Natural Autoimmunity, Apoptosis and ECP

Gregg J. Silverman  MD
Departments of Pathology and Medicine  Musculoskeletal Center of Excellence
NYU School of Medicine
Disclosures

• Consultant for Pfizer, Lilly, GSK, Roche, Takeda, Onyx, EMD Serano

• Not relevant to the current presentation
Learning objectives

• Overview on technical and clinical features of extracorporeal photopheresis (ECP)

• Discuss literature on postulated mechanisms of action of ECP

• Review recent insights into relationships between apoptotic cell infusions and the induction of natural antibodies

• Discuss an emerging hypothesis regarding the anti-inflammatory regulatory properties of IgM-AC complexes
Extracorporeal photopheresis

- Extracorporeal photopheresis (ECP) an immunologic treatment involving a device that enables extracorporeal (outside of the body) exposure of leukocytes to ultraviolet light (UVA) in presence of photosensitizer, 8-methoxypsoralen (8-MOP) (1).

- Introduced by Edelson et al. for treatment of Sezary’s syndrome.


- CTCL is a malignancy of CD4 helper T cells initially seen as pruritic patches, papules or plaques [1].

- CTCL is most common cutaneous lymphoma (0.5 to 1.0/ per 100,000 person-years in US. Male predominance, and typically affects adults 40–50 years age.

- ECP intended to kill the recirculating malignant T cells

2. Edelson et al. NEJM 1997: 316:297-323
Extracorporeal Photopheresis
Graft vs. Host Disease (GVHD), common complication of allogenic bone marrow transplant, has shown good responses to ECP

<table>
<thead>
<tr>
<th>Study</th>
<th>Type</th>
<th>No.</th>
<th>%CR</th>
<th>%PR</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Messina et al. [1]</td>
<td>Retrospect</td>
<td>33</td>
<td>70</td>
<td>NA</td>
<td>5-year overall survival 69% for responding patients vs 12% for non-responders (p = 0.001)</td>
</tr>
<tr>
<td>Greinix et al. [2]</td>
<td>Retrospect</td>
<td>6</td>
<td>67</td>
<td>33</td>
<td>ECP was well tolerated without any significant side effects</td>
</tr>
<tr>
<td>Greinix et al. [3]</td>
<td>Phase II</td>
<td>59</td>
<td>&gt;61</td>
<td>NA</td>
<td>Probability of survival was 59% among patients who responded completely</td>
</tr>
<tr>
<td>Greinix et al. [4]</td>
<td>Prospect</td>
<td>21</td>
<td>60</td>
<td>NA</td>
<td>CR in skin acute GVHD: 100% in grade 2, 67% in grade 3, 12% in grade 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CR in 67% liver involvement</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No CR or PR in gut involvement</td>
</tr>
<tr>
<td>Perfetti et al. [5]</td>
<td>Retrospect</td>
<td>23</td>
<td>NA</td>
<td>NA</td>
<td>CR: 70% in grade 2, 42% in grade 3, 0% in grade 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CR in 66% skin involvement</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CR in 27% liver involvement</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CR in 40% gut involvement</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Patients treated within 35 days of onset of acute GVHD had higher responses (83 vs 47%; p = 0.1)</td>
</tr>
<tr>
<td>Salvanesci et al. [6]</td>
<td>Retrospect</td>
<td>9</td>
<td>NA</td>
<td>77</td>
<td>7/9 children responded to EPC, of which 3 discontinued immunosuppressive treatment</td>
</tr>
<tr>
<td>Perotti et al. [7]</td>
<td>Retrospect</td>
<td>50</td>
<td>NA</td>
<td>68</td>
<td>Response to ECP was inversely associated with death</td>
</tr>
<tr>
<td>Dall’Amico et al. [8]</td>
<td>Retrospect</td>
<td>76</td>
<td>53</td>
<td>NA</td>
<td>CR in 67% skin involvement</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CR in 30% liver involvement</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CR in 54% gut involvement</td>
</tr>
</tbody>
</table>

CR: Complete remission
PR: Partial remission

### ASFA recommendations for extracorporeal photoimmune therapy.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Therapy Type</th>
<th>Recommendation</th>
<th>Evidence Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-erythrodermic</td>
<td>Role is not established</td>
<td></td>
<td>Weak; low quality evidence</td>
</tr>
<tr>
<td>GVHD</td>
<td>Skin (chronic)</td>
<td>Accepted as second-line therapy. ECP is added to corticosteroids for unresponsive cGVHD</td>
<td>Strong; moderate quality evidence</td>
</tr>
<tr>
<td></td>
<td>Skin (acute)</td>
<td>Accepted as second-line therapy, alone or in conjunction with other modes of treatment</td>
<td>Weak; low quality evidence</td>
</tr>
<tr>
<td></td>
<td>Non-skin (acute/chronic)</td>
<td>Role is not established</td>
<td>Weak; low quality evidence</td>
</tr>
<tr>
<td>Cardiac allograft rejection</td>
<td>Prophylaxis</td>
<td>Accepted as first-line therapy</td>
<td>Strong; high quality evidence</td>
</tr>
<tr>
<td></td>
<td>Treatment of rejection</td>
<td>Accepted as second-line therapy</td>
<td>Strong; moderate quality evidence</td>
</tr>
<tr>
<td>Lung allograft rejection</td>
<td></td>
<td>Accepted as second-line therapy</td>
<td>Strong; low quality evidence</td>
</tr>
<tr>
<td>Nephrogenic systemic fibrosis</td>
<td></td>
<td>Role is not established</td>
<td>Weak; low quality evidence</td>
</tr>
<tr>
<td>Pemphigus vulgaris</td>
<td></td>
<td>Role is not established</td>
<td>Weak; low quality evidence</td>
</tr>
<tr>
<td>Scleroderma (progressive SyS)</td>
<td></td>
<td>Published evidence demonstrates ECP to be ineffective</td>
<td>Strong; high quality evidence</td>
</tr>
</tbody>
</table>

ASFA: American Society for Apheresis; cGVHD: Chronic GVHD; CTCL: Cutaneous T-cell lymphoma; ECP: Extracorporeal photopheresis; GVHD: Graft-versus-host disease; MF: Mycosis fungoides; SS: Sézary syndrome; SyS: Systemic sclerosis.

How does ECP work?

- 8-MOPS is activated by the UVA radiation so only treated cells are affected.
- Half-life of 8-MOPS is extremely short, and is not carried into the body.
- Cells are reinfused into a vein.
- Treatment on two sequential days, and then repeated every 2-4 weeks.

Conceptual challenges with ECP

- Only 10-15% of total lymphocytes are in the bloodstream.
- Monocytes are unaffected.
- Empiric findings that potential benefits in other inflammatory and autoimmune diseases are controversial.

In health, dying cells need to be cleared as early as possible!
"Waste Disposal" Hypothesis

Macrophages degrade cell corpse, destroying self antigens, secrete suppressive cytokines.

C1q paradox

Dendritic cells ingest cell corpses to present peptides and pro-inflammatory cytokines that recruit autoreactive T cells. PS in apoptotic membrane suppresses IL-12 secretion.
Mouse model ECP demonstrated immune modulation with IL-10 production.
Mouse model ECP demonstrated immune modulation with T cell changes and expansion of splenic B cells

Capitini et al. Extracorporeal Photopheresis Attenuates Murine Graft-versus-Host Disease via Bone Marrow-Derived Interleukin-10 and Preserves Responses to Dendritic Cell Vaccination Biology of Blood and Marrow Transplantation 17 (6) 2011 790 - 799
Mechanistic Hypothesis based on Mouse model ECP

Goussetis et al. Transfusion and Apheresis Science 4 (2) 2012, pp203–209
Alternate Hypothesis: Does ECP act by induction of immunomodulatory IgM-apoptotic cell complexes?
What is the outcome of AC infusions (i.e., ECP)

Possible outcomes:
- No immune response due to self-tolerance
- Broad polyclonal response to the immunization
- Limited and specific responses

AC = avg ~1.5x10^7 apoptotic thymocytes in PBS

Study Mice
1. Terminal Bleed
2. Binding to Apoptotic cells
3. Phagocytosis by iDCs
Intravenous infusions of apoptotic cells (ACs) induces IgM anti-PC and anti-MDA- antibodies detected by antigen microarrays.

Syngenic apoptotic thymocytes inject 25 x 10^6 i.v. each week x 3

Chen et al. J Immunol 2009a
IV apoptotic thymocyte treatment induces IgM anti-PC and anti-MDA IgM-secreting cells represent >55% of induced IgM-secreting cells

<table>
<thead>
<tr>
<th></th>
<th>anti-IgM</th>
<th>PC-BSA</th>
<th>MDA-BSA</th>
<th>BSA (albumin)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Naïve B6</strong></td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td><strong>AC-treated B6</strong></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
</tbody>
</table>

AC = apoptotic thymocytes

ELISpot analysis of IgM-secreting cells
Energies associated with out-of-plane Fab domain rotations.

Czajkowsky D M, Shao Z. PNAS 2009;106:14960-14965
IgM anti-ACM recognize Apoptotic Cells (not healthy cells) and recruits C1q!

\[ T15 \text{ IgM} - \text{anti-PC NAb from B-1a cells} \]

Chen et al. J Immunol, 2009

IgM anti-ACM also recruits MBL to early ACs (not shown)
T15 and Post-immunized sera (IgM) recognize cells undergoing apoptosis

Annexin V

None

Ig-deficient sera

Naive Sera (1:100)

Post-Immunized Serum (1:100)

T15 IgM + Ig-deficient sera

IgM
Cannabilistic DCs: T15 IgM anti-ACM NAb binds dead DCs in culture, and enhances their phagocytosis by living DCs.

Overnight DC cultures include 10-20% dying DCs,
DC ingestion of apoptotic cells by immature DC enhanced by T15 IgM and serum C1q and MBL

Flow cytometric assay and microscopic assay document T15 IgM increases in AC phagocytosis

Polyclonal IgM enhances Mϕ clearance of ACs
Quartier. et al 2005 Eur J Immunol
Elkon and coworkers 2005 Autoimmunity

Chen et al. J Immunol, 2009
Regulatory NAb IgM anti-PC/ACM provides protection in murine collagen induced arthritis (CIA)
Collagen Induced Arthritis is improved by T15 NAb
CIA is improved by apoptotic cells

Same in IgG2a anti-collagen II model
of immune complex mediated inflammatory arthritis

Chen et al. Journal of Immunol, 2009
CIA/control treatment

Front paw

Hind paw

CIA/ T15 treatment

Chen et al 2009 J Immunol
Regulatory anti-Apoptotic cell Natural Abs

IgM anti-PC recruits C1q & MBL deposition onto dead and dying cells
- serve as “eat me” signals and may stabilize AC synapse on DC & Mφ

Enhances apoptotic cell clearance by Mφ and immature DC

Blunts TLR3, TLR4, TLR7 and TLR9 signaling that mediate pathology

Blocks DC/Mφ activation and production of inflammatory factors

• ↓ expression MHC II, B7 family, CD40
• ↓ IL-1β, TNFα, IL-6, IL-17, IFNγ and IL-12 mediate disease
• ↓ chemokine production that attract cells to disease sites

Ameliorates CIA arthritis and anti-collagen II antibody-mediated arthritis

Prolonged survival in severe murine lupus (NZWxBXSB)F1
How do anti-AC Abs work in DCs?

Dual specificity phosphatases are master regulators of the MAPK system

<table>
<thead>
<tr>
<th>MKP</th>
<th>Species orthologues</th>
<th>Substrate specificity</th>
<th>Subcellular localization</th>
<th>Immediate-early gene</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MKP1</td>
<td>DUSP1, CL100, HVH1, 3CH134, ERP</td>
<td>p38 ~ JNK &gt;&gt; ERK</td>
<td>Nuclear</td>
<td>Yes</td>
<td>15, 93–97</td>
</tr>
<tr>
<td>MKP2</td>
<td>DUSP4, HVH2, TYP1</td>
<td>ERK ~ JNK &gt;&gt; p38</td>
<td>Nuclear</td>
<td>Yes</td>
<td>53, 98, 99</td>
</tr>
<tr>
<td>MKP3</td>
<td>DUSP6, PYST1, RVH6</td>
<td>ERK &gt;&gt; JNK ~ p38</td>
<td>Cytosolic</td>
<td>No</td>
<td>100–102</td>
</tr>
<tr>
<td>MKP4</td>
<td>DUSP9, PYST3</td>
<td>ERK &gt; p38 &gt; JNK</td>
<td>Nuclear and cytosolic</td>
<td>No</td>
<td>103, 104</td>
</tr>
<tr>
<td>MKP5</td>
<td>DUSP10</td>
<td>p38 ~ JNK &gt;&gt; ERK</td>
<td>Nuclear and cytosolic</td>
<td>No</td>
<td>50, 51</td>
</tr>
<tr>
<td>MKP7</td>
<td>MKPM, DUSP16</td>
<td>JNK ~ p38 &gt;&gt; ERK</td>
<td>Cytosolic</td>
<td>No</td>
<td>18, 55</td>
</tr>
<tr>
<td>MKPX</td>
<td>DUSP7, B59, PYST2</td>
<td>ERK &gt;&gt; JNK ~ p38</td>
<td>Cytosolic</td>
<td>No</td>
<td>100, 105, 106</td>
</tr>
<tr>
<td>DUSP2</td>
<td>PAC1</td>
<td>ERK ~ p38 &gt;&gt; JNK*</td>
<td>Nuclear</td>
<td>Yes</td>
<td>53</td>
</tr>
<tr>
<td>HVH3</td>
<td>DUSP5, B23</td>
<td>ERK</td>
<td>Nuclear</td>
<td>Yes</td>
<td>95, 107</td>
</tr>
<tr>
<td>HVH5</td>
<td>DUSP8, M3/M6</td>
<td>JNK ~ p38 &gt;&gt; ERK</td>
<td>Nuclear and cytosolic</td>
<td>No</td>
<td>108–110</td>
</tr>
</tbody>
</table>

*Although DUSP2 shows substrate preference for p38 and ERK in transient transfection assays, it prefers JNK as a substrate in vivo. CL100, human homologue of MKP1; DUSP, dual-specificity protein phosphatase; ERK, extracellular-signal-regulated kinase; ERP, externally regulated phosphatase; HVH, human VH1-like PTPase; JNK, JUN N-terminal kinase; MAPK, mitogen-activated protein kinase; MKP, MAPK phosphatase; PAC1, phosphatase of activated cells 1; RVH, rat VH1-like PTPase; TYP, threonine/tyrosine phosphatase.

Anti-AC Ab + C1q/MBL

Liu et al. Nature Reviews Immunology 7, 202–212 (March 2007) | doi:10.1038/nri2035
IgM anti-AC, in the presence of C1q or mannose binding lectin induces high nuclear MKP-1 and blocks activation of nuclear p38k
In LPS stimulated DCs, IgM antibody to AC membranes induces high early MKP-1 in C1q dependent manner, which blocks primary MAPK and ELK-1 activation.

Gronwall et al. PNAS 2012
IgM antibodies can form complexes with apoptotic cells

Anti-inflammatory effects do not require IL-10 or TGF-β by DCs

Involve a signal transduction pathway that blocks dominant inflammatory signaling pathways

IgM-AC complexes induce MKP-1 (dual specificity phosphatase) also involved in corticosteroid transactivation signaling
Postulated sequence for formation of immunomodulatory AC complexes
AC synapse, with TLR signaling, induces a counter regulatory MKP-1 signal shuts off inflammatory responses

Gronwall et al.
PNAS 2012
Are regulatory natural antibodies relevant to human health disease?
Human IgM anti-PC: Levels vary 100-fold and are stable in health.
In healthy adult, levels of serum IgM anti-PC are proportional to IgM binding of ACs

IgM α-PC negatively correlates with SLICC index

\[ p = 0.0071 \]
\[ r = -0.27 \]
Higher levels of IgM anti-PC NAbs correlate with protection from ASCVD events (MI and CVA) in SLE patients

Caroline Grönwall et al.
Clinical Immunology 2012
Levels of IgM anti-PC drop after limb perfusion with TNFα for sarcoma/melanoma.

Infusions induce massive death of malignant and endothelial cells of tumor vasculature.

Levels of anti-PC IgM before and after limb perfusion with TNFα.

Selective consumption of IgM anti-PC with stable total IgM levels.

IgM anti-malondialdehyde Response

- cGVHD
- No difference
- Levels of control IgM

NYU Langone Medical Center
IgM anti-PC Antibody responses in patients receiving ECP

OA: Osteoarthritis
AS: Ankylosing Spondylitis
CTCL: Cutaneous T-Cell Lymphoma
GvHD: Graft-versus-Host Disease

Significantly lower Levels of anti-PC IgM

(unpublished)
Conclusions

• NAbs to apoptotic cell (AC) epitopes (e.g. phosphorylcholine) have regulatory properties for innate immunity

• Anti-AC IgM may enhance for strong signal pathway that shuts down inflammatory MAPK signaling

• MKP-1 is required for NAb anti-AC effects in DCs:
  • Anti-AC Abs may represent physiologic factors that help to maintain homeostasis.
  • Levels of regulatory IgM anti-ACs may represent a balance between consumption and production.

• May be relevant to ECP treatment, and improving clinical outcomes.
Acknowledgements

Caroline Grönwall
Yifang Chen
Jaya Vas
Carl Goodyear
Sahil Khanna
Y-B Park
Haitao

UCSD
Maripat Corr
David Boyle

TSRI
Dwight Kono

U Penn
Don Siegel
Alain Rook

John Jung
Haitao Niu
Adam Pelzek
Jeff Greenberg
Jose Scher

NIH-NIAMS
NIAID
Alliance for Lupus Research
ACR-REF Within Our Reach
Arthritis Foundation
IgM anti-PC responses and ECP treatments over time
Regulatory NAbs enhance apoptotic cell signaling via dual sets of receptors.

**Phosphorylcholine (PC):** head group in neutral PLs becomes accessible after oxidative modification.

**Malondialdehyde (MDA):** reactive aldehyde formed by reactive oxygen species interactions with PLs.

**Phosphatidylserine (PS):** group flips to the outer leaflet of cell membrane to become exposed for opsonin binding.
Surveys of 120 patients with SLE

Table 4  Associations between IgM antibody levels and prevalence of renal disease or cardiovascular disease.

<table>
<thead>
<tr>
<th>IgM anti-</th>
<th>Renal disease</th>
<th>ASCVD events</th>
</tr>
</thead>
<tbody>
<tr>
<td>(≥ median)</td>
<td>Odds ratio (95% CI)</td>
<td>p-Values</td>
</tr>
<tr>
<td>PC</td>
<td>0.75 (0.32, 1.77)</td>
<td>0.51</td>
</tr>
<tr>
<td>MDA</td>
<td>0.41 (0.17, 1.01)</td>
<td>0.053</td>
</tr>
<tr>
<td>β2-GPI</td>
<td>0.62 (0.26, 1.47)</td>
<td>0.27</td>
</tr>
<tr>
<td>CL</td>
<td>0.37 (0.14, 0.98)</td>
<td>0.046 *</td>
</tr>
<tr>
<td>dsDNA</td>
<td>0.37 (0.14, 0.98)</td>
<td>0.046 *</td>
</tr>
</tbody>
</table>
### Table 3  Correlations between IgM antibody levels and complement factors.

<table>
<thead>
<tr>
<th>IgM anti-</th>
<th>C3</th>
<th></th>
<th>C4</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>PC</td>
<td>0.031</td>
<td>0.7364</td>
<td>0.065</td>
<td>0.4851</td>
</tr>
<tr>
<td>MDA</td>
<td>−0.24</td>
<td>0.0086*</td>
<td>−0.14</td>
<td>0.13</td>
</tr>
<tr>
<td>β2-GPI</td>
<td>−0.22</td>
<td>0.015*</td>
<td>−0.31</td>
<td>0.0007*</td>
</tr>
<tr>
<td>CL</td>
<td>−0.27</td>
<td>0.0078*</td>
<td>−0.41</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>dsDNA</td>
<td>−0.19</td>
<td>0.068</td>
<td>−0.23</td>
<td>0.025*</td>
</tr>
</tbody>
</table>
IgM anti-AC, in the presence of C1q or mannose binding lectin induces high nuclear MKP-1 and blocks activation of nuclear JNK or ERK.