Think Immunodermatology Testing!

Clinical Immunodermatology Laboratory

Kristin M. Leiferman, M.D.
Professor of Dermatology

Co-Director
Immunodermatology Laboratory
University of Utah
History

Late 1800s
Paul Ehrlich put forth the concept of autoimmunity calling it
“horror autotoxicus”
History

Early 1940s
Albert Coons was the first to conceptualize and develop immunofluorescent techniques for labeling antibodies.
History

1945
Robin Coombs (and colleagues) described the **Coombs antiglobulin reaction test**, used to determine if antibodies or complement factors have bound to red blood cell surface antigens *in vivo* causing hemolytic anemia

- Waaler-Rose rheumatoid factor
- Hargraves’ LE cell
- Witebsky-Rose induction of thyroiditis with autologous thyroid gland
History

Mid 1960s
Ernest Beutner and Robert Jordon demonstrated IgG cell surface antibodies in pemphigus, autoantibodies in circulation and bound to the dermal-epidermal junction in bullous pemphigoid
Immunobullous Diseases
Immunobullous Diseases

- Desmogleins / Desmosomes
  - Pemphigus

- BP Ags in hemidesmosomes / lamina lucida
  - Pemphigoid
  - Linear IgA bullous dermatosis

- Type VII collagen / anchoring fibrils
  - Epidermolysis bullosa acquisita
Immunodermatology Tests are Diagnostic Aids in Many Diseases

- Dermatitis herpetiformis & celiac disease
- Drug reactions
- Eosinophil-associated disease
- Epidermolysis bullosa acquisita
- Lichen planus & lichenoid reactions
- Linear IgA bullous dermatosis
- Lupus erythematosus (all types including drug-induced)
- Mixed / undefined connective tissue disease
- Pemphigoid (all types)
- Pemphigus (all types, including paraneoplastic)
- Porphyria & pseudoporphoryria
- Urticaria
- Vasculitis (including Henoch-Schönlein purpura)
Immunodermatology Tests

• Tissue specimens
  – Skin
  – Mucous membranes
• Serum
  – Initial diagnosis
  – Following disease activity
• Tissue and serum together may be helpful in certain diseases
Immunodermatology Tests

• Tissue specimens
  – Direct immunofluorescence
    • IgG
    • IgM
    • IgA
    • C3
    • Fibrinogen
  – Indirect immunofluorescence
    • Epidermal transglutaminase (dermatitis herpetiformis)
    • Eosinophil granule proteins (eosinophil-associated diseases)

• Serum
  – Indirect immunofluorescence
  – Enzyme linked immunosorbent assays (ELISA)
Immunodermatology Tests

- **Tissue specimens**
  - Direct immunofluorescence

  - **IgG** cell surface antibody staining in pemphigus
  - **IgA** granular basement membrane zone antibody staining in dermatitis herpetiformis
  - IgG linear basement membrane zone antibody staining in pemphigoid
Immunodermatology Tests

• Serum
  – Indirect immunofluorescence for circulating antibodies
    • Epithelial cell surface (pemphigus)
    • Basement membrane zone (pemphigoid, epidermolysis bullosa acquisita, linear IgA disease)
    • Cell surface, basement membrane zone and rodent substrates (paraneoplastic pemphigus)
    • Endomysium (dermatitis herpetiformis)
Immunodermatology Tests

• Serum
  – Enzyme linked immunosorbent assays (ELISA)
    • IgG desmoglein 1 (pemphigus foliaceus) and IgG desmoglein 3 (pemphigus vulgaris)
    • IgG BP 180 and IgG BP 230 (bullous pemphigoid, mucous membrane pemphigoid, pemphigoid gestationis)
    • IgG collagen VII (epidermolysis bullosa acquisita)
    • IgG tissue transglutaminase and IgA tissue transglutaminase, IgA epidermal transglutaminase (dermatitis herpetiformis and celiac disease)
Immunodermatology Tests

- Serum
  - Indirect immunofluorescence

Serum IgG cell surface antibodies on monkey esophagus substrate in pemphigus

Serum IgG basement membrane zone antibodies on monkey esophagus substrate in pemphigoid

Serum IgA endomysial antibodies on monkey esophagus substrate in dermatitis herpetiformis
DISEASES

Immunodermatology Testing
Dermatitis Herpetiformis

Associations

- **Celiac Disease**, gluten sensitive enteropathy
  - HLA-DQ2, less commonly –DQ8, association
    - May be important in binding of transglutaminase modified gliadin on antigen presenting cells
  - Prevalence 11-58 per 100,000 population in northern Europe, highest in Ireland; celiac disease prevalence 1 per 250 in US; symptomatic/asymptomatic 1/6

- **Lymphoma**
Dermatitis Herpetiformis

Classical Presentation

- Pruritus is cardinal feature
- Lesions
  - Symmetric
  - Grouped or herpetiform
  - Primary lesion is papule or papulo-vesicle
Dermatitis Herpetiformis

Clinical Presentation

Because of intense pruritus, secondary lesions consist of excoriations with erosions and crusting, chronic eczematoid changes or lichenified, prurigo-like lesions.
Dermatitis Herpetiformis

Clinical Presentation

Ecchymoses may be seen.
Scarring does not occur.
Dermatitis Herpetiformis
Clinical Presentation

Lesional distribution
- Extensor surfaces of elbows, forearms, buttocks, and knees most common
- Back, posterior neck and scalp next most common
- Facial lesions, particularly periorbital, may be seen in patients on adequate suppressive therapy
- Palmar, plantar and oral involvement is rare
Dermatitis Herpetiformis

Location
Location
Location
Immunodermatology Tests
Dermatitis Herpetiformis

- Tissue specimen
- Serum
- Tissue and serum together most helpful
Dermatitis Herpetiformis

Associated Diseases

Celiac disease and lymphoma

- Insulin-dependent diabetes mellitus
- Autoimmune thyroid disease
- Lupus erythematosus
- Sjögren’s syndrome
- Arthritis
- Pernicious anemia
- Hypoadrenalism

- Sclerosing cholangitis
- Primary biliary cirrhosis
- Sarcoidosis
- Vitiligo
- Alopecia areata
- Micronutrient deficiency, anemia and osteoporosis
Bullous Pemphigoid

Characteristic Lesions and Classical Presentation

- Elderly patients
- Pruritus
- Tense bullae on urticarial and/or erythematous bases in flexural areas
Pemphigoid Variants

Dyshidrosiform

Localized
Pemphigoid Variants

- Inflammatory and desquamative gingivitis
- Erythrodermic
- Prurigo nodularis-like
- Noninflammatory
Pemphigoid Variants
Atypical nonbullous presentations

Eczematous lesions

Urticarial

Generalized pruritus (with excoriations), no primary lesions


It is important to include serological and direct immunofluorescence in the diagnostic algorithm of itch.
Pemphigoid *quo vadis*

- Current incidence 4-22 new cases per $10^6$
  - Incidence likely to continue to rise
  - After age 70, incidence significantly increases
  - Relative risk $>90$ years old is 300 fold higher than $<60$ years old
  - New pathogenic mechanisms, IgE
Pemphigoid Variant
Drug-Induced

- Implicated drug: furosemide
- Tense vesicles and bullae on dorsum of foot

- Penicillins
- Ciprofloxacin
- Furosemide
- Hydrochlorothiazide
- ACE inhibitors (captopril, enalapril)
- Chloroquine
- Sulfasalazine
- Phenacetin
- Nifedipine
- Terbinafine
- Spironolactone
Pemphigoid Variants

- Infants and children
- Vulva, in prepubertal children
- Localized, lower extremities
- Vegetating plaques, groin and axillae
- Herpetiform, vesicles
- Dyshidrosiform, palmar and plantar lesions resembling dyshidrotic eczema
- Pemphigoid nodularis, prurigo nodularis-like lesions on extremities
- Lichen planus pemphigoides
- Drug-induced
- Pemphigoid gestationis
Immunodermatology Tests

Pemphigoid

- Tissue specimen
- Serum
- Tissue and serum together most helpful
Mucous Membrane Pemphigoid

- Affects skin and/or mucous membranes
  - Tense bullae  –  Erosions
- Scarring sequelae including ocular conjunctivae
- Immunofluorescence
  - Linear IgG and C3 BMZ
  - Circulating IgG BMZ antibodies in only 20%, epidermal pattern
  - Targeted proteins, BP180 and laminin 332 (laminin V, epiligrren); anti-epiligrren cicatricial pemphigoid
Linear IgA Disease

- Tense bullae similar to pemphigoid
  - “String of pearls” or “Cluster of jewels” sign
Linear IgA Bullous Dermatosis

Immunopathology

Serum on human split skin substrate with anti IgA

Epidermal localization

Roof of separation:

IgA basement membrane zone antibodies

Direct immunofluorescence of perilesional tissue with anti IgA

Linear BMZ staining
Linear IgA Disease

- Oral involvement common along with skin in adult disease
- Neutrophils more common in infiltrate than in pemphigoid which shows predominance of eosinophils
- Antigenic target is LABD97, a portion of BP 180
- Drug induced variant
  - Vancomycin
  - Captopril, lithium, diclofenac, phenytoin, IL-2, somatostatin
Epidemiology and pathogenesis of bullous pemphigoid and mucous membrane pemphigoid

UpToDate

Strong association with underlying malignancy

bullous pemphigoid
linear IgA bullous dermatosis
anti-γ1 pemphigoid
epidermolysis bullosa acquisita
Epidermolysis Bullosa Acquisita

• Tense bullae
• Oral mucosal involvement common
• Occur in areas of trauma or friction
Epidermolysis Bullosa Acquisita

Immunopathology

• Linear IgG and C3 BMZ, rarely IgA, IgM
  – Similar to pemphigoid but may be thicker, u-serrated pattern

• Indirect immunofluorescence of serum has IgG localized to dermal side of split skin

• Antibodies directed to Type VII collagen (anchoring fibrils)
Epidermolysis Bullosa Acquisita Immunopathology

Serum on human split skin substrate with anti IgG

Dermal localization

Floor of separation:

Epidermolysis Bullosa Acquisita

In contrast to…. Bullous Pemphigoid

Roof of separation:

Epidermal localization

EBA
Immunodermatology

- Desmogleins / Desmosomes
  - Pemphigus

- BP Ags in hemidesmosomes / lamina lucida
  - Pemphigoid
  - Linear IgA disease

- Type VII collagen / anchoring fibrils
  - Epidermolysis bullosa acquisita
Pemphigus

- Organ specific autoimmune disorders
- Characterized by development of blisters and erosions on skin and mucous membranes
- Caused by detachment of epithelial cells (acantholysis)
- Severe morbidity and death, as a result of skin loss, oropharyngeal ulcerations and sepsis
Immunodermatology

- Pemphigus
  - Pemphigus vulgaris and vegetans
  - Pemphigus foliaceus and erythematous
  - Fogo selvagem
  - Paraneoplastic pemphigus
Pemphigus Vulgaris

- Severe oral ulcerations
- Inflammation and erosions of other mucosa
  - Ocular
  - Nasal
  - Genital
- 50% develop blisters and erosions of skin, often head and neck
- Without treatment, mortality approaches 100%, current mortality 5-25%
Pemphigus Vulgaris
Pathology and Immunopathology

- Acantholysis
- Dermal inflammation including eosinophils
- Epidermal cell surface IgG
- Serum cell surface and desmoglein 3 antibody titers correlate with disease activity
Pemphigus Foliaceus

- Superficial cutaneous erosions
- Pattern of seborrheic dermatitis
- Mucosal involvement rare
- Cell surface and desmoglein 1 antibodies correlate with disease
Pemphigus

Pemphigus foliaceus

- Graph showing DSG 1 and DSG 3 values over time.

Pemphigus vulgaris

- Graph showing DSG-3 and DSG-1 values over time.

Images of skin lesions for both conditions.
Epitope Migration in Pemphigus
Pemphigus

• In all forms, autoantibodies develop to desmosomal cadherins (DSG1 in PF and FS and DSG3 in PV) and/or other adhesion molecules

• Pemphigus antibodies produce acantholysis in vivo in neonatal mice and in vitro in skin explants and keratinocyte cultures

• Inducing factors unknown
  – Genetics
  – Environmental factors

Pemphigus
Environmental factors

- Drug-induced disease
  - Antibiotics
    - Penicillin/derivatives
    - Rifampin
    - Cephalexin
  - Thiols
    - Penicillamine
    - Captopril
    - Pyritinol
    - Thiopronine
  - Piroxicam
  - Thiamazole
  - Gold sodium thiomalate

- In PNP, hematologic malignancies initiate and drive autoimmunity against DSGs and desmoplakins
Paraneoplastic Pemphigus

- Severe inflammation, ulceration and scarring of mucosa
  - Oral
  - Ocular conjunctivae
  - Lung
  - Gastrointestinal tract
- Ulcerations of skin
Paraneoplastic Pemphigus

- May appear lichenoid, erythema multiforme-like
Paraneoplastic Pemphigus

- Both cell surface and BMZ antibodies
- Simple columnar/transitional epithelial antibodies on rodent substrate—bladder, heart and liver
- Associated hematologic malignancy
  - Non-Hodgkin’s lymphoma
  - Chronic lymphocytic leukemia
  - Castleman’s disease
  - Thymoma
  - Waldenstrom’s macroglobulinemia
  - Sarcomas
Paraneoplastic Pemphigus
Immunopathology

IgG epithelial cell surface and basement membrane zone IgG staining on biopsy

Epithelial cell surface IgG staining on rodent bladder in serum
Immunodermatology Tests are Diagnostic Aids in Many Diseases

- Dermatitis herpetiformis & celiac disease
- Drug reactions
- Eosinophil-associated disease
- Epidermolysis bullosa acquisita
- Lichen planus & lichenoid reactions
- Linear IgA bullous dermatosis
- Lupus erythematosus (all types including drug-induced)

- Mixed / undefined connective tissue disease
- Pemphigoid (all types)
- Pemphigus (all types, including paraneoplastic)
- Porphyria & pseudoporphyria
- Urticaria
- Vasculitis (including Henoch-Schönlein purpura)
Lichen Planus

Nails and scalp
Lupus Erythematosus

Granular immune deposits along the BMZ

IgM = C3 > IgG > IgA
Subacute Cutaneous Lupus Erythematosus

- Drug induced
- Neonatal LE
  - Congenital heart block
Vasculitis

IgA Vasculitis
(Henoch Schoenlein Purpura)
**Immunodermatology Tests are Helpful Diagnostically and in Monitoring Disease Activity**

<table>
<thead>
<tr>
<th>Blistering Skin and Mucous Membrane Diseases</th>
<th>Other Immune-Mediated Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Pemphigoid (all types)</td>
<td>• Lupus erythematosus (all types)</td>
</tr>
<tr>
<td>• Pemphigus (all types, including paraneoplastic)</td>
<td>• Mixed / undefined connective tissue disease</td>
</tr>
<tr>
<td>• Epidermolysis bullosa acquisita</td>
<td>• Lichen planus &amp; lichenoid reactions</td>
</tr>
<tr>
<td>• Linear IgA bullous dermatosis</td>
<td>• Vasculitis (including Henoch-Schönlein purpura)</td>
</tr>
<tr>
<td>• Dermatitis herpetiformis &amp; celiac disease</td>
<td>• Eosinophil-associated diseases</td>
</tr>
<tr>
<td>• Porphyria &amp; pseudoporphyria</td>
<td>• Urticaria</td>
</tr>
<tr>
<td></td>
<td>• Drug reactions</td>
</tr>
</tbody>
</table>

**Perilesional Tissue AND Serum**

**Lesional Tissue**
Immunodermatology Tests

- Biopsy site and transport fixative is critical
  - **Perilesional for blistering diseases** (do NOT biopsy ulcer or erosion, need intact tissue containing epidermis and dermis)
  - **Lesional, active border of new lesion for others**
    - Use Michel’s or Zeus’ transport medium, NOT formalin

- Serum studies along with biopsy or alone
  - Diagnosis (not as sensitive as biopsy but distinguishes subtypes and may be positive when biopsy is not)
  - Monitoring disease activity

http://medicine.utah.edu/dermatology/labservices/immunodermatology/
Immunodermatology Tests

- Postage paid kits available to submit specimens
- Biopsy site is important
  - Information is in kit or on web site
Immunodermatology Tests

Immunodermatology Laboratory
University of Utah

• Co-Directors
  John J. Zone, M.D.
  Kristin M. Leiferman, M.D.

• Web site
  http://medicine.utah.edu/dermatology/labservices/immunodermatology/

• ARUP Laboratories
  arupconsult.com/Topics/ImmunobullousSkinDz.html

• Toll free telephone number 1.866.266.5699

Marjorie Allen, Chief Technician, 11 years plus
Chase Myrick, Aubrey Curvin
**Immunobullous Skin Diseases Testing**

**INDICATIONS FOR TESTING**
Symptomatic adults

---

**Screen for Immunobullous Diseases** (pemphigus, pemphigoid, epidermolysis bullosa acquisita, linear IgA disease, or dermatitis herpetiformis)
- Histology
- Perilesional skin biopsy
- Cutaneous Direct Immunofluorescence, Biopsy
- Serology
  - Pemphigus Antibody Panel - Epithelial Cell Surface Antibodies and Desmoglein 1 and Desmoglein 3 Antibodies, IgG
  - Pemphigoid Antibody Panel - Epithelial Basement Membrane Zone Antibodies, IgG and IgA, BP-180 and BP-230 Antibodies, IgG
  - Tissue Transglutaminase (TG) Antibody, IgA with Reflex to Endomysial Antibody, IgA by IFA (alternative test: Celiac Disease Dual Antigen Screen with Reflex)
  - Epithelial Skin Antibody AND Tissue Transglutaminase (TG) Antibody, IgA with Reflex to Endomysial Antibody, IgA by IFA

---

**Presence of neoplastic disease**

**ORDER**
- Perilesional skin biopsy for Cutaneous Direct Immunofluorescence, Biopsy AND Paraneoplastic Pemphigus Antibody Screen

- Positive
  - Paraneoplastic Pemphigus

- Negative
  - Screen for immunobullous diseases

---

**Pregnant female, typically 2nd or 3rd trimester**

**ORDER**
- Perilesional skin biopsy for Cutaneous Direct Immunofluorescence, Biopsy AND Herpes Gestationis Factor (Complement-Fixing Basement Membrane Zone Antibody IgG) and IgG BP180 antibody level

---

**Monitor for developing immunobullous disease**
Consider porphyria or pseudoporphyria (perilesional skin biopsy is not specific but will not be consistent with immunobullous disease)
Repeat screening:
- In 3-6 months for persistent unexplained disease
- In 6-12 weeks for rapidly evolving disease

**IgA endomysial antibodies***
- IgA basement membrane zone epidermal, dermal or combined epidermal/dermal pattern antibodies
- Cutaneous DIF skin biopsy for granular and/or fibrilar IgA

**Negative**
- Repeat DIF skin biopsy

**Positive, titer >1:10**
- Monitor treatment response with Epithelial Basement Membrane Zone Antibody IgA OR Epithelial Skin Antibody

---

**Linear IgA disease**
- Monitor treatment response with Epithelial Basement Membrane Zone Antibody IgA and/or Epidermal Transglutaminase (etG/TG) Antibody, IgA by ELISA (or IgA deficient Tissue Transglutaminase Antibody, IgG)

---

**Epidermolysis bullosa acquisita**
- Monitor treatment response with Pemphigus Antibody Panel - Epithelial Basement Membrane Zone Antibodies, IgG and IgA, BP-180 and BP-230 Antibodies, IgG

---

**Desmoglein 1 and Desmoglein 3 Antibodies in Pemphigus, IgG**
(Note: Necessary if pemphigoid panel testing was not ordered)
- Monitor treatment response with Epithelial Basement Membrane Zone Antibody IgG AND Collagen Type VII Antibody IgG by ELISA

---

**P. foliaceus** (most common) OR **P. erythrophines**
- Monitor treatment response with Pemphigus Antibody Panel - Epithelial Cell Surface Antibodies and Desmoglein 1 and Desmoglein 3 Antibodies, IgG

---

**IgA Pemphigus**
- Monitor treatment response with Pemphigus Antibody IgA

---

**For Endomysial Antibodies, a screening IgA tissue transglutaminase assay (ELISA) can be ordered OR request Endomysial Antibody test by indirect immunofluorescence (IgA and/or IgG) with or without accompanying IgA and/or IgG tissue transglutaminase assay through the Immunodermatology Laboratory.**

*© 2006 ARUP Laboratories. All Rights Reserved Revised 06/05/2014*
1. Dermatitis Herpetiformis
   Most cases, along with pemphigoid and pemphigus panel tests for mucous membrane involvement, biopsy nonlesional mucosa See Immunobullous Skin Diseases Testing algorithm ...

2. Epidermolysis Bullosa Acquisita
   ...and pemphigus panel tests for skin involvement, biopsy perilesional skin for mucous membrane involvement, biopsy nonlesional mucosa See Immunobullous Skin Diseases Testing algorithm ...

3. Herpes Gestationis
   ...various immunobullous disorders in patients suspected or known to have any type of immunobullous disease See Immunobullous Skin Diseases Testing algorithm ...

4. Immunobullous Skin Diseases Screening
   ...Immunobullous Skin Diseases Screening Dx Background Lab Tests Algorithm Diagnosis Indications for Testing Blistering and other inflammatory disease without obvious etiology See Immunobullous Skin Diseases Testing algorithm Laboratory Testing Initial testing Perilesional skin biopsy for direct immunofluorescence (DIF) plus appropriate serum ...

5. Linear IgA Disease
   ...and pemphigoid panel tests for skin involvement, biopsy perilesional skin for mucous membrane involvement, biopsy nonlesional mucosa See Immunobullous Skin Diseases Testing algorithm ...

6. Paraneoplastic Pemphigus
   ...perform and/or correlate with perilesional skin biopsy; consider evaluation for other immunobullous disease See Immunobullous Skin Diseases Testing Algorithm Perilesional skin biopsy for cutaneous direct immunofluorescence submitted in Michel's (or Zeus) medium ...

7. Pemphigoid
   ...with serum pemphigoid panel for skin involvement, biopsy perilesional skin for mucous membrane involvement, biopsy nonlesional mucosa See Immunobullous Skin Diseases Testing algorithm ...

8. Pemphigus
   ...with serum pemphigoid panel for skin involvement, biopsy perilesional skin for mucous membrane involvement, biopsy nonlesional mucosa See Immunobullous Skin Diseases Testing algorithm ...
Clinical testing algorithm for possible bullous pemphigoid or mucous membrane pemphigoid

Tissue biopsy specimens

- Lesional specimen, formalin fixed, H&E
- Perilesional specimen, transport medium, DIF

Compatible histology

Positive various patterns

- Linear IgA along BMZ
- Granular IgM and C3 and granular or linear IgG and IgA
- Shaggy fibrinogen along BMZ +/- cytid bodies
- Linear IgG +/- linear C3 +/- weak linear IgA along BMZ

Negative consider another perilesional biopsy for DIF

Serum specimen

- IIF with monkey esophagus substrate
- IIF with split human skin substrate
- IgG BP180 ELISA
- IgG BP230 ELISA

Positive various patterns

- IgG BMZ positive
- Follow disease activity with IgG BP180 antibody level

No immunobullous disease or undefined immunobullous disease

Abbreviations:
- BMZ: basement membrane zone
- DIF: direct immunofluorescence
- ELISA: enzyme linked immunosorbent assay
- H&E: hematoxylin and eosin histology stain
- IIF: indirect immunofluorescence

Specimen tests
- Positive
- Negative
Pemphigoid BMZ Antibodies
Direct immunofluorescence
Skin biopsy

BP230 = BPAG1
BP180 = BPAG2
Desmoglein 1 & 3
IgA & IgG tissue transglutaminase
ELISA

IgA Endomysial Antibodies in Dermatitis Herpetiformis

Immunodermatology Testing

LUPUS

DERMATITIS HERPETIFORMIS

Pemphigus Cell Surface Antibodies

Immunodermatology

Pemphigoid BMZ Antibodies
Indirect immunofluorescence Serum