Cutaneous Immunopathology

Paul K. Shitabata, M.D.
Dermatopathologist
APMG
Dermatitis Herpetiformis

- Flesh-colored-to-erythematous vesicles appear in a herpetiform pattern
  - Symmetrically distributed over extensor surfaces including elbows, knees, buttocks, shoulders, and the posterior (nuchal) scalp
  - Erythematous papules and urticarialike plaques occur less frequently, bullae rare
  - Erosions and crusts
  - Burning, stinging, and intense pruritus, often precede new lesions
  - Oral mucosa lesions rare
  - Palms and soles usually spared
Dermatitis Herpetiformis and Sprue

- Majority have some degree of gluten sensitive enteropathy although usually asymptomatic
  - Fewer than 10% of patients have bloating, diarrhea, or symptomatic malabsorption
  - Mild steatorrhea or other signs of mild malabsorption in 20-30%
  - Patients with DH and no apparent gastrointestinal disease can be induced by increased gluten intake
- Gluten-free diet results in normalization of mucosal and skin lesions
  - Resumption of a gluten-containing diet results in recurrence of skin lesions
- Serum tests of IgA endomysial Ab
  - 80% of DH and all of atypical DH
  - Gluten free diet leads to decreased levels
Dermatitis Herpetiformis Histopathology

- Biopsy from normal skin about 3 mm. from the lesion
- Neutrophils may degrade IgA
- DIF necessary, rule out Linear IgA disease and subepidermal bullous dermatoses
Clues in a monkey’s gut!

- Anti-endomysial Ab bind to reticular structures in smooth muscle in primate esophagus
  - 99% specific for gluten sensitive enteropathy
  - Occur in >80% of DH cases
    - >95% of DH cases with villous atrophy
  - Titers not affected by dapsone but decreased with gluten free diet
  - If gluten reintroduced, skin lesions precede AEmA and AEmA reappears before villous atrophy
# Location of Biopsy

<table>
<thead>
<tr>
<th>Skin blister</th>
<th>3 mm biopsy with both the edge of a fresh lesion and some adjacent normal skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucosa</td>
<td>Perilesional with normal intact mucosa</td>
</tr>
<tr>
<td>Screen</td>
<td>Edge of fresh skin and include scale, if possible</td>
</tr>
</tbody>
</table>
**Specific Diseases**

<table>
<thead>
<tr>
<th>Condition</th>
<th>1st biopsy</th>
<th>2nd biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pemphigus or pemphigoid, skin</td>
<td>edge of lesion</td>
<td>3 mm from lesion</td>
</tr>
<tr>
<td>Pemphigus or pemphigoid, oral</td>
<td>3 mm from lesion</td>
<td>at edge</td>
</tr>
<tr>
<td>Purpura/vasculitis</td>
<td>10 mm from lesion</td>
<td></td>
</tr>
<tr>
<td>Stasis</td>
<td>Edge of lesion</td>
<td></td>
</tr>
</tbody>
</table>
## Specific Diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Biopsy Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermatitis herpetiformis</td>
<td>Biopsy normal skin 3mm from lesion</td>
</tr>
<tr>
<td>Porphyria/ Pseudoporphyria</td>
<td>Biopsy from edge of a fresh lesion with edge of normal skin</td>
</tr>
</tbody>
</table>
Disorders Excluded With Negative IF

- IgA pemphigus
- Pemphigus
- Bullous pemphigoid
- DLE
- SLE
- IgA vasculitis/Henoch-Schonlein Purpura
Disorders with Negative/Nonspecific IF

- Subcorneal pustulosis
- Hailey-Hailey disease
- Bullous impetigo
- Grover’s disease
- Acantholytic PR
- Bullous insect bite
- Bullous drug eruption
- Lichen planopilaris
- Drug induced lichenoid photodermatitis
- Non-IgA associated vasculitis
Basement Membrane Components

- Bullous pemphigoid antigens (BP 220/BP180)
- Epiligrin (Laminin 5)
- Uncein
- Ladinin (LAD-1)
- EBA antigen (Noncollagenous domain of type VII collagen)
The dermal-epidermal basement membrane

Intermediate filaments: keratins 4, 15

Hemidesmosome: 200 kD protein, BP180, α6β4 integrin, BP230, HD1

Plasma Membrane

Lamina Lucida: laminin-1, nidogen

Lamina Densa: HSPG, type IV collagen

Anchoring filaments: laminin-5, laminin-6, LAD-1, uncein, LH39 antigen

Anchoring fibrils: type VII collagen

Anchoring plaques: type IV collagen
## Location of Components

<table>
<thead>
<tr>
<th>Location</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma membrane</td>
<td>BP antigen</td>
</tr>
<tr>
<td>Lamina lucida</td>
<td>Laminin</td>
</tr>
<tr>
<td>Lamina densa</td>
<td>Type IV collagen</td>
</tr>
<tr>
<td></td>
<td>EBA antigen</td>
</tr>
<tr>
<td></td>
<td>Heparin sulfate</td>
</tr>
<tr>
<td>Component</td>
<td>Disease</td>
</tr>
<tr>
<td>-----------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Epiligrin</td>
<td>Anti-epiligrin cicatricial pemphigoid</td>
</tr>
<tr>
<td></td>
<td>Some junctional EB</td>
</tr>
<tr>
<td>Uncein</td>
<td>Overlap syndrome with features of CP and EBA</td>
</tr>
<tr>
<td>Ladinin (LAD1)</td>
<td>Chronic bullous disease of childhood</td>
</tr>
<tr>
<td></td>
<td>Linear IgA disease</td>
</tr>
<tr>
<td>EBA antigen</td>
<td>EBA</td>
</tr>
</tbody>
</table>
Technical Considerations

- Storage of slides at room temperature <11 months
  - Storage with antifading agent-2 years
- Biopsy should be placed in Michel’s or Zeus solution and kept from light
Bullous Pemphigoid

- Tense bullae on erythematous base
- Negative Nikolsky
- Subepidermal bullous dermatosis with eosinophils
- DDX: Herpes gestationis, Bullous LE, Cicatricial pemphigoid
Bullous Pemphigoid Antigen

- **BPAg1 (220kd)**
  - Intracellular associated with hemidesmosomes
  - Homology with desmoplakin
  - 70% of BP pts have circulating Ab to this

- **BPAg2 (180kd)**
  - Intra and extracellular with collagen-like domains
  - Also called collagen XVII
  - Extramembranous portion is antigenic epitope site for BP and HG
Cicatricial Pemphigoid

- Brunstig-Perry variant
  - Scarring blisters on head and neck
  - Mucosa rare
- Antiepiligrin variant
  - Associated with malignancy including endometrial, lung, and stomach
  - May be paraneoplastic blistering disease
Cicatricial Pemphigoid-
Histopath/IF

- Subepithelial blister with mixed inflammatory cells
- Perilesional epithelium shows linear IgG and complement
  - Technically difficult
  - 70% have circulating Ab to BMZ material
Pemphigus Vulgaris

- Mucous membranes, usually oral cavity with erosions
- Flaccid and fragile skin blister filled with clear fluid that arises on normal skin or erythematous base
- Vegetating PV frequently in intertriginous areas and scalp or face
- Nikolsky sign
  - Firm sliding pressure with a finger separates normal-appearing epidermis, producing an erosion
- Asboe-Hansen sign
  - Lateral pressure on the edge of a blister may spread the blister into clinically unaffected skin
Pemphigus Vulgaris Histopathology

- Intradermal blister with suprabasal clefting and acantholysis
- May have preceding eosinophilic spongiosis
- DIF intercellular IgG
  - IgG1 and IgG4 subclasses
  - C3 and IgM less frequent
- IIF circulating IgG autoantibodies that bind to epidermis
  - 80-90% of patients
  - Titer of antibody correlates with disease course
Linear IgA Disease

- Vesiculobullous eruption on trunk, inner thigh, and pelvic region
  - Not symmetrical unlike DH
  - No association with gluten sensitivity
  - May involve mucosa with scarring
  - Bullae may be discrete or arranged in a herpetiform pattern (Cluster of jewels sign)
  - Lesions may be seen at the edge of annular or polycyclic lesions (String of beads sign)
Linear IgA Disease

- Childhood lesions (Chronic bullous disease of childhood)
  - Localized to the lower abdomen and anogenital areas with frequent involvement of the perineum
  - Other sites of involvement include the feet, the hands, and the face, particularly the perioral area
- Adults
  - Trunk and the limbs are most commonly affected
  - Perineum and the perioral area is less frequent
- Both
  - Distribution may be symmetric or asymmetric
  - Dermatitis herpetiformis-like involvement of the extensor surfaces of the knees and the elbows infrequently
  - Oral manifestations vesicles, ulcerations, erythematous patches, erosions, desquamative gingivitis, or erosive cheilitis
  - Ocular symptoms, such as grittiness, burning, or discharge
Linear IgA Disease - Clinical

- Drug related
  - Vancomycin
  - Penicillin
  - Lithium
  - Dilantin
  - Diclofenac
- Lesions clear after cessation
- Rechallenge may have more severe changes
Linear IgA Disease-Histology and IF

- Neutrophil rich interface dermatitis
- Homogenous sharp linear band for IgA
- Linear granular variant
  - No deposits within the dermal papillae, resembles DH
  - Low level of HLAB8 and gluten sensitivity unlike DH
Lupus Band Test

- **SLE**
  - Best specificity is to take biopsy of normal skin of sun-exposed forearm (Positive in 67%)
  - Normal unexposed skin will be positive only in severe cases (35-40%)

- **DLE**
  - Biopsy of untreated skin lesion in exposed area that has been present for at least 3 months
Lupus Band Test-Baseline

- Deposition of Ig at the DEJ in lesional and nonlesional skin
  - IgM most frequent deposit
  - IgA least frequent
- Granular pattern most frequent
  - Sharp linear band not accepted
## Baseline

| Sun exposed skin | IgM continuous distribution over at least 50% width of biopsy with moderate intensity  
|                 | 25% of normal skin show weak interrupted linear granular IgM/C1q |
| Non-sun exposed skin | Interrupted IgM of moderate intensity  
|                   | If IgA present, high specificity |
## Sensitivity and Specificity

<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
</tr>
</thead>
</table>
| SLE       | 70-80% of patients with SLE in sun-exposed skin  
Non sun-exposed non lesional skin, only positive in SLE pts with severe extracutaneous disease and positive for DS DNA  
Positive in 90% interface dermatitis |
| DLE       | Negative in non-scarring cases  
Positive in 90% interface dermatitis |
Lupus Band Test and Prognosis

- 70% of patients with active nephritis with LBT on normal skin
- C1q deposits - higher incidence of renal disease
Dermatomyositis

- Heliotrope rash and Gottron papules
- Malar erythema, poikiloderma in a photosensitive distribution, violaceous erythema on the extensor surfaces, and periungual and cuticular changes
- Nail fold changes-periungual telangiectases
- Poikiloderma may occur on exposed skin or the upper part of the back (Shawl sign)
- Photodistributed and photoexacerbated, except for heliotrope rash
- Facial erythema rarely
Scalp involvement in DM is relatively common (coup d’šabre)

Calcinosis of the skin or the muscle
- Unusual in adults
- 40% of children or adolescents
- Calcinsosis cutis manifests as firm yellow nodules
Dermatomyositis

- **Muscle findings include weakness and, sometimes, tenderness.**
  - Muscle disease manifests as a proximal symmetrical muscle weakness.
  - Distal strength is almost always maintained
  - Muscle tenderness variable finding

- **Other systemic features**
  - Joint swelling occurs
  - Small joints of the hands are the most frequently involved
  - Non-deforming arthritis
  - Pulmonary disease with abnormal breath sounds.
  - Patients with an associated malignancy may have physical findings relevant to the affected organs
Dermatomyositis

- Muscle enzyme levels abnormal
  - CK, AST, LDH
  - Myositis-specific antibodies (antisignal recognition protein and anti-Ku)
- ANA positive
  - Anti-Mi-1 is highly specific for DM, but it lacks sensitivity because only 25%
  - Anti-Jo-1 is associated with pulmonary involvement, more common in patients with PM than DM
Dermatomyositis Histopathology

- Cell poor interface dermatitis with dermal mucinosis
- May be identical to DLE, SCLE, SLE
- DIF with Variable Lupus band
- Utilize C5b-9
C5b-9 and Disease

- Terminal complement, membrane attack complex (MAC)
- Formation of membrane pores allow circulating Ab access to nucleus and cytoplasm
- Represent activation of complement pathway within the BMZ
C5b binds C6 and C7
C5b,6,7, complexes bind to membrane via C7
C6 binds to the complex and inserts into the cell membrane
C9 molecules bind to the complex and polymerize
10–16 molecules of C9 bind to form a pore in the membrane

lipid bilayer
Pathogen

C5b,6,7 complex

C9

C8

C7

C5b

C6

C9

water and Naions

K and Cl ions
## MAC Diseases and Patterns

<table>
<thead>
<tr>
<th>Disease</th>
<th>Pattern Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>Intense granular DEJ 80%</td>
</tr>
<tr>
<td>SCLE</td>
<td>Granular DEJ 60%</td>
</tr>
<tr>
<td></td>
<td>Granular nuclear/cytoplasmic epidermal</td>
</tr>
<tr>
<td>DLE</td>
<td>DEJ 60%</td>
</tr>
<tr>
<td>MCTD</td>
<td>Granular nuclear/cytoplasmic epidermal 100%</td>
</tr>
<tr>
<td></td>
<td>DEJ 100%</td>
</tr>
<tr>
<td>Dermatomyositis</td>
<td>DEJ 90%</td>
</tr>
<tr>
<td></td>
<td>Endothelial cells</td>
</tr>
<tr>
<td></td>
<td>Dermal papillary capillaries</td>
</tr>
</tbody>
</table>
Overlap

- Anti-Ro associated SLE, Dermatomyositis, and MCTD
  - Endothelial decoration for C5b-9
  - Endothelial cell necrosis and denudement
  - Reduction in vascular plexus
  - Granular and cytoplasmic decoration within keratinocytes for C5b-9
  - Differentiate by LBT and clinical

- Non-lesional skin
  - Usually negative or very weak
Epidermolysis Bullosa Acquisita

- Noninflammatory or mildly inflammatory form
  - Most common
  - Tense vesicles and bullae, and erosions primarily on the extensor surfaces of hands, knuckles, elbows, knees, and ankles
  - Blisters may be hemorrhagic
  - Blisters on mucus membranes rupture easily
  - Usually heals with significant scar and milia formation
  - Nail dystrophy and scarring alopecia rarely
  - Resembles porphyria cutanea tarda in elderly patients,
  - Resembles the dominantly inherited form of epidermolysis bullosa dystrophica in children
EBA

- **Generalized inflammatory form**
  - Widespread, tense vesicles and bullae (some hemorrhagic)
  - Not localized to trauma-prone sites
  - Generalized erythema, urticarial plaques, and generalized pruritus may occur
  - Usually heals with minimal scarring and milia formation
  - Usually heals with minimal scarring and milia formation
  - Clinically resembles bullous pemphigoid or linear IgA bullous dermatosis
EBA

- Third variant of EBA predominantly involves mucus membranes
  - Affects mucous membranes of buccal, conjunctival, gingival, palatal, nasopharyngeal, rectal, genital, and esophageal mucosa
  - Resembles mucous membrane pemphigoid and can result in significant mucosal scarring and dysfunction
  - Recent international consensus statement (2002) reassigned this group of patients to the category of mucous membrane pemphigoid
EBA-Pathogenesis

- Autoimmune disease with IgG autoantibodies targeting non-collagenous domain of collagen VII in basement membrane
  - Initiating event unknown
- Subset of clinically milder EBA
  - IgA autoantibodies
  - IgG autoantibodies to the collagenous domain, rather than the NC1 domain of collagen VII
EBA Histopathology

- Subepidermal blister with mixed inflammation
- DIF linear thick band of IgG, and to a lesser extent C3 at basement membrane zone
  - Occasionally IgM or IgA
- IIF IgG circulating autoantibodies in the patient's serum that target the skin basement membrane component, type VII collagen.
  - Bind to the dermal floor (lower part) on salt-split normal human skin substrate
Salt-Split Skin Assay

- IIF-utilize patient’s serum
- Incubate normal skin with 1M NaCl
- Separates the epidermis from dermis
- Epidermal half
  - Upper lamina lucida and hemidesmosomes
  - BP antigen
- Dermal half
  - Laminin 5
  - Lamina densa, anchoring fibrils
<table>
<thead>
<tr>
<th>Split</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermal</td>
<td>Bullous pemphigoid</td>
</tr>
<tr>
<td>Dermal</td>
<td>EBA</td>
</tr>
<tr>
<td></td>
<td>Bullous lupus erythematosus</td>
</tr>
<tr>
<td></td>
<td>Anti-epiligrin cicatricial pemphigoid</td>
</tr>
<tr>
<td></td>
<td>Anti-p105 bullous pemphigoid</td>
</tr>
</tbody>
</table>
Porphyria

- Fragility of sun-exposed skin after trauma
  - Erosions and bullae on the dorsal aspects of the hands, the forearms, and the face
  - Healing of crusted erosions and blisters leaves scars, milia, and hyperpigmented and hypopigmented atrophic patches.

- Hypertrichosis
  - Temporal and malar facial areas
  - Arms, legs
  - Indurated, waxy, yellowish sclerodermal-like plaques-upper trunk
  - Melasmalike hyperpigmentation of the face
  - Erythematous suffusion or plethora of face and upper trunk
  - Severe cases with scarring alopecia and onocholysis and contractures
    - Urine sample may have a tea- or wine-colored tint
Porphyria

- Subepidermal bullae with minimal dermal inflammatory infiltrate festooning of dermal papillae
- Thickened upper dermal capillary walls and dermoeipidermal basement membrane zones
- Elastosis and sclerosis
- Trapped basement membrane zone (caterpillar bodies) in epidermal roof (Ab to Collagen IV/laminin)
- DIF with immunoglobulins and complement in and around the dermal capillaries and at the basement membrane zone
The trouble with facts is that there are so many of them.

Samuel McChord Crothers
The Gentle Reader