Immunology of Lupus Nephritis
Recognition of Targets
Natallia Maroz, MD
Goal:
Create a concept of the immunopathogenesis in LN

Objectives:
• Why do we need to know it?

• What are the key levels in immunological process of LN.
  Defects in:
  Apoptosis
  Complement system
  Antigen recognition and presentation
  T-cells stimulation
  B-cell stimulation
  Antibodies production
  Effect of immune complex deposition in the kidney

• Targets of novel therapies
Lupus Nephritis is a major course of morbidity and mortality in patients with SLE

**Statistics**

- SLE - 3-6 cases per 100,000 person-years. Prevalence 40-200 per 100,000 (250,000 in USA)
- Lupus Nephritis – 60% after 5 yrs of f/u
- Progression to ESRD from proliferative LN – 10-30%
- Reoccurrence of LN in patients post Kidney Transplant: Reported incidence 2-9% over the period 1-16 years
“The clinical course of the disease was different 15 years ago, when Keith (1940) wrote that "renal insufficiency does not play an important role in causing death". At that time patients usually died of a "lupus crisis" or as a result of concurrent infection. “

This communication outlines some observations on 34 patients studied intensively during the past 18 months. 61 biopsies, 3 autopsies
### Table

**DIAGNOSTIC CRITERIA USED IN THE SELECTION OF PATIENTS ILL WITH SYSTEMIC LUPUS ERYTHEMATOSUS**

<table>
<thead>
<tr>
<th>Clinical</th>
<th>Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Arthralgia and arthritis</td>
<td>1. Skin or renal biopsy compatible with L.E.</td>
</tr>
<tr>
<td>2. Fever</td>
<td>2. Hargraves’s cells</td>
</tr>
<tr>
<td>3. Serositis</td>
<td>3. Leucopenia, anaemia, or thrombocytopenia</td>
</tr>
<tr>
<td>4. Dermatological lesions (face, hair, nails, body surface, mucous membranes)</td>
<td>4. Urinary abnormalities</td>
</tr>
<tr>
<td>5. Raynaud’s phenomenon</td>
<td>5. Raised erythrocyte sedimentation rate</td>
</tr>
<tr>
<td>6. Sensitivity to sunlight</td>
<td>6. Positive thymol turbidity test; other tests of liver function normal</td>
</tr>
<tr>
<td>7. Splenomegaly and/or hepatomegaly</td>
<td>7. Increased serum globulin levels</td>
</tr>
<tr>
<td>8. Remissions and exacerbations</td>
<td>8. Positive serological tests for syphilis</td>
</tr>
<tr>
<td></td>
<td>9. Positive Coombs’ test</td>
</tr>
</tbody>
</table>
The Classification of Glomerulonephritis in Systemic Lupus Erythematosus Revisited

JAN J. WEENING,* VIVETTE D. D’AGATI,† MELVIN M. SCHWARTZ,‡ SURYA V. SESHAN,§ CHARLES E. ALPERS, GERALD B. APPEL,¶ JAMES E. BALOW,# JAN A. BRUIJN,** TERENCE COOK,†† FRANCO FERRARIO,‡‡ AGNES B. FOGO,§§ ELLEN M. GINZLER, LEE HEBERT,¶¶ GARY HILL,### PRUE HILL,****
J. CHARLES JENNETTE,††† NORELLA C. KONG,‡‡‡ PHILIPPE LESAVRE,§§§ MICHAEL LOCKSHIN,§ LAI-MENG LOOI, HIROFUMI MAKINO,¶¶¶ LUIZ A. MOURA,### and MICHIO NAGATA***** ON BEHALF OF THE INTERNATIONAL SOCIETY OF NEPHROLOGY and RENAL PATHOLOGY SOCIETY WORKING GROUP ON THE CLASSIFICATION OF LUPUS NEPHRITIS

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Class I     Minimal mesangial lupus nephritis
Class II    Mesangial proliferative lupus nephritis
Class III   Focal lupus nephritis
Class IV    Diffuse segmental (IV-S) or global (IV-G) lupus nephritis
Class V     Membranous lupus nephritis
Class VI    Advanced sclerosing lupus nephritis
Immunology of Lupus Nephritis
Loss of Self-Tolerance

Innate Immunity

Adaptive immunity

Environmental And epigenetic factors

Hormones, Sex hormones

Not Monogenetic Disease

Crispin et al. Pathogenesis of human systemic lupus erythematosus: recent advances
Figure 1. SLE-associated loci and genes
The approximate position of SLE-associated loci (red squares) and genes (arrows) in the human genome is shown. Additional studies will identify the risk alleles responsible for these associations. This will allow a more comprehensive understanding of disease pathogenesis and the selection of better biomarkers and therapeutic targets. *FCGR stands for FCGR2A, FCGR3B, and FCGR3A; COMP stands for C2, and C4A, and C4B.
### Murine models for SLE

<table>
<thead>
<tr>
<th>Model</th>
<th>Defect</th>
</tr>
</thead>
<tbody>
<tr>
<td>NZB/WF1</td>
<td>Not recognized</td>
</tr>
<tr>
<td>MRL/lpr</td>
<td>Fas receptor</td>
</tr>
<tr>
<td>BXSB/Yaa</td>
<td>TLR-7</td>
</tr>
<tr>
<td>NZM2410, 2328</td>
<td>Sle1-3, (MHC) class II locus H-2</td>
</tr>
<tr>
<td>Pristane-induced model</td>
<td>Induced model of environmental factor</td>
</tr>
<tr>
<td>cGVHD</td>
<td>Induced model</td>
</tr>
</tbody>
</table>

Daniel Perry, Allison Sang, Yiming Yin, Ying-Yi Zheng, Laurence Morel. *Murine Models of Systemic Lupus Erythematosus* *Journal of Biomedicine and Biotechnology Volume 2011*
Why do We need to know all of it?

Why nephrologists are not comfortable to treat lupus nephritis?
The Classification of Glomerulonephritis in Systemic Lupus Erythematosus Revisited

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The American College of Rheumatology Response Criteria for Proliferative and Membranous Renal Disease in Systemic Lupus Erythematosus Clinical Trials (analysis of 21 clinical trials)

### Table 2. Response criteria for renal function based on the estimated GFR

<table>
<thead>
<tr>
<th>Renal function</th>
<th>Estimated GFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improved†‡</td>
<td>25% increase if baseline estimated GFR is abnormal</td>
</tr>
<tr>
<td>No change</td>
<td>Stable values for the estimated GFR</td>
</tr>
<tr>
<td>Worsened†§¶</td>
<td>25% decline in estimated GFR or ESRD</td>
</tr>
</tbody>
</table>

### Table 3. Response criteria for urinary protein values

<table>
<thead>
<tr>
<th>Urinary protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improved</td>
</tr>
<tr>
<td>Partial response</td>
</tr>
<tr>
<td>Complete response</td>
</tr>
<tr>
<td>No change</td>
</tr>
<tr>
<td>Worsened</td>
</tr>
</tbody>
</table>

### Table 4. Response criteria for urinary sediment

<table>
<thead>
<tr>
<th>Urinary sediment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improved</td>
</tr>
<tr>
<td>Worsened</td>
</tr>
</tbody>
</table>
“Why are Rheumatologists Treating Lupus Nephritis?”

• Well chosen response criteria by ACR?

“ What should be done, then, to improve the management of lupus nephritis?”

“Program directors must ensure that nephrology trainees receive core training in the diagnosis and treatment of this disease and experience in the management of acute and chronic manifestations of lupus nephritis.”

“It would be preferable for nephrologists to work together with other specialists, especially rheumatologists, as this integrative approach may help us to learn from each other, and, most importantly, improve patient care.”
Consultations

- Nephrologist (for renal biopsy or, if desired, for help in management of renal disease)
- Pathologist (for renal biopsy): The experience of pathologists in reading lupus nephritis biopsy specimens varies considerably. The most consistent readers are usually found in larger academic centers with substantial populations of patients with SLE.
Immunology of Lupus Nephritis

- Apoptosis
- Complement
- APS
- T cells
- B cells
- Antibodies
- Renal effect
Apoptosis
“Programmed cell death in multicellular organisms”

Mechanisms:
• Extracellular signals: toxins, cytokine, NO, calcium, hormones..
• Mitochondrial regulation
• Direct signal transduction:
  TNF induced path
  Fas-Fas ligand mediated path
  Apoptosis-inducing factor (caspase independent)

Defective apoptosis:
• Dysregulation of p53/interferon overproduction
• Binding of apoptosis inhibitors (lung cancer)
• Viruses encodes defective pathways (EBV, HIV, Adenovirus)
Chromatin degradation in Apoptosis

Nucleosome, a characteristic DNA fragmentation pattern generated during physiologic cellular apoptosis was discovered in 1973.
Figure 1. Distinct balance of apoptotic cell clearance. Normal clearance of apoptotic cells (left side, blue) involves sequential signals and plays an important role in tolerance induction and maintenance. Inflammatory clearance of apoptotic cells (right side, red) involves multi-inflammatory stimuli, breaks down tolerance, and drives autoimmunity including systemic lupus erythematosus. Blue blebs: early apoptotic cells modify surface markers and release signals to regulate chemotaxis and phagocytosis. Red blebs: later apoptotic cells and necrotic cells lose the cell membrane integrity, leading to the release of danger signals and modified autoantigens. DRP S19, dimer of ribosomal protein S19; HMGB1, high mobility group box 1; IL, interleukin; LTF, lactoferrin; MP, microparticle; NET, neutrophil extracellular trap; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylycerine; TGF-β, transforming growth factor beta.
Targets of Therapy:

- IL-6r MAB Tocilizumab, open-label phase I competed

- IFN-α kinoid vaccination (Sifalimumab, Rontalizumab)
  Phase I trial showed positive result

- TNF-α Etanercept Phase II trial, Infliximab showed both +/-

Immune Tolerance and Apoptosis

Central immune tolerance

- Elimination of T-lymphocytes with receptors to self antigen

Peripheral immune tolerance

- Elimination of T-lymphocytes with de-novo receptors to self antigen
- Elimination of hypermutated B cells recognizing self antigen
Role of Complement in LN

- Clearance of apoptotic particles
- Activation of complement by Ab
- Formation of immune complexes
- Stimulation of lymphocytes
- Chemoattraction of inflammatory mediators

- Targets for the therapy

Bridges innate and adaptive immune responses
Complement regulators and inhibitory proteins

Nature Reviews volume 9 | october 2009

Apoptosis

Complement

APS

T cells

B cells

Antibodies

Renal effect

*‡ and Christine Skerka

Complement regulators and inhibitory proteins

Nature Reviews volume 9 | october 2009
Complement Impairment Leading to Autoimmunity

Deficiencies (innate acquired) → Antibodies formation → Consumption

- Individual components C1Q, C2, C3, C4
- Regulatory proteins C1r, C1s
- Complement receptors eg. C1Qr
- Convertase complexes
Immunofluorescence
IgG, IgA, IgM, C1q, C4, C3, C5b-9 (“full house” pattern)

Complement as target of therapy:
Eculizumab MAB to C5b-C9 (membrane attack complex)
Pattern Recognition Receptors in Apoptosis

Phagocytes (macrophages and DC) express pattern recognition receptors:

- Scavenger receptors (SRs)
- Toll-like receptors (TLRs)
- Nod-like receptors (NLRs)

They recognize and mediate clearance of pathogens along with directing the immune system to a proper response.

These receptors also recognize host-derived ligands, such as apoptotic cells and modified low density lipoprotein.

They can also directly affect B-cells activation.

Emilie DomangeJordö *, Fredrik Wermeling, Yunying Chen, Mikael C. I. Karlsson ** Scavenger receptors as regulators of natural antibody responses and B cell activation in autoimmunity Molecular Immunology (2011)
Robson M. Toll-Like Receptors and Renal Disease
Nephron Exp Nephrol 2009;113:e1–e7

TLR

Apoptosis  Complement  APS  T cells  B cells  Antibodies  Renal effect

Bacterial Cell Wall Components
Flagellin  LPS

Mitochondria
DNA
dT
dsDNA

Endoplasmic Reticulum

Cytoplasm

Golgi

EROSOME

dRNA

mRNA

dtRNA

dsDNA

MYD88
Renal disease could be influenced by stimulation of TLRs on leucocytes or on renal cells.

TLR 7 and TLR 9 were found of particular interest for LN.

Target of therapy: Dual TLR7/9 oligoclonal antibodies was protective in murine models and ameliorated both GN and Ab.
• Fc gammaRII and III polymorphism

Falk Nimmerjahn & Jeffrey V. Ravetch
Fcγ receptors as regulators of immune responses
Nature Reviews Immunology 8, 34-47 (January 2008)
T-cells

- Helper CD4 (Th1, Th2, Th17)
- Cytoxic CD8
- Memory CD4/8
- Regulatory FOXP3
- NKT CD1d
- γδ T cells

**Communication with APC**
**Cell signaling mechanisms**
**Targets of Therapy**
B-cells role:

- APC (recognize free (soluble) antigen in the blood or lymph)
- Plasmacyte
- Memory cells

<table>
<thead>
<tr>
<th>B cell Stages</th>
<th>CD 20 expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progenitor (or pre-pro) B cells</td>
<td>-</td>
</tr>
<tr>
<td>Early Pro (or pre-pre)-B cells</td>
<td>+</td>
</tr>
<tr>
<td>Late Pro (or pre-pre)-B cells</td>
<td>+</td>
</tr>
<tr>
<td>Large Pre-B cells</td>
<td>+</td>
</tr>
<tr>
<td>Small Pre-B cells</td>
<td>+</td>
</tr>
<tr>
<td>Immature B cells</td>
<td>+</td>
</tr>
<tr>
<td>Mature B cells</td>
<td>-</td>
</tr>
</tbody>
</table>
Blys=BAFF

- Expressed on the surface membrane of myeloid cells, dendritic cells, monocytes and macrophages and released as a soluble protein.
- Its expression is regulated by INF-alfa, INF-gamma, IL-10, CD40L.
- Its interaction with a receptor on B-cell promote maturation, differentiation and survival of B-cells.
- Belimumab (Benlysta) monoclonal antibody against BAFF/Blys.

* It is not known if safe and effective for severe active LN.
Basophils and Nephritis in Lupus

Lyn deficient mice
30% pts with LN have high IgE

Activiation of circulating basophils by IgE immune complex
Up-regulated CD62L and MHC class II on basophils and homing to secondary lymphoid organs

Srini V. Kaveri et al. Basophils and Nephritis in Lupus
Mr. X., Case Conference 2 weeks ago,
46 yo AAM who underwent DDKT for LN 2001.
10 years after he had flair of SLE/LN.
Hemolysis. Rise in ds DNA, ANA. Low C3,C4
Active sediment in the UA. Severe TMA on biopsy.
Nephrectomy?

Why the Kidney?
ANTIBODIES in LN

- Anti-dsDNA antibodies were discovered in 1957 and initially thought to be inciting antigen
- Lupus patients produce 2000 different antibodies.
- Not all of them nephritogenic.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANA</td>
<td>&gt;90%</td>
<td>80</td>
</tr>
<tr>
<td>dsDNA</td>
<td>40-60%</td>
<td>74</td>
</tr>
<tr>
<td>Antinucleosome AB</td>
<td>58 - 99%</td>
<td>low</td>
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<td>...</td>
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</table>

DNA antibodies to anti-ds-DNA

Many researchers found:

1) Correlation of antibodies level with nephritis
2) Rising titers correlate with disease activity
3) Concentration in glomerluli higher than in serum
4) Nephritis can be induced by administration of Ab to normal mice

But, above was not always replicated in other studies

1) Ig M and IgG (1 and 3 subtype) have an ability to fix complement. Unique properties of antigen binding region
2) Cationic in charge (high affinity to the anionic antigen)
3) Highly cross-reactive (bind to extracellular antigens) as laminin, heparan sulfate, and α-actinin
Local Renal Antibody production in Lupus Nephritis

- Kidney is a major source of Ab producing PC in animal models with overt LN
- Likely is amplifying mechanism of clinically apparent disease
- Could be due to local changes in the expression of chemotactic molecules (as it is parallel to DC and macrophages)
- PC are fully differentiated long-lived type, not in cell cycle
- PC express prosurvival cytokine BAFF (as CD4 and CD8 T cells)
- Long-lived PC are resistant to immunosuppressive drugs, which target dividing cells. They do not express CD 20 (rituximab target).
- But they express CD 22 – target therapy Epratuzumab (IIIb phase completed)
- Another target Abetimus (binds anti dsDNA Ab and create B-cell tolerance)
Impact of Acquired Renal DNASE 1 Deficiency on Chromatin Exposure In Glomerulopathy

Endonuclease DNase1, produced in tubular and glomerular cells, is responsible for more than 80% of total nuclease activity in the kidney

- Near-absent DNase1 mRNA activity in nephritic kidneys
- Not a systemic loss of DNase1 activity
- Experimental deletion of the DNase1 gene causes lupus like disease and progression to renal failure
- Chronological relationship between reduced fragmentation of extracellular chromatin, deposition of chromatin fragments in GBM, and pathogenicity of anti-chromatin autoantibodies
Nephritogenic Potential of Anti-DNA Antibodies against Necrotic Nucleosomes

Nephritogenic anti-dsDNA and anti-nucleosome antibodies: Origin and effect

1. Apoptotic cell
   - Secondary necrotic chromatin is targeted by nephritogenic antibodies
   - Glomerular capillary membranes and mesangial matrix

2. Secondary necrotic chromatin activates the innate and the adaptive immune system
   - RNA-CpG
   - RNA-TLR7/CpG-TLR9 interaction

3. Exposed necrotic chromatin

4. DNA-specific B cell binds nucleosomes and presents nuc-derived peptides
   - B cell
   - Activated T cell
   - Mature DC
   - Cd4+ T cell

5. Plasma cells secrete anti-dsDNA antibodies

6. Antibodies bind exposed chromatin fragments prior to or after they associate with glomerular membranes

Inflammation

Apoptosis | Complement | APS | T cells | B cells | Antibodies | Renal effect

Elin Synnøve Mortensen and Ole Petter Rekvig
Nephritogenic Potential of Anti-DNA Antibodies against Necrotic Nucleosomes  JASN 2009
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