Pathogenesis of lupus – apoptosis clearance and regulation of self tolerance

Durga Prasanna Misra
Format

- Apoptosis
- Apoptosis in SLE
  - Decreased apoptosis of autoreactive cells
  - Increased apoptosis
- Defective clearance
  - Soluble factors
  - Macrophage/Mφ defects
  - NET degradation defects
- Apoptosis AND SLE pathogenesis
Apoptosis

- Programmed cell death
- Physiologic process
- Leads to generation of apoptotic bodies
- Clearance of apoptotic cells: No inflammation,

1842 – described apoptosis while studying the development of tadpole of the midwife toad
Apoptosis vs necrosis

<table>
<thead>
<tr>
<th>Provoking stimuli</th>
<th>Apoptosis</th>
<th>Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>programmed tissue remodeling</td>
<td>metabolic stresses</td>
</tr>
<tr>
<td></td>
<td>maintenance of cell pool size</td>
<td>absence of nutrients</td>
</tr>
<tr>
<td></td>
<td>genomic damage</td>
<td>changes in pH,</td>
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<tr>
<td></td>
<td></td>
<td>temperature</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hypoxia, anoxia</td>
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<tr>
<td></td>
<td>metabolic derangement</td>
<td></td>
</tr>
<tr>
<td></td>
<td>hypoxia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>imbalances in signaling pathways</td>
<td></td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Morphological changes</th>
<th>Apoptosis</th>
<th>Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affected cells</td>
<td>individual cells</td>
<td>groups of cells</td>
</tr>
<tr>
<td>Cell volume</td>
<td>decreased</td>
<td>increased</td>
</tr>
<tr>
<td>Chromatin</td>
<td>condensed</td>
<td>fragmented</td>
</tr>
<tr>
<td>Lysosomes</td>
<td>unaffected</td>
<td>abnormal</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>morphologically normal initially</td>
<td>morphologically</td>
</tr>
<tr>
<td></td>
<td></td>
<td>aberrant</td>
</tr>
<tr>
<td>Inflammatory response</td>
<td>none</td>
<td>marked</td>
</tr>
<tr>
<td>Cell fate</td>
<td>apoptotic bodies consumed by</td>
<td>lysis</td>
</tr>
<tr>
<td></td>
<td>neighboring cells</td>
<td></td>
</tr>
</tbody>
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<tr>
<th>Molecular changes</th>
<th>Apoptosis</th>
<th>Necrosis</th>
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</thead>
<tbody>
<tr>
<td>Gene activity</td>
<td>required for program</td>
<td>not needed</td>
</tr>
<tr>
<td>Chromosomal DNA</td>
<td>cleaved at specific sites</td>
<td>random cleavage</td>
</tr>
<tr>
<td>Intracellular calcium</td>
<td>increased</td>
<td>unaffected</td>
</tr>
<tr>
<td>Ion pumps</td>
<td>continue to function</td>
<td>lost</td>
</tr>
</tbody>
</table>
Secondary necrotic cell-derived material (SNEC) – pro-inflammatory

A

B

IL-8

IL-1β

TNF-β

p = 0.025

p = 0.025

p = 0.025

p < 0.001

p < 0.001

n.s.
Abnormal Production of Pro- and Anti-Inflammatory Cytokines by Lupus Monocytes in Response to Apoptotic Cells

**A**

TGF-β secretion

- Control monocytes + Apoptotic cells
- SLE monocytes + Apoptotic cells

\[ p = 0.0031 \]

**B**

TNF-α secretion

\[ p = <10^{-6} \]

- Monocyte+UVJ
- Monocyte+UVJ+RNase
- Monocyte+UVJ+DNase
- Monocyte+UVJ+RNase/DNase

**SLE patients**

0 20 40 60 80 100 200 400 600 800 1000 Pgf/ml

700

0 200 400 600 800 1000 Pgf/ml
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• Apoptosis AND SLE pathogenesis
SLE: Role of decreased apoptosis

- MRL/lpr, gld/gld
- ↓apoptosis of autoreactive T and B cells

Altered localisation of dsDNA producing B cells

Mice deficient in Fas develop antinuclear antibodies

J Immunol 2001; 167:2370-2378
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### Evidence of apoptosis

<table>
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<tr>
<th>Reference</th>
<th>Observation</th>
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<tbody>
<tr>
<td>Hargraves et al. (1948)(^8)</td>
<td>Phagocytosed nuclei (LE cells) in bone marrow (characteristic of SLE)</td>
</tr>
<tr>
<td>Hargraves (1949)(^102)</td>
<td>Phagocytosed nuclei (LE cells) in peripheral blood (characteristic of SLE)</td>
</tr>
<tr>
<td>Haserick &amp; Bortz (1949)(^9)</td>
<td>The presence of LE cells depends on sera of patients with SLE</td>
</tr>
<tr>
<td>Klemperer (1950)(^11)</td>
<td>‘Hematoxylin bodies’ in tissues of SLE patients: role of LE cells in the pathogenesis of SLE</td>
</tr>
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</table>
Apoptosis In SLE: Does it Occur?
Apoptosis In SLE: Does it Occur?

Health

UV exposure -24 hrs

UV exposure -72 hrs

SLE
Apoptosis In SLE: Does it Occur?

Dhir et al. Lupus 2009
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Soluble factors
Role of C1q in apoptotic cell clearance

A

C1q binding

Counts

C1q binding

10^0 10^1 10^2 10^3 10^4

B

p=0.01

RFI

normal patient C1q depleted

C

C3 deposition

Counts

C3 deposition

10^0 10^1 10^2 10^3 10^4

Patient: abnormal C1q protein

J Immunol; 2011; 187: 4369-73
Anti-class a scavenger receptor autoAb from SLE patients impair phagocytic clearance of apoptotic cells by Mφ in vitro.

![Graphs showing OD levels for ANTI-SRCR IgG and ANTI-SR-A IgG across different conditions: SLE, pSS, RA, CONTROL. The graphs display statistical data with control and P markers.]

SLE sera: HMGB1–nucleosome complexes

SN from secondary necrotic cells

SN from primary necrotic cells

A

IP:

α-histone

α-histone H2A/H4

α-histone H2B

α-histone H3

α-HMGB1

murine IgG2b

rabbit IgG

human IgG

30 kD

HMGB1

WB:

30 kD

30 kD

PEG (%)

1.5 2 1.5 2 1.5 2 1.5 2 1.5 2 1.5 2 1.5 2 1.5 2 1.5 2 1.5 2

Ig heavy chain

HMGB1

JEM 2008;205(13):3007-18
Mechanisms

- Blocks PS on apoptotic bodies
- Activation of pDC via TLR9 and RAGE
- Activation of auto-reactive B cell via TLR2

Anti-HMGB1 antibodies correlate with lupus activity
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Impaired Uptake of Apoptotic Cells Into Tingible Body Mφ

Green: TUNEL Red: Macrophage (CD68)
Mechanism for decreased uptake

- MFG-E8
Defects in uptake of apoptotic cells

Macrophages from patients with SLE and rheumatoid arthritis have defective adhesion in vitro, while only SLE macrophages have impaired uptake of apoptotic cells.
Defective uptake of late apoptotic cells by SLE sera

A

B

C

Jurkat T cell line
Mechanism of defective uptake by SLE sera: partly thru Fcγ receptors
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Impaired NET degradation in a subset of SLE patients

Higher antidsDNA titres and ANA titres in NET-nondegraders
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Tolerate me signals

NHD

- Early apoptotic cells
- Macrophages
- Production of anti-inflammatory signals; TGF-β and IL-10

SLE

- Defective clearance and accumulation of late apoptotic cells
- Macrophages
- Dendritic cells
- Dendritic cells gain access to autoantigens

Peripheral tissues

No Inflammation - T cell Tolerance

Inflammation - Loss of T cell Tolerance

NHD

- Early apoptotic cells
- Tingible Body Macrophages
- Follicular Dendritic cells
- Sequestration of nuclear autoantigens

SLE

- Defective clearance and accumulation of late apoptotic cells
- Tingible Body Macrophages
- Follicular Dendritic cells
- Sequestration of nuclear autoantigens failed

Lymphoid tissues

No access to autoantigens – B cell Tolerance

Autoantigens are presented to autoreactive B cells – Loss of B cell Tolerance

Rheumatology 2005;44:1101–1107
TAM receptors: tolerance induction

Absence of TAM receptors
- Accumulation of uncleared apoptotic material.
- Production of cytokines
- Severe autoimmune phenotype

Clustering of autoAg on apoptotic surface structures

Unirradiated keratinocytes

Irradiated keratinocytes

Fixed and stained with PI and FITC-anti Ro (52KDa) Yellow= Co-localization

Small blebs:
- Ro (52kDa)
- Ribosomal P
- Calreticulin
- Fodrin
- Jo-1

Apoptotic bodies:
- Nucleosomes
- Ro (60kDa)
- La
- Sm
- U1-70kDa
- Ku/DNA-PK
- Mi-2
- PARP
- NUMA

Entire surface membrane:
- C1q
- PS-protein complexes

Danger signals from secondary necrotic cells

Primary necrotic cell
- Nucleic acids: mRNA, Genomic DNA
- Proteins: U1 snRNP, U2 snRNP, HMGB1, HDGF, S100 proteins, Heat-shock proteins
- Metabolic intermediates: ATP, MSU
- Cytokines: IL-1β, IL-18, IL-6

Secondary necrotic cell
- Nucleosomes: dsDNA, Histones, HMGB1
- Caspase/granzyme B-generated autoantigens
- Metabolic intermediates: MSU

Endosome
- Mincle, TLR2/4, RAGE
- CD14, CD40, CD91, Scavenger receptor

Activation of NFκB and the inflammasome
Recruitment of monocytes, macrophages, neutrophils and DCs
Production of proinflammatory cytokines and chemokines
Upregulation of co-stimulatory molecules

Apoptosis AND SLE

1. Apoptosis
   - 'Eat-me' signal
   - Clearance deficiency

2. Germinal center
   - Secondary necrosis
   - Accumulation of SNEC, induction of autoantibodies

3. Break in self tolerance

4. SNEC-containing immune complex formation

5. SNEC uptake, cytokine production, chronic inflammation
   - Macrophages
   - PMNs
   - Monocytes
   - Blood and tissue

6. Organ damage

SLE
THANK YOU