Pathogenesis of Atopic Dermatitis: Rationale for Barrier Repair Therapy

Peter M. Elias, M.D.
Department of Dermatology, UCSF & Dermatology Service, VAMC, San Francisco
March 30, 31 & April 1, 2010

Atopic Dermatitis

- Common disease
- Associated with other atopic diseases
  - Asthma, Allergic rhinitis
  - IgE sensitization to food and airborne allergens (note: these antigens initiate disease after penetrating the skin!)

Atopic Dermatitis

- Incidence and severity are increasing

Atopic Dermatitis

- Common disease
- Associated with other atopic diseases
  - Multigenic (Some shared with asthma (T(h2))
  - More common in Asians (over 50%)

Atopic Disease: A Modern Epidemic

Why Is Prevalence Rising?

- Hygiene hypothesis (HH)
  - ‘inside-outside’ perspective

Why is Prevalence Rising?

- Hygiene hypothesis (HH)
  - ‘inside-outside’ perspective

  - HH= ‘outside-inside’ perspective:
    - Crowded urban environment—sustained dust mite exposure
    - Percutaneous absorption across a defective barrier
    - Excessive hygiene damages barrier

The Traditional View of Atopic Dermatitis: “From the Inside-Out”

- An Immunologic Disorder
  - IgE response to antigens
  - T_{H2} cytokine production
  - Epidermis is a downstream participant in the battlefront of the immune response

Atopic Dermatitis: ‘Outside-Inside’ Paradigm

- Genetic/ Constitutional
  - Vulnerable Barrier

“Immune Dysregulation”

Busse WW, et al. NEJM 2001;344:350-362
Genetic + Acquired Stressors

Atopic Dermatitis: ‘Outside-Inside’ Paradigm

Vulnerable Barrier + Inflammation

But What Do We Mean by ‘Barrier’?

Barrier Functions of Stratum Corneum
(Abnormal in Atopic Dermatitis)

- Permeability barrier
  - (also excludes noxious chemicals & allergens)
- Mechanical barrier
- Antimicrobial defense
- Integrity & cohesion (desquamation)
- Antioxidant defense
- Cytokine activation
- Ultraviolet light barrier
- Hydration (pliability)

Stratum Corneum

“Normal Basket Weave” = artifact of lipid extraction during tissue processing

Frozen section stained with hydrophobic dye

“Normal Basket Weave” = artifact of lipid extraction during processing

Stratum Corneum Structure

- Bricks and Mortar Analogy
- Bricks = anucleate corneocytes

Stratum Corneum Structure

- Bricks and Mortar Analogy
- Bricks = anucleate corneocytes
- Filled with keratin macrofibrils
- Osmotically-active small molecules derived from breakdown of filaggrin
**Stratum Corneum Structure**

- Bricks and Mortar Analogy
- Bricks = anucleate corneocytes
- Surrounded by a highly cross-linked protein shell, the cornified envelope

**Ceramide**

Cer (sphingol + fatty acid)

\[
\text{Ceramide} = \text{Cer} (\text{sphingol + fatty acid})
\]

\[
\text{C16-24 (epidermis C16-34)}
\]

**Permeability Barrier**

- Non-polar lipid bilayers fill intercellular domain
- Repeating arrays of lamellar sheets
- Lipids are very hydrophobic

**Requirements for a Competent Permeability Barrier**

- Correct 3 Lipids (cholesterol, free fatty acids, & ceramides)
- Sufficient amounts of lipid (10% of weight of SC)
- Correct Proportion (1:1:1 molar ratio)
- Lamellar structures in intercellular domains
Non-polar Products
Lipid Precursors
Phospholipids
Glucosylceramide
Cholesterol
Catabolic Enzymes
Antimicrobial Proteins

SG-SC INTERFACE

Lipid Precursors → Non-polar Products
Free Fatty Acids → pH
Sphingosine → Ceramides

LOWER SC

hBD2, LL-37
Yellow = Antimicrobial Activity

Permeability Barrier in Atopic Dermatitis
• Severity of barrier dysfunction parallels severity of disease

Molecular Genetics Shows That AD Is Initiated by a Defect in Barrier Function
Broader Implication Is That Barrier Function Is Clinically Relevant!

Genes Highly-Associated with Atopic Dermatitis Affect the Barrier
- Loss of Filaggrin, a Structural Protein of the Stratum Corneum (AD & Ichthyosis Vulgaris)
- Excessive Serine Protease Activity
  1) Reduced Expression of the Serine Protease Inhibitor, LEKTI (Netherton syndrome)
  2) Acquired LEKTI Deficiency in AD
  3) KLK 5 Activation of Th2 Cytokines

Ichthyosis vulgaris
- Autosomal dominant
- Mild to moderate scale
- Assoc. w/ Atopic dermatitis
  - >50%
- Common
  - 1/250 school kids w/ “dry skin” (pre-genotype era)
  - Actual incidence must be much higher (“uninvolved” skin of AD=IV)
Same Filaggrin Mutations Underlie Both AD and Ichthyosis Vulgaris (IV)

- Same loss-of-function mutations in FILAGGRIN in both IV and AD
- In large IV kindreds, if:
  - One allele affected → Most have (mild) IV and some have AD (later onset, mild disease)
  - Both alleles affected → All have IV and most have AD (early onset, severe)*

*Even some double-allele IV do not develop AD—why not?

How Does Loss of Filaggrin (an intracellular protein) Provoke a Barrier Abnormality?

- Structurally-defective corneocyte? (No)
- Decreased SC hydration-accentuates barrier abnormality (Yes)
- ↑pH (Yes)

Consequences of Filaggrin Deficiency

FLG mutations ↓
↓ Profilaggrin
↓ Filaggrin
↓ Corneocyte osmolytes
↓ Organic acids (Urocanic acid; Pyrrolidone carboxylic acid)
↓ Corneocyte hydration “Dry Skin”

Increased Water Loss
Contributes To Barrier Abnormality

Filaggrin Proteolytic Pathway: How Deficiency Contributes to AD Pathogenesis

Direct Evidence for Importance of pH in AD

Maintenance of an Acidic pH Prevents Development of AD!
(Hatano, et al, JID 2009)
Netherton Syndrome: 2nd Genetic Link to AD

- Atopic dermatitis
- ↑ IgE levels
- Anaphylactic reactions to food antigens
- Severe barrier abnormality
  → Fluid & Electrolyte Abnormalities
  → Growth Failure

PATHOGENESIS OF NETHERTON SYNDROME

- Mutations in $\text{SPINK5}$
- Encodes LEKTI 1
  - Serine protease inhibitor
  - Epidermis
    - Normally localizes to lamellar bodies & SC interstices

Netherton Syndrome: Pathophysiology

- Gene defect leads to unopposed serine protease activity
- How is this relevant for atopic dermatitis?
  Increased serine protease activity also in AD
  (Due to ↑ pH)
Increased Serine Protease Activity in AD

Atopic Dermatitis

Normal Epidermis

Relationship of Ichthyosis Vulgaris and Netherton Syndrome to Atopic Dermatitis

Serine protease activity

pH-Dependence of Serine Protease Activation

SP Bind To and Activate PAR2, a G-Protein-Coupled Plasma Membrane Receptor

Serine Proteases Bind To PAR2, Which Is Expressed in Outer Nucleated Epidermis

‘Superbase’-Induced Increase in Primary Cytokine Production Is Reversible by SPI
**Basis for Lipid Abnormality in Atopic Dermatitis**

- Lipids are in wrong place (↓Total Lipids)
  - ↓ lamellar body secretion → entombed in corneocytes
- Further ↓ in ceramide content (3 reasons)
  - ↑Th2 cytokines → ↓ceramide synthesis
  - ↑pH → ↓ activity of Cer-generating hydrolases
  - ↑pH → SP-mediated degradation of Cer-generating hydrolases

**Abnormal Lipids in AD Lead To**

↓ Lamellar Membranes

Normal (Intact) Membranes Restrict Allergen Penetration

Decreased & Fragmented Membranes in AD Allow Allergen Ingress

**Generation of Ceramides: Role of pH**

<table>
<thead>
<tr>
<th>SG/SC</th>
<th>Lower SC</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Diagram" /></td>
<td><img src="image" alt="Diagram" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>pH</th>
<th>7.3</th>
<th>5.0</th>
</tr>
</thead>
</table>

**Consequences of ↑pH in Atopic Dermatitis**

- Both Acquired Stressors & FLG Deficiency
  - ↑pH
  - ↑ Serine Protease Activity
  - ↓ Corneodesmosomes
  - ↓ SC Cohesion
  - ↓ Permeability Barrier
  - Ceramides
  - Acid Sphingomyelinase
  - β-Glucocerebrosidase

**The ‘Outside-to-Inside-(Back) to-Outside’ View**

**OUTSIDE**

H₂O → H₂O → Antigen → Stratum corneum → H₂O → H₂O

**INSIDE**

TH2 cytokines: IL 4, 5, 13 → Histamine → IgE production

Histamine
Distinctive Lipid Abnormality in AD

- Total quantities of SC lipid are reduced
- Further ↓ in ceramide content

Provides the rationale for therapy with ceramide-dominant mixture of the 3 key physiologic lipids

Conflict of Interest Statement

Barrier Repair Therapy Is Subject of a UC Patent (Dr. Elias is an inventor)

Licensed to Promius Pharma in US

Basis for Lipid Abnormalities in Atopic Dermatitis

Decreased extracellular lipids:
- Lipids are in wrong place due to SP → PAR2
- entombed in corneocytes
- ↓ delivery to extracellular domains
- ↓ Ceramide content
  - ↑ Th2 cytokines → ↓ ceramide synthesis (Oita group)
  - ↑ pH → ↓ activity of Cer-generating acid hydrolases
  - Sustained ↑ pH → proteolytic degradation of Cer-generating hydrolases

Sustained ↑ pH Eventually Destroys Ceramide-Generating Enzymes

De-Activation and Degradation of Lipid Processing Enzymes Results in ↓ & Immature Lamellar Bilayers
Atopic Dermatitis: New ‘Outside-Inside’ Paradigm

Inherited + Acquired

Acquired Triggers: ↑pH soaps, ↓ambient humidity, ↑stress

Vulnerable Barrier

Inherited Defects Alone May Produce Only IV: Acquired Insults, Which Further Degrade Barrier, May Also Be Required

↓ FLG  ↓ PCA  ↑ pH  →  ↑ SP
↓ Barrier

↑LB secretion
PAR2 \rightarrow Th_1 \rightarrow 2
Inflammation
↓ FLG
↓ PCA
↑ pH
↑ SP
\rightarrow Barrier

IL-4, 13

↓ FLG
↓ PCA
↑ pH
↑ SP
\rightarrow Barrier

IL-4, 13

↓ FLG
↓ PCA
↑ pH
↑ SP
\rightarrow Barrier

IL-4, 13

↓ FLG
↓ PCA
↑ pH
↑ SP
\rightarrow Barrier

IL-4, 13

↓ FLG
↓ PCA
↑ pH
↑ SP
\rightarrow Barrier

IL-4, 13

↓ FLG
↓ PCA
↑ pH
↑ SP
\rightarrow Barrier

IL-4, 13

Consequences for Barrier of Th2 Inflammation

- ↓ Filaggrin → ↑ pH (and defective corneocytes)
- ↓ Ceramides
- ↓ Desmoglein → ↓ SC Cohesion
- ↓ Antimicrobial peptides

Acquired Stressors

- ↓ Filaggrin → ↓ pH
- ↓ Ceramides
- ↓ Desmoglein → ↓ SC Cohesion
- ↓ Antimicrobial peptides
Current Therapy Is Directed at Inflammatory Infiltrate

Genetic/ Constitutional | Disease Trigger | Clinical Disease
Vulnerable Barrier → Defective Barrier → Inflammation

As AD Improves, Barrier Function Deteriorates

Genetic/ Constitutional | Disease Trigger | Clinical Disease
Vulnerable Barrier → Defective Barrier → ↓ Inflammation
Corticosteroid or Immunomodulator

Likely Explanation for Rebound Flares & Tachyphylaxis

Concerns about Immunomodulators

- Increased Infections, esp. Eczema Herpeticum
- Photocarcinogenicity
- Other Tumors

Topical Immunomodulators Carcinogenic?

Tacrolimus and pimecrolimus are immunosuppressants
Both show blood levels after topical administration, which can be as high as in organ transplant patients
Topical pimecrolimus and tacrolimus enter the lymphatic system (don’t need blood levels)
Lymphoma signal evident in mouse carcinogenicity studies

Tacrolimus Blood Levels Following Application of 0.1% Ointment

<table>
<thead>
<tr>
<th>Days</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max Observed C (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Adults (n=32)
- Peds (n=20)
Blood Levels after 0.03% Tacrolimus Ointment in Children

Max Observed C (ng/ml)

Days

New Awareness of Importance of Barrier Function

- New opportunities for dermatologic therapy: Treat inflammatory skin diseases by correcting (primary) abnormalities in epidermal structure and function

Cutaneous Tumors with Topical Pimecrolimus

(reported to FDA as of 2006)

<table>
<thead>
<tr>
<th>Tumor type</th>
<th># cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphomas</td>
<td>14</td>
</tr>
<tr>
<td>Squamous cell CA</td>
<td>5</td>
</tr>
<tr>
<td>Basal Cell CA</td>
<td>1</td>
</tr>
<tr>
<td>Paget disease (breast CA)</td>
<td>1</td>
</tr>
<tr>
<td>Melanoma, metastatic</td>
<td>1</td>
</tr>
<tr>
<td>Other tumors</td>
<td>8</td>
</tr>
</tbody>
</table>

Approaches to Treat AD

By Fixing the Barrier

- Educate (soaps, hydration, ↓stress)
- Hydrate (emollients→↓steroid usage)
- ↓Staph carriage
- Break Itch-Scratch cycle (antihistamines-also good for the barrier)
- Topical barrier repair (optimal ratio of Cer, FFA, Chol)
- Lower SC pH
- Serine protease inhibitors
- PAR2 inhibitors

Barrier Repair Therapy

- Standard emollients
  - Aquaphor®, Eucerin®, etc.
- Non-physiologic lipids
  - Petroleum based, lanolin, etc.
- Remain on skin surface
  - Temporary ↓TEWL
  - Not incorporated into SC lamellar membranes
  - Do not correct underlying barrier defect
Corrective Barrier Repair Therapy

- Physiologic lipid-based formulations
  - Ceramides, FFA, cholesterol
    - Incorporated into lamellar bodies → secreted
    - Repair barrier ONLY if present in correct molar ratios
    - e.g., ceramides alone will make barrier worse!

Physiologic Lipids Traverse the SC & Enter the Nucleated Layers

Anti-inflammatory Mechanisms of Barrier-Corrective Therapy in AD

- Normalizing Barrier → ↓ Cytokine Cascade
- Prevents Allergen/Hapten Ingress
- ↑ Permeability Barrier → ↑ Antimicrobial Defense
- Certain Free Fatty Acids Are Anti-inflammatory
- Normalizing pH → ↓ Serine Protease Activity
  (↓ Th2 inflammation; ↓ IL-1 activation; ↓ PAR2-mediated pruritus)

Therapy That Corrects the Barrier Abnormality Is Anti-Inflammatory

By Which Mechanisms?

‘OUTSIDE-INSIDE’ PATHOGENESIS OF AD:
Barrier Abnormality Stimulates a Cytokine Cascade

Barrier Repair Therapy

- Physiologic lipid-based formulations
  - Ceramides, FFA, cholesterol
    - Incorporated into SC lamellar membranes
    - Repair barrier ONLY if present in correct molar ratios
  - CAVEATS
    - “ceramides”, “barrier repair” functioning as “Buzz words”
Barrier Repair Therapy

- Physiologic lipid-based formulations
  - Ceramides, FFA, cholesterol
  - Incorporated into SC lamellar membranes
  - Repair barrier ONLY if present in correct molar ratios

- CAVEATS
  - Many “barrier repair” formulations on market
  - Often little data to support claim
    - “ceramides”, “barrier repair” functioning as “buzz words”

EpiCeram emulsion®

- Physiologic lipid-based formulations
  - “Optimal” molar ratio of 3 key lipids
  - Developed at UCSF (Elias & Feingold labs)

- EpiCeram® emulsion
  - High content of physiologic lipids (5.1%)
  - Ceramide-dominant
  - ↓ pH
  - Slow-release delivery system (nanospheres)
  - Certain lipids (PPAR activators) add potency & prevent side effects of GC

Efficacy of EpiCeram in Comparison To Mid-Strength Steroid – Moderate-to-Severe Childhood AD

- Comparable ↓ SCORAD scores
- Comparable ↓ Reduction of Itch
- Comparable Improvement in Sleep Habits
- Comparable % Patients “Clear or Almost Clear” by Physicians’ Global Assessment
- Comparable % Patients with >75% Reduction in SCORAD scores

Sugarman & Parish, J. Drugs Dermatol 2009
EPIC study

Open Label, 37 Centers
Over 250 patients enrolled to date
Ages 2 months to 86 years

Bottom Line:
BARRIER FUNCTION IS CLINICALLY RELEVANT!

Effects of Anti-Inflammatories Differ in Diseased vs. Normal Skin
- Diseased skin: initially improve barrier function by decreasing inflammation
- Treated skin: as inflammation resolves, negative effects on barrier function become evident

Over-Arching Theme: (Re)Capturing Respect for Dermatology
- End Domination of Immuno-Centric Ideation
- “Basta” (= enough already) to ‘Hand-Me-Down’ Therapies from Other Medical Specialties
- Identify Organ (Skin)-Specific Therapies
- Reorganize & Reshape Cosmeceuticals as Pharmaceuticals (and validate accordingly)

Possible End-Result: Regain our self-respect & recapture portions of specialty lost to other medical and surgical subspecialties

Relationship of Ichthyosis Vulgaris and Netherton Syndrome to Atopic Dermatitis

The “Atopic March”

Barnetson & Rogers, BMJ 2002, 324:1376-9
Can Barrier Repair Strategies Prevent Progression of the Atopic March?
EpiCeram Usage In the Treatment of Atopic Dermatitis (AD)

Mild-to-Moderate

- EpiCeram BID (Affected Area) +/- Steroid or TIM
- Remission in 2 wks
- EpiCeram QD to Affected Areas and Emollient to Unaffected Areas

Moderate-to-Severe

- EpiCeram BID (Affected Area) + Steroid / TIM
- Remission in 2 wks
- EpiCeram QD to Affected Areas +/- Emollient to Unaffected Areas

Additional Suggestions

- EpiCeram should be applied prior to a steroid or TIM
- Suggested use is EpiCeram + steroid/TIM in the mornings (after bathing) & evenings
- Emollient should not overlap areas of EpiCeram application

---

Posttransplant Cutaneous T-Cell Lymphoma

Case Reports and Review of the Association of Calcineurin Inhibitor Use With Posttransplant Lymphoproliferative Disease Risk

Rebecca L. Parmet, MD, Lauren J. Campbell, MD, Eyeran M. Jalko, MD, Patricia L. Lataro, MD, T. Golak, MD

Dept of Dermatology, University of Pittsburgh School of Medicine, Pittsburgh, PA

<table>
<thead>
<tr>
<th>Condition</th>
<th>B-Cell Lymphoma</th>
<th>T-Cell Lymphoma</th>
<th>PT-CTL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Association with PT-CTL</td>
<td>80%</td>
<td>50%</td>
<td>30%</td>
</tr>
<tr>
<td>DEXA</td>
<td>Off</td>
<td>On</td>
<td>Off</td>
</tr>
<tr>
<td>Association with Epstein Barr virus</td>
<td>60%</td>
<td>30%</td>
<td>10%</td>
</tr>
<tr>
<td>Time of development after transplantation</td>
<td>&lt;1</td>
<td>&gt;1</td>
<td>&gt;1</td>
</tr>
<tr>
<td>Response to reduced immunosuppression</td>
<td>Poor</td>
<td>Good</td>
<td>Good</td>
</tr>
</tbody>
</table>

Abbreviation: PT-CTL, posttransplant cutaneous T-cell lymphoma

Arch Dermatol 146: May 2010

---

Department of Veterans Affairs Medical Center
San Francisco, California
Thank you for your attention!