IgA Nephropathy (Berger’s Disease)

Mechanisms of Disease

Jean Berger
circa 1968

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IgA Nephropathy
The most common primary glomerulonephritis in the world

IgA1 (with C3, IgG, or IgM)
Mesangial Immunodeposits

Expansion of Extracellular Matrix
Proliferation of Mesangial Cells

Immunofluorescence
Periodic acid-Schiff stain
IgA Nephropathy
Electron Microscopy

Mesangial Deposits
IgA Nephropathy
Cause is Extra-Renal
Kidney is under attack from systemic process

I. Kidney donor, Subclinical IgAN
   Non-IgAN recipients
   Clearance of IgA, within weeks

II. Kidney donor, Healthy
    IgAN recipients
    IgAN recurrence ~50%, at 2 years
IgA Nephropathy
Possible Factors Initiating Disease

Genetic factors
Aberrancy of structure of IgA1 molecules
  Glycosylation aberrancy -> aggregation, CIC formation,
Increased levels of IgA and IgA-containing complexes
  Overproduction or defective clearance
Endogenous or exogenous antigens
  CIC formation, IgA renal deposition
Immunological defects
  (allergy, complement, coagulation, ...)

Modified from Julian BA, et al., Adv Nephrol 1999
IgA Nephropathy

The most common primary glomerulonephritis in the world

Prevalence: 25-50 / 100,000

Percentage of native-kidney biopsies showing IgA nephropathy
Familial IgA Nephropathy
Loci Linked with Familial IgAN

Chr 6q22-23 (*IgAN1*)
  Some Kentucky (USA) and Italian families

Chr 4q26-31
  Some Italian families

Chr 7q12-22
  Some Italian families

Chr 2q36
  Canadian Caucasian family

Other loci (not defined)
  Lebanese family
IgA Nephropathy
Genetic Influence(s) in the Clinical Expression

The mode of presentation and clinical course of patients with familial IgAN do not differ from that of patients with the sporadic forms.

IgA nephropathy is a phenotype that likely results from interactions of multiple susceptibility and progression genes and environmental influences.

Nonetheless, despite the lack of success in characterizing the IgAN gene(s), the familial aggregation of IgA nephropathy (10%-15% cases) suggests that some disease mechanisms are genetically determined.
Human IgA1
Structure and glycosylation

O-linked glycans

N-linked glycans

Hinge Regions of Human IgA1 and IgA2

IgA1

CH1α1  225  228  230  232  236  CH2α1
Pro-Val-Pro-Ser-Thr-Pro-Pro-Thr-Pro-Ser-Thr-Pro-Pro-Thr-Pro-Ser-Pro-Ser-Cys-
(CHO)  CHO  CHO  CHO  (CHO)

IgA2m(1)

CH1α2  CH2α2
Pro-Val-Pro-Pro-Pro-Pro-Pro-Pro-Pro-Pro-Pro-
Human IgA1
Structure and Glycosylation

Molecular forms:
Monomer
Dimer (J chain)
Higher polymer (J chain)
Secretory IgA (J chain + SC)
IgA1 Hinge-region O-glycans
Possible Structures

Most common forms
normal serum IgA1

Greater frequency
IgAN serum IgA1

SA = sialic acid

Gal
\\beta^{1,3}
GalNAc
Ser/Thr

Gal
\\beta^{1,3}
GalNAc
Ser/Thr

α^{2,3}

SA

α^{2,6}

Gal

GalNAc

GalNAc
Ser/Thr

GalNAc
Ser/Thr

-Ser/Thr-
IgA1 Hinge-region O-glycans
Possible Structures & Lectin Binding

Most common forms
normal serum IgA1

Greater frequency
IgAN serum IgA1

Lectin
PNA-reactive

Gal
β1,3
GalNAc
Ser/Thr

Lectin
HAA-reactive

GalNAc
GalNAc
Ser/Thr

Lectin
gacalin-reactive

β1,3
Gal
Ser/Thr

Lectin
SNA-reactive

α2,3
GalNAc
Ser/Thr

SA

SA

SA

SA = sialic acid
IgA Nephropathy

Serum Levels of Galactose-deficient IgA
Caucasian Adult Patients with IgAN and Healthy Controls

Levels in 74% of patients >90th percentile of healthy controls

Serum galactose-deficient IgA is within circulating immune complexes bound to IgG.


IgA Nephropathy
Familial Disease and Increased Serum Galactose-deficient IgA1 Levels

IgA1-secreting Cell Lines from IgA Nephropathy Patients

Secrete Greater Amounts of Galactose-deficient IgA1

Measured as ratio of HAA lectin-reactive IgA1 (GalNAc-specific) to total IgA1,
Expressed relative to standard Gal-deficient IgA1 myeloma protein

Serum Levels of Gal-deficient IgA1
Correlation with HAA Reactivity of IgA1 Secreted by Corresponding Cell Lines

Supernatant HAA-IgA1 (%)
Serum HAA-IgA1 (%)

$P < 0.001$
$R^2 = 0.883$

Suzuki H et al., J Clin Invest 2008
IgA1 Hinge-region O-glycans
Enzymes Facilitate their Construction

- Ser/Thr- GalNAc- transferase2
- Ser/Thr- - Ser/Thr- 
  β1,3 galactosyltransferase
  GalNAc
  Cosmc
  β1,3 galactosyltransferase
  Ser/Thr
  sialyltransferases
  β1,3
  GalNAc
  α2,3
  Gal
  α2,6
  SA
  SA
  GalNAc
  Ser/Thr
  α2,6 sialyltransferase
  GalNAc
  Ser/Thr
  α2,3
  Gal
Transcription of Specific Glycosyltransferases

IgA1-producing Cell Lines

11 IgAN Patients and 11 Healthy Controls

Relative mRNA expression

GalNAcT2 C1GalT1 Cosmc ST6GalNAcII

ST6GalNAcI not expressed

J chain and IgA alpha chain did not differ

- IgAN patients
- Healthy controls

Complex Changes in Biosynthetic Pathways of IgA1 O-glycans

Patients with IgA Nephropathy

\[
\begin{align*}
\text{Gal} & \quad \text{GalNAc} \\
\downarrow 1,3 & \quad \uparrow 1,3 \\
\text{Ser/Thr} & \quad \text{Ser/Thr} \\
\text{GalNAc} & \quad \text{GalNAc} \\
\text{Gal} & \quad \text{GalNAc} \\
\downarrow 1,3 & \quad \uparrow 1,3 \\
\text{Ser/Thr} & \quad \text{Ser/Thr} \\
\text{GalNAc} & \quad \text{GalNAc} \\
\text{Gal} & \quad \text{GalNAc} \\
\downarrow 1,3 & \quad \uparrow 1,3 \\
\text{Ser/Thr} & \quad \text{Ser/Thr} \\
\text{GalNAc} & \quad \text{GalNAc} \\
\text{Gal} & \quad \text{GalNAc} \\
\downarrow 1,3 & \quad \uparrow 1,3 \\
\text{Ser/Thr} & \quad \text{Ser/Thr} \\
\text{GalNAc} & \quad \text{GalNAc} \\
\text{Gal} & \quad \text{GalNAc} \\
\text{ST6GALNAC2} & \quad \text{2,6-GalNAc-sialyltransferase II} \\
\end{align*}
\]

SA = sialic acid
Model for Pathogenesis of IgA Nephropathy

- Defect in IgA1-producing cells
- Increased amount of Gal-deficient IgA1 (Ag)
- Production of anti-glycan IgG/IgA1 (Ab)
- Formation of circulating immune complexes
- Mesangial deposition
- Glomerular injury
IgA Nephropathy

Levels of Antigen-specific IgG in Sera
Against Aberrantly Glycosylated IgA1 are Elevated

Serum antigen-specific IgG

IgAN (n = 16) Control (n = 16)

IgG concentration of each sample was normalized

Suzuki H. J Clin Invest in press
Serum IgG from IgA Nephropathy Patients
Binds More Galactose-deficient IgA1 than does Serum IgG from Controls

1. Degalactosylated and desialylated IgA1 (dd-IgA1)
2. Degalactosylated IgA1
3. Enzymatically galactosylated dd-IgA1
4. Enzymatically sialylated dd-IgA1

Suzuki H. J Clin Invest in press
IgA Nephropathy
Levels of Antigen-specific IgG Secreted by IgG-producing Cells Against Aberrantly Glycosylated IgA1 are Elevated

Serum antigen-specific IgG

Antigen-specific IgG secreted by cells

IgG concentration of each sample was normalized

Suzuki H. *J Clin Invest* in press
Binding of Cell-line IgG to Aberrantly Glycosylated IgA1 Inhibited by GalNAc-specific Lectin (HAA)

Densitometric analysis

Gal-deficient IgA1 (Ag) + HAA lectin + IgG (Ab) + IgG from IgAN + Anti-IgG + Anti-IgA

HAA-lectin GalNAc Gal

Suzuki H. J Clin Invest in press
Analysis of Monoclonal IgG by Single-cell PCR

Recombinant IgG (rIgG) cloned from an IgAN patient binds to Galactose-deficient IgA1

Gal-deficient IgA1 (Ag) + rIgG (Ab) + IgAN ↓ IgAN controls ↓

Anti-IgG

IgA heavy chain

Dot-blot analysis

Patient #

1123 1023 3081 1139 3061 2047

IgAN Controls

OD (490 nm)

0.0 1.0 2.0 3.0 4.0

rIgG against Fab-IgA1

Suzuki H. J Clin Invest in press
Analysis of Monoclonal IgG by Single-cell PCR

How to Explain Patient #3081

Notable Change from Alanine to Serine in CDR3 Region

Heavy Chain of IgA1-Glycan-specific IgG

Cells from IgAN patients
1023 &SXXXXXXXXXXXXXXXXXXXXX
1123 &SXXXXXXXXXXXXXXXXXXXXX
1139 &SXXXXXXXXXXXXXXXXXXXXXXXXX
2047 &SXXXXXXXXXXXXXXXXXX
3061 &SXXXXXXXXXXXXX
3081 &AXXXXXXXXXXXXXXXXXX

Cells from healthy controls
3066 &AXXXXXXX
3070 &ASXXXXXXXXXXXXXXXXXX
3064 &AXXXXXXXXXXXXXXXXX
8043 &AXXXXXXXXXX
9017 &AXXXXXXXXXXXXXXXXXXX
9035 &AXXXXXXXXXXXXXXXXXX

Suzuki H. J Clin Invest in press
Mutagenesis for Amino Acid Substitution
A to S Substitution: Major Role in Binding of IgG to Galactose-deficient IgA1

Cells from IgAN patient
---
Mutation: A

Cells from healthy control
---
Mutation: S

Suzuki H. J Clin Invest in press
Novel Assay for Serum IgA1-Glycan-specific IgG Antibody

Dot-blot analysis

IgG concentration of each sample was normalized

Specificity: 88%
Sensitivity: 95%

Area under the curve: 0.9644

Suzuki H. J Clin Invest in press
Why do Humans have Circulating IgG Antibodies Specific for Galactose-Deficient IgA1 Glycans?

Antibodies synthesized in response to microorganisms that express GaINAc epitopes on surface structures

- **Viruses**
  - Respiratory syncitial virus
  - Epstein-Barr virus
  - Herpes viruses

- **Bacteria**
  - Streptococcus

This exposure to galactose-deficient IgA1 may further boost anti-GaINAc antibodies in patients with IgA nephropathy
Galactose-deficient IgA1
Role in the Mechanism of Disease in IgA Nephropathy

- Serum levels of galactose-deficient IgA1 elevated. This aberrant IgA1 is predominantly in immune complexes.
- Galactose-deficient IgA1 likely originates from an imbalance of the activities of the key enzymes in the synthesis of O-linked glycans in IgA1-secreting cells.
- Galactose-deficient IgA1 in immune complexes is bound to anti-glycan IgG. This binding IgG often has a unique amino-acid sequence in the antigen-binding portion of its heavy chain.
Proposal for Pathogenesis of IgA Nephropathy

IgA1-secreting Cells

Healthy Individual

IgAN Patient

plgA1

Space of Disse

ASGP-R

Hepatocytes

Immune complex

IgG or IgA1

Glomerulus

KIDNEY

Mesangium

Val - Pro - Ser - Thr - Pro -
GalNAc

Fab

IgG or IgA1

KIDNEY

500 -1000 Å

Mesangium
Is there evidence for role of galactose-deficient IgA1 to cause IgA nephropathy?
Circulating Immune Complexes with Galactose-deficient IgA1 Activate Proliferation of Human Mesangial Cells in Culture

Size-exclusion chromatography
Stimulation of Proliferation of Mesangial Cells
Circulating Immune Complexes Obtained during Acute and Convalescent Phases

![Graph showing relative proliferation against fraction number.](image)
Mesangial IgA1 in IgA nephropathy exhibits aberrant O-glycosylation: Observations in three patients.

Background. In IgA nephropathy (IgAN), circulating IgA1 molecules display an abnormal pattern of O-glycosylation. This is usually extended with galactose to form the disaccharide Galβ1,3GalNAc, which may in turn be further extended with one or two sialic acid units [4]. Thus, each O-glycan branch could be as long as 4 to 6 saccharide units. Each IgA1 monomer has...
IgA1 Myeloma Protein: HSP Nephritis
Galactose-deficient O-linked Glycans in the Hinge Region


IgA myeloma presenting as Henoch-Schönlein purpura with nephritis.

Zickerman AM, Allen AC, Talwar V, Olczak SA, Brownlee A, Holland M, Furness PN, Brunskill NJ, Feehally J.

Departments of Nephrology and Pathology, Leicester General Hospital, United Kingdom.

IgA nephropathy (IgAN) and Henoch-Schönlein purpura (HSP) are both characterized by IgA-mediated tissue injury, including mesangial proliferative glomerulonephritis. Abnormalities of IgA1 glycosylation are described in IgA nephropathy and HSP nephritis. IgA-antineutrophil cytoplasmic antibodies (ANCA) have been inconsistently described in the serum of patients with HSP. In IgA myeloma, the paraprotein-mediated renal lesion is typically cast nephropathy; IgAN or HSP have only rarely been reported in myeloma even when an IgA paraprotein is circulating in large concentrations. We report the case of a 50-year-old man with IgA myeloma who presented with HSP including nephritis and rapidly progressive renal failure. His IgA1 had altered O-glycosylation in the pattern seen in IgAN and also contained an IgA-ANCA. This case adds further weight to the evidence that IgA1 O-glycosylation abnormalities predispose to mesangial IgA deposition and also that IgA-ANCA may have a pathogenic role in the development of HSP.

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Serum Levels of IgA1-Glycan-specific IgG
Correlate with Urinary Parameters
Contemporaneous Sampling

Proteinuria

Signal intensity / rIgG (%) vs. UP/Cr ratio (g/g)
- P < 0.0001
- R² = 0.743

Urinary Excretion
IgA-IgG Immune Complexes

Signal intensity / rIgG (%) vs. UlgA-IgG IC/Cr ratio
- P = 0.0082
- R² = 0.485

Suzuki H. J Clin Invest in press
IgA1-Associated Glomerulonephritis
Mechanisms of Renal Injury

Podocyte
Capillary lumen
Bowman's space
Glomerulus
Nephron
Proximal tubules and capillaries

IgA1 immune complex
Mesangial cell

Mesangial cell proliferation
Mesangial cell output
Angiotensin II
Complement components
ECM components
Cytokines
PDGF
IL-6, IL-8
MCP-1
TNFα
TGFβ
MIF

Gluertubulotubular crosstalk
Glomerulotubular crosstalk

PDGF
MIF
MCP-1
IL-8

Podocyte injury
Glomerular scarring
Mesangioproliferative glomerulonephritis
Loss of podocytes

Damaged Nephron
Interstitial fibrosis
Interstitial inflammation
Epithelial-mesenchymal transformation

Urinary protein
Cytokines

nucleus
Substantial progress has been made in understanding genetics and pathology.

IgA nephropathy can be labeled an autoimmune disease, with galactose-deficient IgA1 as the antigen and IgG as the antibody. Still, information about specific molecular mechanisms remains incomplete.

Current “hot topics” for research in the pathogenesis and clinical expression of disease:
- Specific site of IgA1 glycosylation aberrancy
- Function of IgAN-linked genetic loci
- Binding of IgA1-immune complexes to mesangial cells
- Why some transplant patients never develop recurrent disease
- Urinary / serum biomarkers for diagnosis or monitoring disease
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