Post-Translational Protein Modifications in the Biology of Health and Human Disease
Dendritic cell presents antigen to T cells

Other B cells make antibodies that bind instead to the body’s own proteins; they die

Killer T cell

Some mature into killer cells

Helper T cell

Others become helper cells and stimulate B cells to mature into plasma cells

B cells proliferate and mutate; some make antibodies that bind strongly to the foreign antigens and proliferate further

PLASMA CELL
Systemic Lupus Erythematosus: 1982 Revised Diagnostic Criteria

Patients must have 4 of the following criteria simultaneously or serially

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malar rash</td>
<td>Fixed erythema, flat or raised, sparing the nasolabial folds</td>
</tr>
<tr>
<td>Discoid rash</td>
<td>Raised patches, adherent keratotic scaling, follicular plugging; older lesions may cause scarring</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>Unusual reaction to sunlight</td>
</tr>
<tr>
<td>Oral ulcers</td>
<td>Usually painless, may be nasopharyngeal</td>
</tr>
<tr>
<td>Arthritis</td>
<td>Nonerosive, inflammatory in 2 or more peripheral joints</td>
</tr>
<tr>
<td>Serositis</td>
<td>Pleuritis or pericarditis</td>
</tr>
<tr>
<td>Renal disorder</td>
<td>Persistent proteinuria or cellular casts</td>
</tr>
<tr>
<td>Neurologic disorder</td>
<td>Seizures or psychosis</td>
</tr>
<tr>
<td>Hematologic disorder</td>
<td>Hemolytic anemia or leukopenia or lymphopenia or thrombocytopenia</td>
</tr>
<tr>
<td>Immunologic disorder</td>
<td>LE cells or antibodies to DNA or Sm or false positive BFP-STS</td>
</tr>
<tr>
<td>Antinuclear antibody</td>
<td>Elevated titer</td>
</tr>
</tbody>
</table>

## Frequency of Autoantibodies in Systemic Autoimmunity

<table>
<thead>
<tr>
<th>Autoantigen</th>
<th>Systemic Lupus</th>
<th>Rheumatoid Arthritis</th>
<th>Scleroderma</th>
<th>Sjögren's Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>dsDNA</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ssDNA</td>
<td>70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histones</td>
<td>70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sm</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nRNP</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ro (SS-A)</td>
<td>35</td>
<td></td>
<td></td>
<td>60</td>
</tr>
<tr>
<td>La (SS-B)</td>
<td>15</td>
<td></td>
<td></td>
<td>40</td>
</tr>
<tr>
<td>IgG (RF)</td>
<td>5</td>
<td></td>
<td>90</td>
<td>10-20</td>
</tr>
<tr>
<td>Scl-70 (Topo I)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centromere</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Wegener’s Nucleolar CREST Rim pattern Speckled
Dendritic cell presents antigen to T cells

KILLER T CELL

Some mature into killer cells

OTHER T CELL

HELPER T CELL

OTHER T CELL

OTHER T CELL

CYTOKINES RELEASED

OTHER B CELLS MAKE ANTIBODIES THAT BIND INSTEAD TO THE BODY'S OWN PROTEINS; THEY DIE

B CELLS PROLIFERATE AND MUTATE; SOME MAKE ANTIBODIES THAT BIND STRONGLY TO THE FOREIGN ANTIGENS AND PROLIFERATE FURTHER

PLASMA CELL

OTHER B CELLS MAKE ANTIBODIES THAT BIND STRONGLY TO THE FOREIGN ANTIGENS AND PROLIFERATE FURTHER

B CELL

OTHER B CELLS MAKE ANTIBODIES THAT BIND STRONGLY TO THE FOREIGN ANTIGENS AND PROLIFERATE FURTHER

PLASMA CELL

OTHER B CELLS MAKE ANTIBODIES THAT BIND STRONGLY TO THE FOREIGN ANTIGENS AND PROLIFERATE FURTHER

PLASMA CELL

OTHER B CELLS MAKE ANTIBODIES THAT BIND STRONGLY TO THE FOREIGN ANTIGENS AND PROLIFERATE FURTHER

PLASMA CELL

OTHER B CELLS MAKE ANTIBODIES THAT BIND STRONGLY TO THE FOREIGN ANTIGENS AND PROLIFERATE FURTHER

PLASMA CELL
How is autoimmunity initiated by self proteins?

Mouse cytochrome c as a model self antigen: Is there any unique biochemistry of autoantigens?
T cell Immunity to Self Cyt c

Searching For Cryptic Self Peptides

Mouse cyt c Peptide Immunogens

Antigenic Stimulus
- peptide
- whole cyt c

delta cpm

Mouse cyt c Peptide Immunogens

1-20 21-40 41-60 61-80 81-104

0 20000 40000 60000 80000 100000 120000

0 5000 10000 15000 20000 25000 30000
A. Immunization with Mouse cyt c 90-104 (peptide#1)

- Antigenic Stimulus:
  - cyt c 90-104
  - whole Mouse cyt c
  - cyt c 81-104

B. Immunization with Mouse cyt c 90-104 (peptide#2)

- Antigenic Stimulus:
  - cyt c 90-104
  - whole mouse cyt c
Analysis of Cyt c 90-104 peptides

1. Identical mass spec (molecular weight)
2. Identical amino acid analysis
3. Elute at distinct peaks by HPLC
4. Amino acid sequencing (?)
5. NMR analysis
Asn peptide

Asp peptide

NH₃

H₂O

Cyclic imide

70-80%

15-30%

Isoaspartyl peptide

Aspartyl peptide
Protein carboxyl methyltransferase (PCMT) Repair Mechanism

Isoaspartyl peptide

Aspartyl peptide

Methyl ester

Cyclic intermediate

PCMT REPAIR PATHWAY

SAM $\rightarrow$ SAH

70-80%

rapid

H$_3$COH

slow

15-30%
Isoaspartyl Modifications Form Spontaneously in All Cells

- Enhanced levels in aging and stressed cells
- Non-enzymatic modification
- Occurs at physiologic pH and temperature
- Arises most frequently at Asp/Asn-Gly
- May modify the biological functions of proteins
- PCMT repair (conserved in prokaryotes and eukaryotes)
Primary Peptide Structure

Aspartyl

Isoaspartyl
Altered antigen processing as a consequence of protein modification
Isoaspartyl cytochrome c is more efficiently digested by cathepsin D.
\( \alpha \text{PCC 88-104} \)
\( \beta \text{PCC 88-104} \)
\( \alpha \text{PCC 88-104} + \text{CatD} \)
\( \beta \text{PCC 88-104} + \text{CatD} \)
Peptides generated by cathepsin D digest of \(\alpha/\beta\)PCC 88-104 (MALDI-MS)

<table>
<thead>
<tr>
<th>PCC</th>
<th>Peptide Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha)PCC</td>
<td>KAERADLIA YL K</td>
</tr>
<tr>
<td></td>
<td>DL IAYLK QATAK</td>
</tr>
<tr>
<td></td>
<td>KAERADLIA</td>
</tr>
<tr>
<td></td>
<td>IA YLK QATAK</td>
</tr>
<tr>
<td>Intact PCC</td>
<td>KAERADLIA YL K</td>
</tr>
<tr>
<td></td>
<td>K QATAK</td>
</tr>
<tr>
<td>(\beta)PCC</td>
<td>IAYLK QATAK</td>
</tr>
<tr>
<td></td>
<td>KAERADLIA YL</td>
</tr>
<tr>
<td></td>
<td>KL K Q (A)</td>
</tr>
</tbody>
</table>

**Legend:**
- **Red Text:** Peptides generated by \(\alpha\)PCC
- **Blue Text:** Peptides generated by \(\beta\)PCC
- **Black Text:** Intact PCC
Isoaspartyl Proteins

- beta amyloid
- snRNP autoantigen
- histone H2B
- insulin
- human hemoglobin
- calmodulin
- myosin
- human growth hormone
- mouse Ig kappa chains
- IL-1
- CD4
Beta amyloid (Aβ) and isoAsp in Alzheimer’s Disease

Aβ1-42 subject to posttranslational modifications

Asp-1 and Asp-7 in Aβ subject to isoAsp formation
senile plaques - isoAsp 20%
L-isoAsp 55.7%
D-isoAsp 19.2%

isoAsp-7 increased fibrillogenesis
isoAsp 1+7 resistance to enzymatic degradation

Repair enzyme PCMT does not appear deficient in AD brains

Number of isoAsp-7 Aβ positive plaques increases in parallel with disease severity
Peptide
- immunity to peptides

Protein
- immunity to modified whole protein

Cell
- role of intra/extracellular modifications
- signaling defects
- immunity to cellular target

Animal
- altered T/B cell responses
- altered selection/tolerance
- spontaneous autoimmunity
Do modified self proteins trigger lupus autoimmunity?
Immunization of normal mice with Isoasp modified lupus antigen (snRNP D) elicits autoantibodies

Isoasp snRNP peptide immunization

Asp snRNP peptide immunization
What is the profile of protein modifications in normal cells?

Selective accumulation of isoaspartyl modification
In histone H2B among intracellular proteins
(J. Biol. Chem. 276:37161; 2001)

Histone 2B p21-35

Why should immunologists care about post-translational modifications?

- 50-90% of proteins are modified
- 140 flavors of amino acids
- Some probes of immune responses (synthetic peptides) may not reflect real-life antigens
- Modified proteins are important in autoimmune diseases
Dendritic cell
presents antigen
to T cells

T CELL

KILLER T CELL

HELPER T CELL

Some mature into
killer cells

Others become helper
cells and stimulate
B cells to mature into
plasma cells

CYTOKINES RELEASED

DENDRITIC CELL

Other B cells make
antibodies that bind
instead to the body's
own proteins; they die

B cells proliferate and
mutate; some make
antibodies that bind
strongly to the foreign
antigens and proliferate
further

PLASMA CELL
### Common posttranslational modifications associated with autoimmune responses

<table>
<thead>
<tr>
<th>Disease</th>
<th>Modification</th>
<th>Antigen</th>
<th>Immune Response</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Multiple Sclerosis</strong></td>
<td>Phosphorylation, Deimination, Acetylation</td>
<td>αB-crystallin, MBP, MBP Ac 1-11</td>
<td>Specific, Specific</td>
<td>Mouse Rat</td>
</tr>
<tr>
<td><strong>EA E</strong></td>
<td></td>
<td></td>
<td>ND, ND</td>
<td></td>
</tr>
<tr>
<td><strong>Collagen-Induced arthritis (CIA)</strong></td>
<td>Glycosylation, Hydroxylation</td>
<td>Type II Collagen</td>
<td>Specific</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ND, ND</td>
<td>Mouse Rat</td>
</tr>
<tr>
<td><strong>Rheumatoid Arthritis</strong></td>
<td>Deimination</td>
<td>Filaggrin</td>
<td>ND, Specific</td>
<td>Human</td>
</tr>
<tr>
<td><strong>SLE</strong></td>
<td>Phosphorylation, Isoaspartyl formation</td>
<td>Various SnRNP D</td>
<td>ND, Specific</td>
<td>Human Mouse</td>
</tr>
<tr>
<td></td>
<td>Mannose sDMA</td>
<td>Various Sm D1, D3</td>
<td>ND, Specific</td>
<td>Mouse Human</td>
</tr>
<tr>
<td><strong>Celiac Disease</strong></td>
<td>Deamidation</td>
<td>Wheat gliadin</td>
<td>Specific</td>
<td>Human</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Specific to tTG</td>
<td></td>
</tr>
<tr>
<td><strong>Atherlosclerosis</strong></td>
<td>Lipid Peroxidation</td>
<td>LDL, Others</td>
<td>Specific</td>
<td>Diverse Mouse</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Diverse</td>
<td></td>
</tr>
</tbody>
</table>

Anti-cyclic citrullinated peptide antibodies (CCP)

- Autoantibodies directed against citrulline residues formed in post translational modification of arginine.
- Highly specific for RA (98%?), and moderately sensitive (68%). Important diagnostic tool in RF neg RA.
- Potential diagnostic value either alone or with RFs in prognosis and disease severity.

CRP, ESR for monitoring RA in addition to RFs.
A variety of post-translational protein modifications are important in the onset of many human diseases

- Protein modifications alter immunogenicity of self protein.
- Modifications alter the processing and biochemical proteolysis of self proteins leading to altered (different) peptide products.
- How does one identify individual protein/peptide modifications linked with disease?
- Modified proteins are important targets in autoimmune diseases. Important to understand modifications in the laboratory diagnostics of disease.
- Protein modifications can be used as vaccines to break immune tolerance to normal self proteins.
Immunologic Implications of Protein Modification

Lupus autoimmunity may be amplified by two synergistic mechanisms:

1. Isoaspartyl modified self proteins are immunogenic (altered cleavage products and/or presentation).
2. Increased intracellular isoaspartyl modification leads to T cell hyperproliferation.

Step 1: Production and release of increased modified self proteins

Step 2: Increased intracellular Isoasp leads to hyperproliferation

Lupus autoimmunity

Normal tolerance
Post-translational protein modifications can enhance immunity to *some* self proteins (or foreign proteins?). The inability to repair isoaspartyl self peptides leads to autoimmunity.

**Effects of Posttranslational Protein Modifications:**
1. Altered protein biological functions
2. Altered protein catabolism/metabolism
3. Altered antigen processing (novel cryptic peptides, epitope spreading)
4. Synthetic peptides (GAD65) may not reflect modifications that occur in vivo
Diabetes Autoantigens

1. GAD 65
Oxidative modifications enhance recognition by human Abs
GAD65 has four Asx-Gly isomerization sites within known recognized epitopes

2. Insulin/proinsulin
Insulin A-chain disulfides required for CD4 T cell response
Insulin prone to isomerization (isoaspartyl formation)

3. Islet specific glucose 6-phosphatase

4. Insulinoma-associated protein-2 (IA-2)

5. Reg family proteins (PAP; pancreatic assoc. proteins)
Posttranslational protein modifications in T1D: A role for the repair enzyme protein isoaspartate methyltransferase (PIMT).


1. High levels of PIMT (isoasp repair enzyme) is observed in human beta cells.

2. Diabetes delay in BB rats treated with high dose CGP3466B (PIMT inhibitor, apoptosis inhibitor)

3. Non-beta cell Ags may become modified by changes in the beta cell ‘environment’.
Can isoaspartyl modified tumor antigens break immune tolerance?
Murine B16 melanoma

1. tyrosinase-related protein 2 (TRP-2) and glycoprotein 100 (gp100) are expressed on B16 cells.
2. Both proteins are normal, nonmutated self tissue differentiation antigens.
3. Both proteins and peptides are NON-immunogenic in the C57 BL/6 mouse.
TRP-2\textsubscript{181-188} : VY\textcolor{red}{D}FFVWL
H-2K\textsuperscript{b}-restricted

gp100\textsubscript{25-33} : EGSRNQ\textcolor{red}{D}WL
H-2D\textsuperscript{b}-restricted
Draining LN cells from isoAsp TRP-2 immunized mice restimulated by isoAsp TRP-2 peptide in vitro for 5 days.
Immunization with Isoasp Melanoma peptides elicits killer T cells
4 week B16 melanomas from Isoasp and Asp TRP-2 Immune GFP mice

IsoAsp TRP-2 Immune

Asp TRP-2 Immune
IsoAspartyl Modified TRP-2 triggers anti-tumor immunity (CD8, CD4 and antibody)

Complete immune tolerance to unmodified TRP-2

[Doyle et. al., J. Biol Chem. 281:32676 (2006)]
General Characteristics of Autoimmunity
(SLE and/or MS, Th1-mediated diseases)

• Excessive activation of B and T lymphocytes
• Altered cytokine release in unique physiologic sites
• Differentiation of B cells to plasma cells and autoantibody production

Do protein modifications alter cellular function?

We next analyzed the intracellular isoaspartyl content of immune cells in a mouse model of lupus (MRL lupus).
The inability to repair isoaspartyl modification amplifies autoimmune response (?)
Adoptive Transfer of PIMT-/- bone marrow induces lupus like autoimmunity

CD4+ T cell hyperproliferation through TCR

T cell hyperproliferation --- common phenomenon found in MRL.AND mice and SLE patients

B10.BR Mice reconstituted with PIMT-/- bone marrow develop autoantibody

JI 15:171, 2003
Are there any abnormalities in iso-Asp generation or metabolism (repair) in spontaneous murine lupus?

Mouse models used:

**B10.BR** (as normal) versus **MRL+/-** and **MRL/lpr** (lupus-prone)

Initial screening of erythrocyte lysates:

An excellent source for studying nonenzymatic protein damage

The repair of damaged protein does not occur in erythrocytes (no *de novo* protein synthesis).
Fig. 1(A) Amount of Isoaspartyl (Iso-Asp) residues is higher in erythrocytes of the lupus-prone mice and elevated over age. (*, p<0.001 and **, p<0.0001)
Fig.1(B) PIMT activity keeps stable in both of B10.BR and the lupus-prone mice over age.
Do iso-Asp self proteins accumulate in tissues of lupus-prone mice?

Iso-Asp level in tissue lysate (Brain and Kidney)

<table>
<thead>
<tr>
<th>Tissue extract</th>
<th>Iso-Asp residue (pmol/mg protein)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8- 10 weeks</td>
</tr>
<tr>
<td><strong>B10.BR</strong></td>
<td>brain 329.45 ± 57.94</td>
</tr>
<tr>
<td></td>
<td>kidney 147.58 ± 22.82</td>
</tr>
<tr>
<td><strong>MRL+/+</strong></td>
<td>brain 300 ± 50.4</td>
</tr>
<tr>
<td></td>
<td>kidney 137.49 ± 34.61</td>
</tr>
</tbody>
</table>

*: n=5; **p< 0.05

Table 1.(A) The Iso-Asp concentration in brain or kidney was not changed by age in B10.BR mice. The Iso-Asp concentration in brain or kidney was increased in older MRL +/- mice.
PIMT enzyme repair activity is normal in wildtype and lupus prone animals (Brain and Kidney)

<table>
<thead>
<tr>
<th>Tissue extract</th>
<th>8- 10 weeks</th>
<th>18-20 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B10.BR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>brain</td>
<td>195.9 ± 42.27</td>
<td>196.16 ± 27.49</td>
</tr>
<tr>
<td>kidney</td>
<td>122.02 ± 15.03</td>
<td></td>
</tr>
<tr>
<td><strong>MRL+/+</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>brain</td>
<td>209.93 ± 11.37</td>
<td>206.8 ± 14.99</td>
</tr>
<tr>
<td>kidney</td>
<td>161.59 ± 33.5</td>
<td>156.08 ± 47.8</td>
</tr>
</tbody>
</table>

*: n=5; **p= 0.001

**Table 1.(B)** The PIMT enzyme activity in brain was not changed by age in B10.BR mice.
The PIMT enzyme activity in brain or kidney was not changed by age in MRL +/+ mice.
Iso-Asp level in purified T cells

FIG. 3(A) Amount of intracellular Iso-Asp residues is higher in T cells of the lupus-prone mice and elevated over age. (*, p<0.05, **, p<0.01 and ***, p<0.001)
Iso-Asp level in CD4$^+$ T cells (from older mice)

Fig. 4(C) Intracellular iso-Asp contents were increased in CD4$^+$ T cells from MRL$^+$/+.AND and MRL/lpr.AND mice after antigenic stimulation. (*, p<0.05)
Summary

1. The amount of isoAsp modified proteins were significantly higher and accumulated over age in erythrocytes, brain, kidney and T cells from lupus-prone mice compared to levels observed in normal mice.

2. In contrast, the specific activity of isoAsp repaired enzyme, PIMT, remained stable over age in normal or lupus-prone mice.

3. The amount of isoAsp-modified proteins also increases dramatically in lupus-prone CD4\(^+\) T cells after antigenic stimulation.