa (HAA) binding to terminal N-acetyl galactosamine on serum IgA1 in IgAN in relation to IgA subclasses in the kidney biopsies.

The IgAN patients whose biopsies stained positive for both IgA1 and IgA2 had marginally higher levels of defectively galactosylated IgA1 (by HAA) in serum than the IgAN patients whose biopsies stained positive for IgA1 only (fig. 33, p=0.032).

# DISCUSSION

The main aim of this study was to to evaluate whether defective glycosylation of serum IgA1 in IgAN correlates with the levels or activity of the lectin pathway of complements, and whether such association might promote the pathogenesis of the disease.

Two types of glycan deviations in the IgA1 hinge region were observed: a) increased GalNAc in terminal position, and b) increased sialylation masking degalactosylated GalNAc. This is in accordance with work of others (Leung*, et al.*2002, Suzuki*, et al.*2008). Another striking finding was a difference between IgAN and HSP regarding lectin pathway proteins. Thus, MBL, L-ficolin and MASP-3 were all found to be significantly elevated in HSP compared to IgAN. This has to my knowledge not been reported before.

**The lectin pathway**

Table 9 summarizes the results for the lectin pathway proteins in IgAN and HSP in relation to the healthy control group. Taken together the results do not provide a conclusive evidence for the notion that the lectin pathway of the complement system is abnormal or a major initiating factor in the pathogenesis of IgAN. Thus, although the levels of serum C3d were significantly increased in the IgAN patients, the serum levels of C4d did not differ from those found in the healthy controls.

**Table 9:** Summary of lectin pathway findings in IgAN and HSP. The results are compared to the control group.

| **Levels of:** | **IgAN** | **HSP** |
|---|---|---|
| **C3d** | ↑ | nd |
| **C4d** | 0 | ↓ |
| **MBL** | 0 | ↑ |
| **L-ficolin** | 0 | ↑ |
| **H-ficolin** | (↑) | 0 |
| **MASP-2** | 0 | 0 |
| **MASP-3** | 0 | ↑↑ |

0 = no difference compared to controls
↑ = elevated compared to controls
↓ = reduced compared to controls
nd = not measured

Furthermore, the levels of the lectins and associated proteins that induce activation of the lectin pathway, namely MBL, L-ficolin and MASP-2 were not raised in the IgAN patients.

These findings do, however, not completely exclude the involvement of the lectin pathway in the pathogenesis of IgAN as we have not yet measured the function of these components or analyzed the kidney biopsies for deposition of MBL, L- and H-ficolins or complement activation fragments.

Deposits of lectin pathway proteins in kidney biopsies of IgAN and HSP patients have previously been reported. Endo and co-workers reported MBL and MASP-1 associated with C3c and MAC in 24.4 % of IgAN patient but did not detect C4 (Endo*, et al.*1998a). Matsuda

observed MBL in 9.5 % of biopsies tested (Matsuda, *et al.*1998). The MBL co-localized with C2 and C4 in 87.8% of these patients compared with only 20% of biopsies that were negative for MBL. Furthermore, MBL positive patients are reported to have more mesangial cell proliferation, lower creatinine clearance and more proteinuria than MBL negative patients (Endo, *et al.*1998a, Matsuda, *et al.*1998). More recent biopsy studies of both HSP and IgAN patients have shown MBL to associate with MASP-1, L-ficolin, C4d and fibrinogen (Hisano, *et al.*2005, Roos, *et al.*2006). Both studies concluded that the presence of lectin pathway proteins in deposits indicate a more severe disease while they differ with respect to co-location of IgA2 in the IgA1 deposits. In the study reported here IgA2 was detected in 30.7 % of the biopsies tested, always together with IgA1.

Although considerably fewer HSP patients compared to IgAN develop chronic or end stage kidney disease, the pathological findings in the HSP and IgAN kidneys have been reported to be identical (Barratt, *et al.*2005). In contrast in the present study some striking differences in lectin pathway proteins between HSP and IgAN were observed (table 9). Thus, the HSP patients were observed to have higher blood levels of MBL, L-ficolin and MASP-3 than both the IgAN patients and the controls. Most striking is a marked increase in the serum levels of the MASP-3 in HSP patients combined with relatively low levels of the C4d activation fragment. To my knowledge this is the first time that such differences in the lectin pathway have been reported between IgAN and HSP. It is tempting to speculate that these differences might be responsible for some of the divergence that has been reported in these

related disorders, including larger immune complexes, systemic vasculitis, and perhaps also a more benign renal disease in HSP.

MASP-3 has been reported to inhibit activation of the lectin pathway of the complement system (Dahl, et al.2001, Moller-Kristensen, et al.2007). This together with reduced serum levels of both intact C4 and the C4 activation fragment C4d in the HSP patients (Stefansson Thors*, et al.*2005), might indicate that the lectin pathway is impared in HSP. It has been consistently demonstrated that a normal classical complement pathway can prevent the formation of large IgM and IgG containing immune complexes and activation of C4 is central in that process (Arason, et al.1999). IgA antibodies, however, do not activate the classical complement pathway and clearance of immune complexes containing mainly IgA may therefore be highly dependent upon an intact lectin pathway. Impaired function of the lectin pathway might also be associated with the formation of relatively large complexes that may be deposited in a systemic fashion as is observed in HSP.

Furthermore, if the lectin pathway is involved in the glomerular pathogenesis in IgAN, a combination of low C4 and high MASP-3 in the HSP patients might to some extent protect their kidneys. Increased production of MASP-3 might also take place in their kidneys as expression of MASP-3 mRNA has been reported in the kidneys (Seyfarth*, et al.*2006) .

An alternative and more trivial explanation of the difference between IgAN and HSP patients might be that MBL, L-ficolin and MASP-3 components all behave like acute phase reactants and higher levels in HSP are secondary to the more widespread vasculitis observed in these patients. While it is well documented that MBL is an acute phase

reactant this has not been established for L-ficolin or MASP-3. It should also be noted in this context that the HSP patients were all in clinical remission when their blood samples were obtained (Stefansson Thors*, et al.*2005) while the activity of the glomerular disease of the IgAN patients was not assessed when their samples were collected. Therefore these findings call for a further and preferably a prospective study on HSP and IgAN. Special emphasis should be on potential differences in the size and complement content of circulating and deposited IgA-ICs in IgAN and HSP patients.

**C4 haplotypes**

The present study was initiated to follow up a previous study showing HSP patients to have an increased frequency of the C4B null allele (Stefansson Thors*, et al.*2005). In that study the C4B null allotype was associated with a marked decrease in serum levels of C4 in the HSP patients. In the present study C4B null alleles were slightly more frequent in the IgAN patients compared to controls (24.5% vs. 17.4%), but this did not reach statistical significance.

The physiological function of the two different C4 allotypes has not been sufficiently studied, but in the previous study on HSP it was concluded that C4B might have a bigger role in IgA immune complex clearance than hitherto considered. The present study on IgAN patients shows that both patients and controls carrying the C4B null allele have lower levels of the C4d activation product than those with other C4B allotypic variants. Nevertheless, frequency of raised immune complexes in the C4B null carriers did not differ from the non-null C4B patients. In contrast, IgAN patients carrying the C4B1-1 haplotype had an increased

frequency of elevated IgA-ICs (37.5% vs 17.5% (table 6)). MBL from these patients associated strongly with IgA-ICs and C3d and somewhat less to C4d. C3d alone related even more strongly with IgA-ICs and L-ficolin. No association in this direction was observed for the cohort at large. The C4B1-1 patients had significantly lower MBL compared to the non-null patients even when C4B null carriers were included (table 6). Low MBL could therefore account for the diminished immune complex clearance.

However, MASP-1 activates the lectin pathway through C2 and can possibly also activate C3 directly while MASP-2 effectively activates C4 (Matsushita*, et al.*1995, Moller-Kristensen*, et al.*2007). The efficacy of C3 activation may therefore depend on sufficient amounts of both MASP-1 and MASP-2 in the serum. MBL composed of small oligomers associates preferentially with MASP-1 while larger MBL oligomers tend to associate more with MASP-2 (Dahl*, et al.*2001). Sera with low MBL levels may contain disproportionately high amounts of small MBL oligomers, and thereby associate with MASP-1 rather than MASP-2 molecules. This might impare complement clearance through lack of C4 needed for the opsonization of the complexes (Dahl*, et al.*2001). As neither MBL oligomerization nor MASP-1 was measured in this study this possibility could not be ruled out. Previously, before the lectin pathway was discovered complement activation in IgAN was attributed to the alternative pathway. The relative significance of alternative pathway versus lectin pathway activation in both IgAN and HSP needs to be addressed further.

**IgA glycosylation**

Attempts to use conventional methods for lectin detection of glycans as previously described in the literature failed due to high background staining. The most substantial element of the present study is therefore perhaps isolation of IgA and development of an assay for evaluation of glycan motifs in serum IgA. The isolation steps followed a protocol described by Sandin and co-workers based on the use of streptococcal IgA-binding protein attatched to a chromatography column (Sandin*, et al.*2002). Due to limited time and funds further purification of the IgA isolate was not carried out. This drawback had to be taken into account when developing the ELISA assay for detection of different glycan motifs. Glycosylation measurements of IgA1 from HSP patients could not be undertaken due to inadequate sample volume and low serum concentration of IgA.

The glycan composition of the IgA in the isolate was detected by an indirect method using various glycan specific lectins. This process turned out to be more cumbersome than expected as some of the lectins did not show binding to the IgA glycans. This was especially noticeable for the lectins *Vicia villosa* and Peanut Agglutinin. These lectins turned out to be faulty due to a defective production. These and other problems are well known even by far more experienced investigators in this field (Moore*, et al.*2007). This called for a revision of the lectins used. *Helix arspesa* then became the primary choice for GalNAc detection and for further verification of this glycan *Helix pomatia* was selected.

It has been suggested that increased frequency of terminal GalNAc may be the basic defect leading to kidney deposition of immune complexes (Allen*, et al.*2001, Allen*, et al.*1998, Mestecky*, et al.*1993).

Therefore it was surprising that only five IgAN patients had HAA binding to GalNAc above that of the reference value, indicating that only 10% of the patients were inflicted with this glycan defect. This conclusion was later revised when removal of sialic acid from IgA1 by neuraminidase exposed considerable more terminal GalNAc; increasing significantly the number of patients with degalactosylated GalNAc. Thus, two types of glycan defects in the IgAN patients were demonstrated: 1) degalactosylated GalNAc and 2) oversialylated glycans. Both glycan defects may contribute to the immune complex formation, while sialic acid is considered a major contributor to mesangial cell uptake of complexes (Leung*, et al.*2007, Leung*, et al.*2001).

In a number of patients galactosylation did not differ from that of controls, making interpretations difficult without information about disease activity when the blood samples were obtained..

It has been reported that the levels of degalactosylated GalNAc vary according to disease activity, being highest in active disease and during bouts of enhanced disease activity (Novak*, et al.*2005b). Normal galactosylation may therefore indicate a state of remission. As clinical data for the IgAN patients in the present study are not available such associations can not be made. Furthermore, IgA glycan status was not determined for the HSP patients. This study could therefore not confirm earlier claims that increase in degalactosylated GalNAc is restricted to HSP patients with severe renal disease (Allen*, et al.*1998).

**IgA and IgA containing complexes**

Increased levels of IgA have been noted in many studies on IgAN. However, neither IgA or immune complex levels have been found to correlate with disease activity or progression (Droz*, et al.*1984).

It is interesting that in the present study increased levels of terminal GalNAc were associated with low levels of serum IgA and that such patients did not have increased circulating IgA-ICs. This may indicate that the production of IgA is not increased in these patients but rather that their clearance of immune complexes may be relatively high. This phenomen has not been reported before, but may, circuitously, support the findings of Peterman who showed that elevated total IgA in IgAN patients with normal renal function was of the mIgA1 type (Peterman*, et al.*1991), leading him to conclude that mIgA1 served a protective role in the disease. In the present study the balance of mIgA and pIgA in the circulation was not analysed. In order to elucidate this, the composition of serum IgA should be analyzed, i.e. the size and charge of the IgA1 molecules with regard to both the glycosylation status and MASP-3 levels. The same applies for the composition of immune complexes in HSP patients as there are indications that they may be of yet another order.

The size and charge of complexes is of interest as only polymeric anionic IgA is considered pathogenic in IgAN and this is true for HSP nephritis as well (Leung*, et al.*2007). Analyzes of the charge of IgA in the serum of the participants in our study was not carried out, but indirect information could be attained from our study on sialylation of the IgA. Not only did the IgAN patients have significantly higher sialylation than the control group, but their sialic acid was also to a significantly greater

extent connected through α2,6 ST linkage. It can therefore be concluded that a number of the IgAN patients had both of the deviant glycan forms reported to be at the heart of complex formation and mesangial cell uptake (Leung, *et al.*1999, Moura, *et al.*2005, Novak, *et al.*2005b).

Discussion on immune complexes in IgAN can not be abandoned without mentioning some of the theories that have been developed on the subject. In earlier publications elevated IgA-RF was thought to be a marker for both IgAN and HSP. Therefore RF was measured in all the IgAN patients in the present study. Unexpectedly only one IgAN patient was found to be positive for IgA-RF and this patient also had elevation of IgG RF and IgM RF isotype. The RF ELISA assay used in this study has been extensively compared in terms of sensitivity and specificity with other methods used for detecting RF, and it has been in routine use for over 25 years. Therefore, the results are likely to reflect the true RF status of the patients tested. However, these results are in clear conflict with earlier studies that implicated RF in the pathogenesis of IgAN. Czerkinsky and co-workers reported IgA-RF in more than one third of IgAN patients and also found that 87% of immune complexes in IgAN were composed of IgA1-IgG (Czerkinsky, *et al.*1986). Another early report suggested that IgA-RF antibodies participated in IgAN pathogenesis by decreasing solubilization of circulating immune complexes (Schena, *et al.*1988). Touching on these reports, two theories have emerged concerning IgA1-IgG complexes in IgAN, both from a research team in Birmingham, Alabama. The first theory suggested that IgG complexed with IgA1 could hinder normal clearance of the complexes due to longer survival in the circulation (Tomana, *et al.*1997),

while the second theory suggested that the overall size and structure of IgA1-IgG complexes prevented entrance through the space of Disse, thereby hindering their disposal by Kupffer cells (Mestecky, *et al.*2008).

**Immune deposits in IgAN kidneys**

The presence of IgA2 deposits in kidney biopsies from IgAN patients may be a critical marker for identification of IgAN patients that are likely to proceed to more severe disease (Hisano, *et al.*2005, Roos, *et al.*2006). In the present study a total of 26 kidney biopsies were examined for IgA1 and IgA2 deposits. In agreement with results from other investigators IgA1 was found in all the biopsies, with IgA2 co-localized in eight biopsies (30.7%). This is somewhat less than Hisano and co-workers reported (50%) but, interestingly, his study indicated that evidence for activation of the lectin pathway activation was confined to this particular subgroup. Roos et al reported that 24.4% of IgAN biopsies contained lectin pathway components, but they could not demonstrate any IgA2. Espinosa, on the other hand, focused on activation pathways and stained IgAN kidney biopsies for both C4d and C1q. Of the biopsies 32.2% stained positive for C4d in mesangial areas, while none of the biopsies stained positive for C1q (Espinosa, *et al.*2009). The diversity of reported findings may reflect different criteria used for deciding when to perform biopsy. In the present study, staining for lectin pathway proteins in the mesangial deposits remains to be completed.

The IgAN patients staining positive for IgA2 in mesangial deposits had significantly increased degalacosylated GalNAc in their serum compared to the patients having only IgA1 in their deposits.

Comparison of kidney deposits and glycan status of serum IgA1 has not to my knowledge been presented before. The results may support previous observations suggesting that increased GalNAc in terminal position is associated with more severe IgAN. Should that be the case it would also support the claim of Hisano that IgA2 in IgAN deposits indicate a more severe disease.

**Concluding remarks**

Glycosylation status of serum IgA in IgAN patients has not previously been analyzed in the context of complement components, including lectin pathway proteins. Neither have IgAN and HSPN patients been compared before in terms of serum complement components, glycosylation status and IgA isotype deposits. The results reveal a complex pattern of complement activation, glycan defects and immunoglobulin deposits that reflect a complicated clinical picture.

The prevelance of IgAN is low in the population and although all patients with biopsy proven IgAN in Iceland were invited to participate in the study, the cohort was relatively small. This together with the fact that IgAN is clinically not a homogenious disease makes interpretation of the results somewhat difficult. The problem is further enhanced as clinical information was not collected at the outset of the study. In order to strengthen the study further, permission has now been attained to: 1) increase the size of the study cohort and: 2) assess retrospectively the clinical status of the IgAN patients. This would be useful with regard to both the serological and immunohistological results where under-representation of each group is a problem.

The strength of this study rests on the information gained by evaluating complement proteins, in particular the lectin pathway, and glycoylation status in the two diseases under observation. Lectin pathway proteins have not previously been studied in connection with IgAN or HSP. These results may provide a clue for the different manifestations of the two overlapping diseases; IgAN and HSP.

# CONCLUSIONS

- Decreased levels of lectin pathway complement components were not found in patients with IgAN. On the other hand, defective IgA1 glycosylation correlated negatively with MBL and MASP-3 levels.

- Patients with HSP had higher levels of the lectin pathway complement components MBL and L-ficolin and especially MASP-3 than both the control group and the IgAN patients.

- A significant increase in the C4BQ*0 allotype has been found in patients with HSP compared to a control group. No such increase was found for IgAN.

- Two types of defective IgA1 glycosylation were observed in the IgAN patients: 1) Increased degalactosylation of the hinge region O-linked glycans and 2) Oversialylation of the O-linked glycans. A negative association was observed between IgA1 degalactosylation and both MASP-3 and MBL.

- Patients with increased degalactosylation of the hinge region O-linked glycans had significantly lower serum IgA levels than the other IgAN patients. However, no association was found between increased IgA1 degalactosylation and IgA containing immune complexes.

- All IgAN patients had IgA1 deposits, while only 30.7% had IgA2. Patients with IgA2 in their deposits had significantly higher levels of degalactosylated IgA1 compared to those with deposits of only IgA1.

Overall conclusion: The study confirms previous reports showing increased defective IgA1 glycosylation in IgAN. The significantly increased levels of several lectin pathway components observed in patients with HSP, but not in IgAN, may indicate a major difference in the pathogenesis of these two IgA related diseases. Preferably these findings should be followed by a prospective study focusing on the relation between lectin pathway components and clinical and prognostic factors of these two diseases.

# REFERENCE LIST

Abe, K, M Miyazaki, T Koji, A Furusu, K Shioshita, S Tsukasaki, Y Ozono, T Harada, H Sakai and S Kohno.Intraglomerular synthesis of complement C3 and its activation products in IgA nephropathy.Nephron.2001.87.3.231-9

Akaiwa, M, Y Yae, R Sugimoto, SO Suzuki, T Iwaki, K Izuhara and N Hamasaki.Hakata antigen, a new member of the ficolin/opsonin p35 family, is a novel human lectin secreted into bronchus/alveolus and bile.J Histochem Cytochem.1999.47.6.777-86

Allen, AC.Methodological approaches to the analysis of IgA1 O-glycosylation in IgA nephropathy.J Nephrol.1999.12.2.76-84

Allen, AC, EM Bailey, J Barratt, KS Buck and J Feehally.Analysis of IgA1 O-glycans in IgA nephropathy by fluorophore-assisted carbohydrate electrophoresis.J Am Soc Nephrol.1999.10.8.1763-71

Allen, AC, EM Bailey, PE Brenchley, KS Buck, J Barratt and J Feehally.Mesangial IgA1 in IgA nephropathy exhibits aberrant O-glycosylation: observations in three patients.Kidney Int.2001.60.3.969-73

Allen, AC, SJ Harper and J Feehally.Galactosylation of N- and O-linked carbohydrate moieties of IgA1 and IgG in IgA nephropathy.Clin Exp Immunol. 1995. 100.3.470-4

Allen, AC, FR Willis, TJ Beattie and J Feehally.Abnormal IgA glycosylation in Henoch-Schonlein purpura restricted to patients with clinical nephritis.Nephrol Dial Transplant.1998.13.4.930-4

Almogren, A and MA Kerr.Irreversible aggregation of the Fc fragment derived from polymeric but not monomeric serum IgA1--implications in IgA-mediated disease.Mol Immunol.2008.45.1.87-94

Andre, PM, P Le Pogamp and D Chevet.Impairment of jacalin binding to serum IgA in IgA nephropathy.J Clin Lab Anal.1990.4.2.115-9

Arason, GJ, MS D'Ambrogio, T Vikingsdottir, A Sigfusson and H Valdimarsson. Enzyme immunoassays for measuring complement-dependent prevention of immune precipitation (PIP) and solubilization of preformed antigen-antibody complexes (SOL).J Immunol Methods.1999.223.1.37-46

Arnold, JN, RA Dwek, PM Rudd and RB Sim.Mannan binding lectin and its interaction with immunoglobulins in health and in disease.Immunol Lett.2006.106.2.103-10

Atkinson, AP, M Cedzynski, J Szemraj, A St Swierzko, L Bak-Romaniszyn, M Banasik, K Zeman, M Matsushita, ML Turner and DC Kilpatrick.L-ficolin in children with recurrent respiratory infections.Clin Exp Immunol.2004. 138.3.517-20

Ault, BH, FB Stapleton, ML Rivas, FB Waldo, S Roy, 3rd, RH McLean, JA Bin and RJ Wyatt.Association of Henoch-Schonlein purpura glomerulonephritis with C4B deficiency.J Pediatr.1990.117.5.753-5

Awdeh, ZL, D Raum and CA Alper.Genetic polymorphism of human complement C4 and detection of heterozygotes.Nature.1979.282.5735.205-7

Barratt, J and J Feehally.IgA nephropathy.Journal of the American Society of Nephrology.2005.16.7.2088-97

Barratt, J, AC Smith and J Feehally.The pathogenic role of IgA1 O-linked glycosylation in the pathogenesis of IgA nephropathy.Nephrology (Carlton).2007.12.3.275-84

Batra, A, AC Smith, J Feehally and J Barratt.T-cell homing receptor expression in IgA nephropathy.Nephrol Dial Transplant.2007.22.9.2540-8

Batten, MR, BW Senior, M Kilian and JM Woof.Amino acid sequence requirements in the hinge of human immunoglobulin A1 (IgA1) for cleavage by streptococcal IgA1 proteases.Infect Immun.2003.71.3.1462-9

Bene, MC, G Faure and J Duheille.IgA nephropathy: characterization of the polymeric nature of mesangial deposits by in vitro binding of free secretory component. Clin Exp Immunol.1982.47.3.527-34

Bene, MC, B Hurault de Ligny, M Kessler, B Foliguet and GC Faure.Tonsils in IgA nephropathy.Contrib Nephrol.1993.104.153-61

Berger, J and N Hinglais.[Intercapillary deposits of IgA-IgG].J Urol Nephrol (Paris).1968.74.9.694-5

Bisceglia, L, G Cerullo, P Forabosco, DD Torres, F Scolari, M Di Perna, M Foramitti, A Amoroso, S Bertok, J Floege, PR Mertens, K Zerres, E Alexopoulos, D Kirmizis, M Ermelinda, L Zelante and FP Schena.Genetic heterogeneity in Italian families with IgA nephropathy: suggestive linkage for two novel IgA nephropathy loci.Am J Hum Genet.2006.79.6.1130-4

Buck, KS, EM Foster, D Watson, J Barratt, IZ Pawluczyk, JF Knight, J Feehally and AC Allen.Expression of T cell receptor variable region families by bone marrow gammadelta T cells in patients with IgA nephropathy.Clin Exp Immunol. 2002.127.3.527-32

Cederholm, B, J Wieslander, P Bygren and D Heinegard.Circulating complexes containing IgA and fibronectin in patients with primary IgA nephropathy.Proc Natl Acad Sci U S A.1988.85.13.4865-8

Chen, WP, A Chen and CY Lin.In vitro effects of interleukins on human mesangial cells: implications for glomerulonephritis.J Pathol.1995.175.3.327-37

Chintalacharuvu, KR, PD Chuang, A Dragoman, CZ Fernandez, J Qiu, AG Plaut, KR Trinh, FA Gala and SL Morrison.Cleavage of the human immunoglobulin A1 (IgA1) hinge region by IgA1 proteases requires structures in the Fc region of IgA.Infect Immun.2003.71.5.2563-70

Christiansen, FT, RL Dawkins, G Uko, J McCluskey, PH Kay and PJ Zilko. Complement allotyping in SLE: association with C4A null.Aust N Z J Med.1983.13.5.483-8

Clarkson, AR, AE Seymour, AJ Thompson, WD Haynes, YL Chan and B Jackson.IgA nephropathy: a syndrome of uniform morphology, diverse clinical features and uncertain prognosis.Clin Nephrol.1977.8.5.459-71

Collard, CD, A Vakeva, MA Morrissey, A Agah, SA Rollins, WR Reenstra, JA Buras, S Meri and GL Stahl.Complement activation after oxidative stress: role of the lectin complement pathway.Am J Pathol.2000.156.5.1549-56

Colomb, MG, GJ Arlaud and CL Villiers.Activation of C1.Philos Trans R Soc Lond B Biol Sci.1984.306.1129.283-92

Coons, AH and MH Kaplan.Localization of antigen in tissue cells; improvements in a method for the detection of antigen by means of fluorescent antibody.J Exp Med.1950.91.1.1-13

Coppo, R and A Amore.Aberrant glycosylation in IgA nephropathy (IgAN).Kidney Int.2004.65.5.1544-7

Coppo, R, A Amore, B Gianoglio, A Reyna, L Peruzzi, D Roccatello, D Alessi and LM Sena.Serum IgA and macromolecular IgA reacting with mesangial matrix components.Contrib Nephrol.1993.104.162-71

Coppo, R, B Basolo, G Piccoli, G Mazzucco, MR Bulzomi, D Roccatello, M De Marchi, AO Carbonara and G Barbiano di Belgiojoso.IgA1 and IgA2 immune complexes in primary IgA nephropathy and Henoch-Schonlein nephritis.Clin Exp Immunol.1984.57.3.583-90

Coppo, R and G D'Amico.Factors predicting progression of IgA nephropathies.J Nephrol.2005.18.5.503-12

Coppo, R, G Mazzucco, L Cagnoli, A Lupo and FP Schena.Long-term prognosis of Henoch-Schonlein nephritis in adults and children. Italian Group of Renal Immunopathology Collaborative Study on Henoch-Schonlein purpura.Nephrol Dial Transplant.1997.12.11.2277-83

Cortesio, CL and W Jiang.Mannan-binding lectin-associated serine protease 3 cleaves synthetic peptides and insulin-like growth factor-binding protein 5.Arch Biochem Biophys.2006.449.1-2.164-70

Czerkinsky, C, WJ Koopman, S Jackson, JE Collins, SS Crago, RE Schrohenloher, BA Julian, JH Galla and J Mestecky.Circulating immune complexes and immunoglobulin A rheumatoid factor in patients with mesangial immunoglobulin A nephropathies.J Clin Invest.1986.77.6.1931-8

Daha, MR, DT Fearon and KF Austen.Isolation of alternative pathway C3 convertase containing uncleaved B and formed in the presence of C3 nephritic factor (C3neF).J Immunol.1976.116.2.568-70

Dahl, MR, S Thiel, M Matsushita, T Fujita, AC Willis, T Christensen, T Vorup-Jensen and JC Jensenius.MASP-3 and its association with distinct complexes of the mannan-binding lectin complement activation pathway.Immunity. 2001. 15.1.127-35

Davies, EJ, N Snowden, MC Hillarby, D Carthy, DM Grennan, W Thomson and WE Ollier.Mannose-binding protein gene polymorphism in systemic lupus erythematosus.Arthritis Rheum.1995.38.1.110-4

Davin, JC, IJ Ten Berge and JJ Weening.What is the difference between IgA nephropathy and Henoch-Schonlein purpura nephritis?Kidney Int.2001a. 59.3.823-34

Davin, JC and JJ Weening.Henoch-Schonlein purpura nephritis: an update.Eur J Pediatr.2001b.160.12.689-95

Di Rosa, F.T-lymphocyte interaction with stromal, bone and hematopoietic cells in the bone marrow.Immunol Cell Biol.2009.87.1.20-9

Dommett, RM, N Klein and MW Turner.Mannose-binding lectin in innate immunity: past, present and future.Tissue Antigens.2006.68.3.193-209

Donadio, JV and JP Grande.IgA nephropathy.N Engl J Med.2002.347.10.738-48

Dornelles, LN, L Pereira-Ferrari and I Messias-Reason.Mannan-binding lectin plasma levels in leprosy: deficiency confers protection against the lepromatous but not the tuberculoid forms.Clin Exp Immunol.2006.145.3.463-8

Droz, D, A Kramar, T Nawar and LH Noel.Primary IgA nephropathy: prognostic factors.Contrib Nephrol.1984.40.202-7

Endo, M, H Ohi, I Ohsawa, T Fujita and M Matsushita.Complement activation through the lectin pathway in patients with Henoch-Schonlein purpura nephritis.Am J Kidney Dis.2000.35.3.401-7

Endo, M, H Ohi, I Ohsawa, T Fujita and M Matsushita.Glomerular deposition of mannose-binding lectin (MBL) indicates a novel mechanism of complement activation in IgA nephropathy.Nephrol Dial Transplant.1998a.13.8.1984-90

Endo, M, H Ohi, I Ohsawa, T Fujita, M Matsushita and T Fujita.Glomerular deposition of mannose-binding lectin (MBL) indicates a novel mechanism of complement activation in IgA nephropathy.Nephrology Dialysis Transplantation. 1998b. 13.8.1984-90

Endo, Y, Y Liu, K Kanno, M Takahashi, M Matsushita and T Fujita.Identification of the mouse H-ficolin gene as a pseudogene and orthology between mouse ficolins A/B and human L-/M-ficolins.Genomics.2004.84.4.737-44

Endo, Y, M Takahashi and T Fujita.Lectin complement system and pattern recognition. Immunobiology.2006.211.4.283-93

Espinosa, M, R Ortega, JM Gomez-Carrasco, F Lopez-Rubio, M Lopez-Andreu, MO Lopez-Oliva and P Aljama.Mesangial C4d deposition: a new prognostic factor in IgA nephropathy.Nephrol Dial Transplant.2009.24.3.886-91

Feehally, J and AC Allen.Pathogenesis of IgA nephropathy.Ann Med Interne (Paris). 1999.150.2.91-8

Floege, J.Recurrent IgA nephropathy after renal transplantation.Semin Nephrol.2004. 24.3.287-91

Fujita, T, M Matsushita and Y Endo.The lectin-complement pathway--its role in innate immunity and evolution.Immunol Rev.2004.198.185-202

Gadjeva, M, K Takahashi and S Thiel.Mannan-binding lectin--a soluble pattern recognition molecule.Mol Immunol.2004.41.2-3.113-21

Galla, JH.IgA nephropathy.Kidney Int.1995.47.2.377-87

Galla, JH.Molecular genetics in IgA nephropathy.Nephron.2001.88.2.107-12

Galla, JH, EC Kohaut, R Alexander and J Mestecky.Racial difference in the prevalence of IgA-associated nephropathies.Lancet.1984.2.8401.522

Gharavi, AG, Z Moldoveanu, RJ Wyatt, CV Barker, SY Woodford, RP Lifton, J Mestecky, J Novak and BA Julian.Aberrant IgA1 glycosylation is inherited in familial and sporadic IgA nephropathy.J Am Soc Nephrol.2008.19.5.1008-14

Gharavi, AG, Y Yan, F Scolari, FP Schena, GM Frasca, GM Ghiggeri, K Cooper, A Amoroso, BF Viola, G Battini, G Caridi, C Canova, A Farhi, V Subramanian, C Nelson-Williams, S Woodford, BA Julian, RJ Wyatt and RP Lifton.IgA nephropathy, the most common cause of glomerulonephritis, is linked to 6q22-23.Nature Genetics.2000.26.3.354-7

Haas, M.Histology and immunohistology of IgA nephropathy.Journal of Nephrology. 2005.18.6.676-80

Hansen, S and U Holmskov.Structural aspects of collectins and receptors for collectins. Immunobiology.1998.199.2.165-89

Harper, SJ, AC Allen, MC Bene, JH Pringle, G Faure, I Lauder and J Feehally.Increased dimeric IgA-producing B cells in tonsils in IgA nephropathy determined by in situ hybridization for J chain mRNA.Clin Exp Immunol. 1995.101.3.442-8

Harper, SJ, AC Allen, JH Pringle and J Feehally.Increased dimeric IgA producing B cells in the bone marrow in IgA nephropathy determined by in situ hybridisation for J chain mRNA.J Clin Pathol.1996.49.1.38-42

Harper, SJ, JH Pringle, AC Wicks, J Hattersley, L Layward, A Allen, A Gillies, I Lauder and J Feehally.Expression of J chain mRNA in duodenal IgA plasma cells in IgA nephropathy.Kidney Int.1994.45.3.836-44

Hayday, AC, S Roberts and E Ramsburg.gammadelta cells and the regulation of mucosal immune responses.Am J Respir Crit Care Med.2000.162.4 Pt 2.S161-3

Hiki, Y, A Horii, H Iwase, A Tanaka, Y Toda, K Hotta and Y Kobayashi.O-linked oligosaccharide on IgA1 hinge region in IgA nephropathy. Fundamental study for precise structure and possible role.Contrib Nephrol.1995.111.73-84

Hiki, Y, A Tanaka, T Kokubo, H Iwase, J Nishikido, K Hotta and Y Kobayashi. Analyses of IgA1 hinge glycopeptides in IgA nephropathy by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.J Am Soc Nephrol.1998.9.4.577-82

Hisano, S, M Matsushita, T Fujita, Y Endo and S Takebayashi.Mesangial IgA2 deposits and lectin pathway-mediated complement activation in IgA glomerulonephritis. Am J Kidney Dis.2001.38.5.1082-8

Hisano, S, M Matsushita, T Fujita and H Iwasaki.Activation of the lectin complement pathway in Henoch-Schonlein purpura nephritis.Am J Kidney Dis.2005. 45.2.295-302

Hudson, BG, ST Reeders and K Tryggvason.Type IV collagen: structure, gene organization, and role in human diseases. Molecular basis of Goodpasture and Alport syndromes and diffuse leiomyomatosis.J Biol Chem.1993.268.35.26033-6

Hummelshoj, T, L Munthe-Fog, HO Madsen, T Fujita, M Matsushita and P Garred.Polymorphisms in the FCN2 gene determine serum variation and function of Ficolin-2.Hum Mol Genet.2005.14.12.1651-8

Ikezumi, Y, T Suzuki, N Imai, M Ueno, I Narita, H Kawachi, F Shimizu, DJ Nikolic-Paterson and M Uchiyama.Histological differences in new-onset IgA nephropathy between children and adults.Nephrol Dial Transplant. 2006. 21.12.3466-74

Imai, H, A Chen, RJ Wyatt and A Rifai.Composition of IgA immune complexes precipitated with polyethylene glycol. A model for isolation and analysis of immune complexes.J Immunol Methods.1987.103.2.239-45

Iobst, ST, MR Wormald, WI Weis, RA Dwek and K Drickamer.Binding of sugar ligands to Ca(2+)-dependent animal lectins. I. Analysis of mannose binding by site-directed mutagenesis and NMR.J Biol Chem.1994.269.22.15505-11

Isenman, DE, DI Kells, NR Cooper, HJ Muller-Eberhard and MK Pangburn.Nucleophilic modification of human complement protein C3: correlation of conformational changes with acquisition of C3b-like functional properties.Biochemistry.1981.20.15.4458-67

Ivan, CM, B Marc, L Pierre, V FranÃ§ois, B Ulrich and CM Renato.The Glomerular Response to IgA Deposition in IgA Nephropathy.Seminars in nephrology. 2008.28.1.88-95

Iwaki, D, K Kanno, M Takahashi, Y Endo, NJ Lynch, WJ Schwaeble, M Matsushita, M Okabe and T Fujita.Small mannose-binding lectin-associated protein plays a regulatory role in the lectin complement pathway.J Immunol.2006.177.12.8626-32

Iwase, H, A Tanaka, Y Hiki, T Kokubo, T Sano, I Ishii-Karakasa, K Toma, Y Kobayashi and K Hotta.Aggregated human serum immunoglobulin A1 induced by neuraminidase treatment had a lower number of O-linked sugar chains on the hinge portion.J Chromatogr B Biomed Sci Appl.1999.724.1.1-7

Jack, DL, NJ Klein and MW Turner.Mannose-binding lectin: targeting the microbial world for complement attack and opsonophagocytosis.Immunol Rev.2001a. 180.86-99

Jack, DL, RC Read, AJ Tenner, M Frosch, MW Turner and NJ Klein.Mannose-binding lectin regulates the inflammatory response of human professional phagocytes to Neisseria meningitidis serogroup B.J Infect Dis.2001b.184.9.1152-62

Jackson, S, Z Moldoveanu, KA Kirk, BA Julian, TF Patterson, AL Mullins, T Jilling, J Mestecky and JH Galla.IgA-containing immune complexes after challenge with food antigens in patients with IgA nephropathy.Clin Exp Immunol. 1992. 89.2.315-20

Jackson, S, RI Montgomery, BA Julian, JH Galla and C Czerkinsky.Aberrant synthesis of antibodies directed at the Fab fragment of IgA in patients with IgA nephropathies.Clin Immunol Immunopathol.1987a.45.2.208-13

Jackson, S, RI Montgomery, J Mestecky, BA Julian, JH Galla and C Czerkinsky. Antibodies directed at Fab of IgA in the sera of normal individuals and IgA nephropathy patients.Adv Exp Med Biol.1987b.216B.1537-44

Jonsson, T, JA Arnason and H Valdimarsson.Enzyme-linked immunosorbent assay (ELISA) screening test for detection of rheumatoid factor.Rheumatol Int.1986. 6.5.199-204

Jordan, JE, MC Montalto and GL Stahl.Inhibition of mannose-binding lectin reduces postischemic myocardial reperfusion injury.Circulation.2001.104.12.1413-8

Julian, BA and J Novak.IgA nephropathy: an update.Current Opinion in Nephrology & Hypertension.2004.13.2.171-9

Julian, BA, PA Quiggins, JS Thompson, SY Woodford, K Gleason and RJ Wyatt.Familial IgA nephropathy. Evidence of an inherited mechanism of disease.N Engl J Med.1985.312.4.202-8

Julian, BA, M Tomana, J Novak and J Mestecky.Progress in the pathogenesis of IgA nephropathy.Adv Nephrol Necker Hosp.1999.29.53-72

Julian, BA, SY Woodford, RW Baehler, RG McMorrow and RJ Wyatt.Familial clustering and immunogenetic aspects of IgA nephropathy.Am J Kidney Dis.1988.12.5.366-70

Junqueira, LCU and J Carneiro.2005.Basic histology : text & atlas.11th.McGraw-Hill Medical,.New York ; London.2005

Kashtan, CE.Familial hematurias: what we know and what we don't.Pediatr Nephrol.2005.20.8.1027-35

Kemper, C and JP Atkinson.T-cell regulation: with complements from innate immunity. Nat Rev Immunol.2007.7.1.9-18

Kerr, MA.The structure and function of human IgA.Biochem J.1990.271.2.285-96

Kilpatrick, DC.Mannan-binding lectin: clinical significance and applications.Biochim Biophys Acta.2002.1572.2-3.401-13

Kincaid-Smith, PS.Mesangial IgA nephropathy.Br Med J (Clin Res Ed). 1985. 290.6462.96-7

Koch, A, M Melbye, P Sorensen, P Homoe, HO Madsen, K Molbak, CH Hansen, LH Andersen, GW Hahn and P Garred.Acute respiratory tract infections and mannose-binding lectin insufficiency during early childhood. JAMA .2001.285.10.1316-21

Kokubo, T, K Hashizume, H Iwase, K Arai, A Tanaka, K Toma, K Hotta and Y Kobayashi.Humoral immunity against the proline-rich peptide epitope of the IgA1 hinge region in IgA nephropathy.Nephrol Dial Transplant.2000.15.1.28-33

Kokubo, T, Y Hiki, H Iwase, A Tanaka, K Toma, K Hotta and Y Kobayashi.Protective role of IgA1 glycans against IgA1 self-aggregation and adhesion to extracellular matrix proteins.J Am Soc Nephrol.1998.9.11.2048-54

Koskimies, O, S Mir, J Rapola and J Vilska.Henoch-Schonlein nephritis: long-term prognosis of unselected patients.Arch Dis Child.1981.56.6.482-4

Krarup, A, DA Mitchell and RB Sim.Recognition of acetylated oligosaccharides by human L-ficolin.Immunol Lett.2008.118.2.152-6

Krarup, A, UB Sorensen, M Matsushita, JC Jensenius and S Thiel.Effect of capsulation of opportunistic pathogenic bacteria on binding of the pattern recognition molecules mannan-binding lectin, L-ficolin, and H-ficolin.Infect Immun. 2005. 73.2.1052-60

Kuraya, M, Z Ming, X Liu, M Matsushita and T Fujita.Specific binding of L-ficolin and H-ficolin to apoptotic cells leads to complement activation. Immunobiology. 2005.209.9.689-97

Lau, YL, CS Lau, SY Chan, J Karlberg and MW Turner.Mannose-binding protein in Chinese patients with systemic lupus erythematosus.Arthritis Rheum.1996. 39.4.706-8

Launay, P, B Grossetete, M Arcos-Fajardo, E Gaudin, SP Torres, L Beaudoin, N Patey-Mariaud de Serre, A Lehuen and RC Monteiro.Fcalpha receptor (CD89) mediates the development of immunoglobulin A (IgA) nephropathy (Berger's

disease). Evidence for pathogenic soluble receptor-Iga complexes in patients and CD89 transgenic mice.J Exp Med.2000.191.11.1999-2009

Leung, JC, LY Chan, SC Tang, PC Tam, J Fenn and KN Lai.Glycosylation profile of differently charged IgA1 and their binding characteristics to cultured mesangial cells in IgA nephropathy.Nephron Exp Nephrol.2007.107.3.e107-18

Leung, JC, PY Poon and KN Lai.Increased sialylation of polymeric immunoglobulin A1: mechanism of selective glomerular deposition in immunoglobulin A nephropathy?J Lab Clin Med.1999.133.2.152-60

Leung, JC, SC Tang, DT Chan, SL Lui and KN Lai.Increased sialylation of polymeric lambda-IgA1 in patients with IgA nephropathy.J Clin Lab Anal.2002.16.1.11-9

Leung, JC, SC Tang, LY Chan, AW Tsang, HY Lan and KN Lai.Polymeric IgA increases the synthesis of macrophage migration inhibitory factor by human mesangial cells in IgA nephropathy.Nephrol Dial Transplant.2003a.18.1.36-45

Leung, JC, SC Tang, LY Chan, AW Tsang, HY Lan and KN Lai.Polymeric IgA increases the synthesis of macrophage migration inhibitory factor by human mesangial cells in IgA nephropathy.Nephrology Dialysis Transplantation. 2003b. 18.1.36-45

Leung, JC, SC Tang, MF Lam, TM Chan and KN Lai.Charge-dependent binding of polymeric IgA1 to human mesangial cells in IgA nephropathy.Kidney Int.2001.59.1.277-85

Leung, JC, AW Tsang, DT Chan and KN Lai. Absence of CD89, polymeric immunoglobulin receptor, and asialoglycoprotein receptor on human mesangial cells.J Am Soc Nephrol.2000.11.2.241-9

Lhotta, K, R Wurzner and P Konig.Glomerular deposition of mannose-binding lectin in human glomerulonephritis.Nephrol Dial Transplant.1999.14.4.881-6

Lipscombe, RJ, DW Beatty, M Ganczakowski, EA Goddard, T Jenkins, YL Lau, AB Spurdle, M Sumiya, JA Summerfield and MW Turner.Mutations in the human mannose-binding protein gene: frequencies in several population groups.Eur J Hum Genet.1996.4.1.13-9

Liu, Y, Y Endo, D Iwaki, M Nakata, M Matsushita, I Wada, K Inoue, M Munakata and T Fujita.Human M-ficolin is a secretory protein that activates the lectin complement pathway.J Immunol.2005.175.5.3150-6

MacKay, SL and JR Dankert.Bacterial killing and inhibition of inner membrane activity by C5b-9 complexes as a function of the sequential addition of C9 to C5b-8 sites.J Immunol.1990.145.10.3367-71

Madsen, HO, ML Satz, B Hogh, A Svejgaard and P Garred.Different molecular events result in low protein levels of mannan-binding lectin in populations from southeast Africa and South America.J Immunol.1998.161.6.3169-75

Mancini, G, AO Carbonara and JF Heremans.Immunochemical quantitation of antigens by single radial immunodiffusion.Immunochemistry.1965.2.3.235-54

Matousovic, K, J Novak, T Yanagihara, M Tomana, Z Moldoveanu, R Kulhavy, BA Julian, K Konecny and J Mestecky.IgA-containing immune complexes in the urine of IgA nephropathy patients.Nephrol Dial Transplant.2006.21.9.2478-84

Matsuda, M, K Shikata, J Wada, H Sugimoto, Y Shikata, T Kawasaki and H Makino.Deposition of mannan binding protein and mannan binding protein-mediated complement activation in the glomeruli of patients with IgA nephropathy.Nephron.1998.80.4.408-13

Matsushita, M, Y Endo and T Fujita.Cutting edge: complement-activating complex of ficolin and mannose-binding lectin-associated serine protease.J Immunol. 2000a. 164.5.2281-4

Matsushita, M, Y Endo, S Taira, Y Sato, T Fujita, N Ichikawa, M Nakata and T Mizuochi.A novel human serum lectin with collagen- and fibrinogen-like domains that functions as an opsonin.J Biol Chem.1996.271.5.2448-54

Matsushita, M and T Fujita.Cleavage of the third component of complement (C3) by mannose-binding protein-associated serine protease (MASP) with subsequent complement activation.Immunobiology.1995.194.4-5.443-48

Matsushita, M, S Thiel, JC Jensenius, I Terai and T Fujita.Proteolytic activities of two types of mannose-binding lectin-associated serine protease.J Immunol. 2000b. 165.5.2637-42

Mattu, TS, RJ Pleass, AC Willis, M Kilian, MR Wormald, AC Lellouch, PM Rudd, JM Woof and RA Dwek.The glycosylation and structure of human serum IgA1, Fab, and Fc regions and the role of N-glycosylation on Fc alpha receptor interactions.J Biol Chem.1998.273.4.2260-72

McDonald, KJ, AJ Cameron, JM Allen and AG Jardine.Expression of Fc alpha/mu receptor by human mesangial cells: a candidate receptor for immune complex deposition in IgA nephropathy.Biochem Biophys Res Commun.2002.290.1.438-42

McLean, RH, RJ Wyatt and BA Julian.Complement phenotypes in glomerulonephritis: increased frequency of homozygous null C4 phenotypes in IgA nephropathy and Henoch-Schonlein purpura.Kidney Int.1984.26.6.855-60

Medof, ME, T Kinoshita and V Nussenzweig.Inhibition of complement activation on the surface of cells after incorporation of decay-accelerating factor (DAF) into their membranes.J Exp Med.1984.160.5.1558-78

Meri, S.Loss of self-control in the complement system and innate autoreactivity.Ann N Y Acad Sci.2007.1109.93-105

Mestecky, J, M Tomana, PA Crowley-Nowick, Z Moldoveanu, BA Julian and S Jackson.Defective galactosylation and clearance of IgA1 molecules as a possible etiopathogenic factor in IgA nephropathy.Contrib Nephrol.1993.104.172-82

Mestecky, J, M Tomana, Z Moldoveanu, BA Julian, H Suzuki, K Matousovic, MB Renfrow, L Novak, RJ Wyatt and J Novak.Role of aberrant glycosylation of IgA1 molecules in the pathogenesis of IgA nephropathy.Kidney Blood Press Res.2008.31.1.29-37

Miyazaki, M, K Abe, T Koji, A Furusu, Y Ozono, T Harada, PK Nakane, M Yagame, M Endoh, Y Nomoto and H Sakai.Intraglomerular C3 synthesis in human kidney detected by in situ hybridization.J Am Soc Nephrol.1996.7.11.2428-33

Moldoveanu, Z, RJ Wyatt, JY Lee, M Tomana, BA Julian, J Mestecky, WQ Huang, SR Anreddy, S Hall, MC Hastings, KK Lau, WJ Cook and J Novak.Patients with IgA nephropathy have increased serum galactose-deficient IgA1 levels.Kidney Int.2007.71.11.1148-54

Molina, H, VM Holers, B Li, Y-F Fang, S Mariathasan, J Goellner, J Strauss-Schoenberger, RW Karr and DD Chaplin.Markedly impaired humoral immune response in mice deficient in complement receptors 1 and 2.Proceedings of the National Academy of Sciences.1996.93.8.3357-3361

Moller-Kristensen, M, S Thiel, A Sjoholm, M Matsushita and JC Jensenius.Cooperation between MASP-1 and MASP-2 in the generation of C3 convertase through the MBL pathway.Int Immunol.2007.19.2.141-9

Monteiro, RC, L Halbwachs-Mecarelli, MC Roque-Barreira, LH Noel, J Berger and P Lesavre.Charge and size of mesangial IgA in IgA nephropathy.Kidney Int.1985. 28.4.666-71

Monteiro, RC, H Kubagawa and MD Cooper.Cellular distribution, regulation, and biochemical nature of an Fc alpha receptor in humans.J Exp Med. 1990. 171.3.597-613

Monteiro, RC, IC Moura, P Launay, T Tsuge, E Haddad, M Benhamou, MD Cooper and M Arcos-Fajardo.Pathogenic significance of IgA receptor interactions in IgA nephropathy.Trends in Molecular Medicine.2002.8.10.464-468

Moore, JS, R Kulhavy, M Tomana, Z Moldoveanu, H Suzuki, R Brown, S Hall, M Kilian, K Poulsen, J Mestecky, BA Julian and J Novak.Reactivities of N-acetylgalactosamine-specific lectins with human IgA1 proteins.Mol Immunol. 2007.44.10.2598-604

Moura, IC, M Arcos-Fajardo, A Gdoura, V Leroy, C Sadaka, N Mahlaoui, Y Lepelletier, F Vrtovsnik, E Haddad, M Benhamou and RC Monteiro. Engagement of transferrin receptor by polymeric IgA1: evidence for a positive feedback loop involving increased receptor expression and mesangial cell proliferation in IgA nephropathy.J Am Soc Nephrol.2005.16.9.2667-76

Moura, IC, M Arcos-Fajardo, C Sadaka, V Leroy, M Benhamou, J Novak, F Vrtovsnik, E Haddad, KR Chintalacharuvu and RC Monteiro.Glycosylation and size of IgA1 are essential for interaction with mesangial transferrin receptor in IgA nephropathy.J Am Soc Nephrol.2004a.15.3.622-34

Moura, IC, M Arcos-Fajardo, C Sadaka, V Leroy, M Benhamou, J Novak, F Vrtovsnik, E Haddad, KR Chintalacharuvu and RC Monteiro.Glycosylation and size of IgA1 are essential for interaction with mesangial transferrin receptor in IgA nephropathy.Journal of the American Society of Nephrology.2004b.15.3.622-34

Moura, IC, M Benhamou, P Launay, F Vrtovsnik, U Blank and RC Monteiro.The glomerular response to IgA deposition in IgA nephropathy.Semin Nephrol. 2008. 28.1.88-95

Moura, IC, MN Centelles, M Arcos-Fajardo, DM Malheiros, JF Collawn, MD Cooper and RC Monteiro.Identification of the transferrin receptor as a novel immunoglobulin (Ig)A1 receptor and its enhanced expression on mesangial cells in IgA nephropathy.J Exp Med.2001.194.4.417-25

Muller-Eberhard, HJ.Molecular organization and function of the complement system.Annu Rev Biochem.1988.57.321-47

Munro, JM.Endothelial-leukocyte adhesive interactions in inflammatory diseases.Eur Heart J.1993.14 Suppl K.72-7

Nakamoto, Y, Y Asano, K Dohi, M Fujioka, H Iida, H Kida, Y Kibe, N Hattori and J Takeuchi.Primary IgA glomerulonephritis and Schonlein-Henoch purpura nephritis: Clinicopathological and immunohistological characteristics.Q J Med.1978.47.188.495-516

Nielsen, CH, EM Fischer and RG Leslie.The role of complement in the acquired immune response.Immunology.2000.100.1.4-12

Novak, J, BA Julian, M Tomana and J Mesteck.Progress in molecular and genetic studies of IgA nephropathy.J Clin Immunol.2001.21.5.310-27

Novak, J, BA Julian, M Tomana and J Mestecky.IgA glycosylation and IgA immune complexes in the pathogenesis of IgA nephropathy.Semin Nephrol. 2008. 28.1.78-87

Novak, J, M Tomana, K Matousovic, R Brown, S Hall, L Novak, BA Julian, RJ Wyatt and J Mestecky.IgA1-containing immune complexes in IgA nephropathy differentially affect proliferation of mesangial cells.Kidney Int.2005a.67.2.504-13

Novak, J, M Tomana, K Matousovic, R Brown, S Hall, L Novak, BA Julian, RJ Wyatt and J Mestecky.IgA1-containing immune complexes in IgA nephropathy differentially affect proliferation of mesangial cells.Kidney International. 2005b. 67.2.504-13

Novak, J, HL Vu, L Novak, BA Julian, J Mestecky and M Tomana.Interactions of human mesangial cells with IgA and IgA-containing immune complexes.Kidney Int.2002.62.2.465-75

Odani, H, Y Hiki, M Takahashi, A Nishimoto, Y Yasuda, H Iwase, T Shinzato and K Maeda.Direct evidence for decreased sialylation and galactosylation of human serum IgA1 Fc O-glycosylated hinge peptides in IgA nephropathy by mass spectrometry.Biochem Biophys Res Commun.2000.271.1.268-74

Odum, J, CA Peh, AR Clarkson, KM Bannister, AE Seymour, D Gillis, AC Thomas, TH Mathew and AJ Woodroffe.Recurrent mesangial IgA nephritis following renal transplantation.Nephrol Dial Transplant.1994.9.3.309-12

Ohsawa, I, H Ohi, M Endo, T Fujita and M Matsushita.Evidence of lectin complement pathway activation in poststreptococcal glomerulonephritis.Kidney Int. 1999. 56.3.1158-9

Olive, C, AC Allen, SJ Harper, AC Wicks, J Feehally and MC Falk.Expression of the mucosal gamma delta T cell receptor V region repertoire in patients with IgA nephropathy.Kidney Int.1997.52.4.1047-53

Oortwijn, BD, PJ van der Boog, A Roos, RN van der Geest, JW de Fijter, MR Daha and C van Kooten.A pathogenic role for secretory IgA in IgA nephropathy.Kidney Int.2006.69.7.1131-8

Pangburn, MK and HJ Muller-Eberhard.Relation of putative thioester bond in C3 to activation of the alternative pathway and the binding of C3b to biological targets of complement.J Exp Med.1980.152.4.1102-14

Pepys, MB.Role of complement in induction of antibody production in vivo. Effect of cobra factor and other C3-reactive agents on thymus-dependent and thymus-independent antibody responses.J Exp Med.1974.140.1.126-45

Peterman, JH, BA Julian, KA Kirk and S Jackson.Selective elevation of monomeric IgA1 in IgA nephropathy patients with normal renal function.Am J Kidney Dis.1991.18.3.313-9

Petersen, SV, S Thiel and JC Jensenius.The mannan-binding lectin pathway of complement activation: biology and disease association.Mol Immunol. 2001. 38.2-3.133-49

Podack, ER, KT Preissner and HJ Muller-Eberhard.Inhibition of C9 polymerization within the SC5b-9 complex of complement by S-protein.Acta Pathol Microbiol Immunol Scand Suppl.1984.284.89-96

Presanis, JS, K Hajela, G Ambrus, P Gal and RB Sim.Differential substrate and inhibitor profiles for human MASP-1 and MASP-2.Mol Immunol. 2004. 40.13.921-9

Rebmann, V, I Doxiadis, BS Kubens and H Grosse-Wilde.Quantitation of the human component C4: definition of C4 Q0 alleles and C4A duplications.Vox Sang.1992.62.2.117-23

Roitt, IM and DK Male.2006.Immunology.7th.Mosby Elsevier,.Edinburgh.2006

Roos, A, LH Bouwman, DJ van Gijlswijk-Janssen, MC Faber-Krol, GL Stahl and MR Daha.Human IgA activates the complement system via the mannan-binding lectin pathway.J Immunol.2001.167.5.2861-8

Roos, A, MP Rastaldi, N Calvaresi, BD Oortwijn, N Schlagwein, DJ van Gijlswijk-Janssen, GL Stahl, M Matsushita, T Fujita, C van Kooten and MR Daha. Glomerular activation of the lectin pathway of complement in IgA nephropathy is associated with more severe renal disease.J Am Soc Nephrol.2006.17.6.1724-34

Roos, MH, E Mollenhauer, P Demant and C Rittner.A molecular basis for the two locus model of human complement component C4.Nature.1982.298.5877.854-6

Rossi, V, S Cseh, I Bally, NM Thielens, JC Jensenius and GJ Arlaud.Substrate specificities of recombinant mannan-binding lectin-associated serine proteases-1 and -2.J Biol Chem.2001.276.44.40880-7

Ruef, C, M Kashgarian and DL Coleman.Mesangial cell-matrix interactions. Effects on mesangial cell growth and cytokine secretion.Am J Pathol.1992.141.2.429-39

Rupert, KL, JM Moulds, Y Yang, FC Arnett, RW Warren, JD Reveille, BL Myones, CA Blanchong and CY Yu.The molecular basis of complete complement C4A and C4B deficiencies in a systemic lupus erythematosus patient with homozygous C4A and C4B mutant genes.J Immunol.2002.169.3.1570-8

Saevarsdottir, S, H Kristjansdottir, G Grondal, T Vikingsdottir, K Steinsson and H Valdimarsson.Mannan-binding lectin and complement C4A in Icelandic multicase families with systemic lupus erythematosus.Ann Rheum Dis. 2006. 65.11.1462-7

Saevarsdottir, S, K Steinsson, BR Ludviksson, G Grondal and H Valdimarsson. Mannan-binding lectin may facilitate the clearance of circulating immune complexes--implications from a study on C2-deficient individuals.Clin Exp Immunol.2007.148.2.248-53

Sanders, JT and RJ Wyatt.IgA nephropathy and Henoch-Schonlein purpura nephritis. Curr Opin Pediatr.2008.20.2.163-70

Sandin, C, S Linse, T Areschoug, JM Woof, J Reinholdt and G Lindahl.Isolation and detection of human IgA using a streptococcal IgA-binding peptide.J Immunol. 2002. 169.3.1357-64

Santos, IK, CH Costa, H Krieger, MF Feitosa, D Zurakowski, B Fardin, RB Gomes, DL Weiner, DA Harn, RA Ezekowitz and JE Epstein.Mannan-binding lectin enhances susceptibility to visceral leishmaniasis.Infect Immun.2001.69.8.5212-5

Saulsbury, FT.Alterations in the O-linked glycosylation of IgA1 in children with Henoch-Schonlein purpura.J Rheumatol.1997.24.11.2246-9

Schedl, A.Renal abnormalities and their developmental origin.Nat Rev Genet. 2007. 8.10.791-802

Schena, FP, L Gesualdo and V Montinaro.Immunopathological aspects of immunoglobulin A nephropathy and other mesangial proliferative glomerulo-nephritides. J Am Soc Nephrol.1992.2.10 Suppl.S167-72

Schena, PF, A Pastore, RA Sinico, V Montinaro and A Fornasieri.Polymeric IgA and IgA rheumatoid factor decrease the capacity of serum to solubilize circulating immune complexes in patients with primary IgA nephropathy.J Immunol. 1988.141.1.125-30

Schonermark, S, EW Rauterberg, ML Shin, S Loke, D Roelcke and GM Hansch.Homologous species restriction in lysis of human erythrocytes: a membrane-derived protein with C8-binding capacity functions as an inhibitor.Journal of Immunology.1986.136.5.1772-6

Scolari, F.Familial IgA nephropathy.J Nephrol.1999.12.4.213-9

Seyfarth, J, P Garred and HO Madsen.Extra-hepatic transcription of the human mannose-binding lectin gene (mbl2) and the MBL-associated serine protease 1-3 genes.Mol Immunol.2006.43.7.962-71

Sharon, N and H Lis.Lectins as cell recognition molecules.Science.1989.246.4927.227-34

Sinniah, R, AR Javier and G Ku.The pathology of mesangial IgA nephritis with clinical correlation.Histopathology.1981.5.5.469-90

Smith, AC, JF de Wolff, K Molyneux, J Feehally and J Barratt.O-glycosylation of serum IgD in IgA nephropathy.J Am Soc Nephrol.2006a.17.4.1192-9

Smith, AC and J Feehally.New insights into the pathogenesis of IgA nephropathy. Pathogenesis of IgA nephropathy.Springer Semin Immunopathol.2003.24.4.477-93

Smith, AC, K Molyneux, J Feehally and J Barratt.O-glycosylation of serum IgA1 antibodies against mucosal and systemic antigens in IgA nephropathy.J Am Soc Nephrol.2006b.17.12.3520-8

Sorensen, R, S Thiel and JC Jensenius.Mannan-binding-lectin-associated serine proteases, characteristics and disease associations.Springer Semin Immuno-pathol. 2005.27.3.299-319

Stefansson Thors, V, R Kolka, SL Sigurdardottir, VO Edvardsson, G Arason and A Haraldsson.Increased frequency of C4B*Q0 alleles in patients with Henoch-Schonlein purpura.Scand J Immunol.2005.61.3.274-8

Steinberger, P, A Szekeres, S Wille, J Stockl, N Selenko, E Prager, G Staffler, O Madic, H Stockinger and W Knapp.Identification of human CD93 as the phagocytic C1q receptor (C1qRp) by expression cloning.J Leukoc Biol.2002.71.1.133-40

Sternberg, SS.1992.Histology for pathologists.Raven Press.New York.1992

Stewart, M, JM Savage, B Bell and B McCord.Long term renal prognosis of Henoch-Schonlein purpura in an unselected childhood population.Eur J Pediatr. 1988. 147.2.113-5

Stockert, RJ.The asialoglycoprotein receptor: relationships between structure, function, and expression.Physiol Rev.1995.75.3.591-609

Sullivan, KE, C Wooten, D Goldman and M Petri.Mannose-binding protein genetic polymorphisms in black patients with systemic lupus erythematosus.Arthritis Rheum.1996.39.12.2046-51

Suzuki, H, R Fan, Z Zhang, R Brown, S Hall, BA Julian, WW Chatham, Y Suzuki, RJ Wyatt, Z Moldoveanu, JY Lee, J Robinson, M Tomana, Y Tomino, J Mestecky and J Novak.Aberrantly glycosylated IgA1 in IgA nephropathy patients is recognized by IgG antibodies with restricted heterogeneity.J Clin Invest.2009.119.6.1668-77

Suzuki, H, Z Moldoveanu, S Hall, R Brown, HL Vu, L Novak, BA Julian, M Tomana, RJ Wyatt, JC Edberg, GS Alarcon, RP Kimberly, Y Tomino, J Mestecky and J Novak.IgA1-secreting cell lines from patients with IgA nephropathy produce aberrantly glycosylated IgA1.J Clin Invest.2008.118.2.629-39

Teh, C, Y Le, SH Lee and J Lu.M-ficolin is expressed on monocytes and is a lectin binding to N-acetyl-D-glucosamine and mediates monocyte adhesion and phagocytosis of Escherichia coli.Immunology.2000.101.2.225-32

Terai, I, K Kobayashi, JP Vaerman and N Mafune.Degalactosylated and/or denatured IgA, but not native IgA in any form, bind to mannose-binding lectin.J Immunol. 2006.177.3.1737-45

Tesch, GH, HY Lan, RC Atkins and DJ Nikolic-Paterson.Role of interleukin-1 in mesangial cell proliferation and matrix deposition in experimental mesangio-proliferative nephritis.Am J Pathol.1997.151.1.141-50

Thiel, S.Complement activating soluble pattern recognition molecules with collagen-like regions, mannan-binding lectin, ficolins and associated proteins.Mol Immunol.2007.44.16.3875-88

Tolkoff-Rubin, NE, AB Cosimi, T Fuller, RH Rublin and RB Colvin.IGA nephropathy in HLA-identical siblings.Transplantation.1978.26.6.430-3

Tomana, M, K Matousovic, BA Julian, J Radl, K Konecny and J Mestecky.Galactose-deficient IgA1 in sera of IgA nephropathy patients is present in complexes with IgG.Kidney Int.1997.52.2.509-16

Tomana, M, J Novak, BA Julian, K Matousovic, K Konecny and J Mestecky. Circulating immune complexes in IgA nephropathy consist of IgA1 with galactose-deficient hinge region and antiglycan antibodies.J Clin Invest. 1999. 104.1.73-81

Torres, VE and PC Harris.Mechanisms of Disease: autosomal dominant and recessive polycystic kidney diseases.Nat Clin Pract Nephrol.2006.2.1.40-55; quiz 55

Toyabe, S, W Harada and M Uchiyama.Oligoclonally expanding gammadelta T lymphocytes induce IgA switching in IgA nephropathy.Clin Exp Immunol. 2001.124.1.110-7

Truedsson, L, Z Awdeh, EJ Yunis, S Mrose, B Moore and CA Alper.Quantitative variation of C4 variant proteins associated with many MHC haplotypes. Immunogenetics.1989.30.6.414-21

Tsujimura, M, T Miyazaki, E Kojima, Y Sagara, H Shiraki, K Okochi and Y Maeda.Serum concentration of Hakata antigen, a member of the ficolins, is

linked with inhibition of Aerococcus viridans growth.Clin Chim Acta. 2002. 325.1-2.139-46

Turner, MW.The role of mannose-binding lectin in health and disease.Mol Immunol. 2003.40.7.423-9

Valdimarsson, H, T Vikingsdottir, P Bang, S Saevarsdottir, JE Gudjonsson, O Oskarsson, M Christiansen, L Blou, I Laursen and C Koch.Human plasma-derived mannose-binding lectin: a phase I safety and pharmacokinetic study.Scand J Immunol.2004.59.1.97-102

van der Boog, PJ, G van Zandbergen, JW de Fijter, N Klar-Mohamad, A van Seggelen, P Brandtzaeg, MR Daha and C van Kooten.Fc alpha RI/CD89 circulates in human serum covalently linked to IgA in a polymeric state.J Immunol. 2002.168.3.1252-8

van Es, LA, AW van den Wall Bake, RM Valentijn and MR Daha.Composition of IgA-containing circulating immune complexes in IgA nephropathy.Am J Kidney Dis.1988.12.5.397-401

van Kooyk, Y, A Engering, AN Lekkerkerker, IS Ludwig and TB Geijtenbeek. Pathogens use carbohydrates to escape immunity induced by dendritic cells.Curr Opin Immunol.2004.16.4.488-93

Walport, MJ.Complement. First of two parts.N Engl J Med.2001.344.14.1058-66

Weiss, V, C Fauser and J Engel.Functional model of subcomponent C1 of human complement.J Mol Biol.1986.189.3.573-81

Welch, TR, LS Beischel and EM Choi.Molecular genetics of C4B deficiency in IgA nephropathy.Hum Immunol.1989.26.4.353-63

Welch, TR, A Berry and LS Beischel.C4 isotype deficiency in IgA nephropathy.Pediatr Nephrol.1987.1.2.136-9

Yan, Y, LX Xu, JJ Zhang, Y Zhang and MH Zhao.Self-aggregated deglycosylated IgA1 with or without IgG were associated with the development of IgA nephropathy. Clin Exp Immunol.2006.144.1.17-24

Yang, YH, YH Chuang, LC Wang, HY Huang, ME Gershwin and BL Chiang.The immunobiology of Henoch-Schonlein purpura.Autoimmun Rev.2008.7.3.179-84

Yano, N, M Endoh, Y Nomoto, H Sakai, K Fadden and A Rifai.Phenotypic characterization of cytokine expression in patients with IgA nephropathy.J Clin Immunol.1997.17.5.396-403

Yasuda, Y, A Horie, H Odani, H Iwase and Y Hiki.Application of mass spectrometry to IgA nephropathy: structural and biological analyses of underglycosylated IgA1 molecules.Contrib Nephrol.2004.141.170-88

Yoo, EM and SL Morrison.IgA: an immune glycoprotein.Clin Immunol.2005.116.1.3-10

Zarate, YA and RJ Hopkin.Fabry's disease.Lancet.2008.372.9647.1427-35