

Clinical Immunodermatology **Immunodermatology Testing!** **Laboratory**

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History

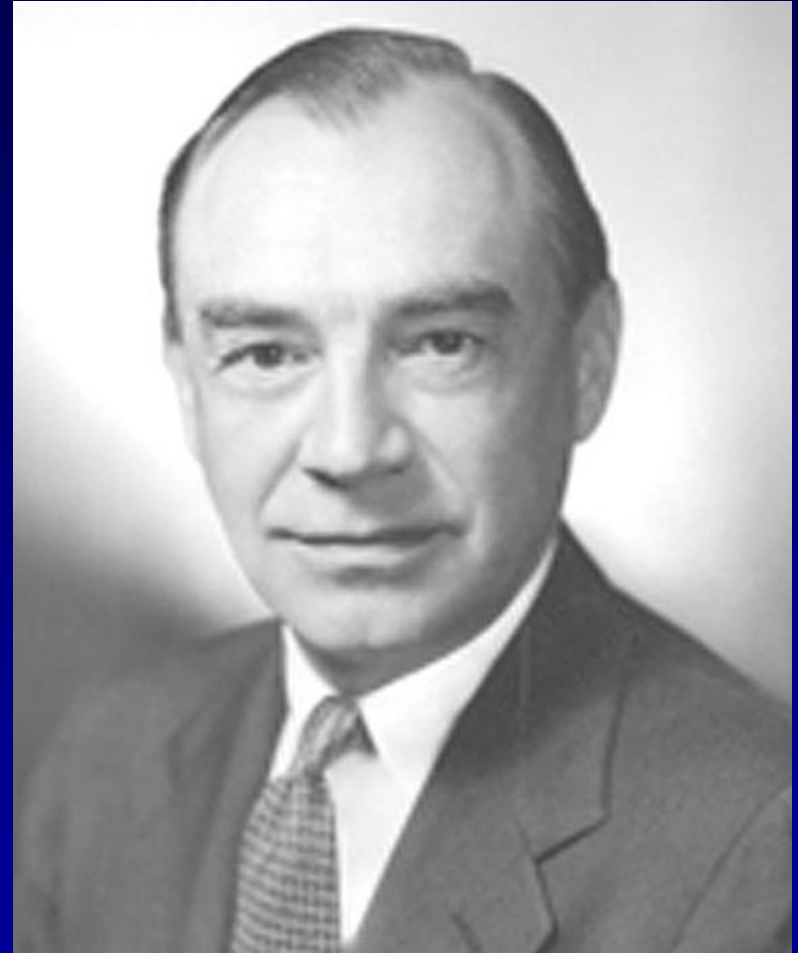
Late 1800s
Paul Ehrlich put
forth the concept
of **autoimmunity**
calling it
**“horror
autotoxicus”**



History

Early 1940s

Albert Coons was
the first to
conceptualize and
develop
immunofluorescent
techniques for
labeling antibodies



History

1945

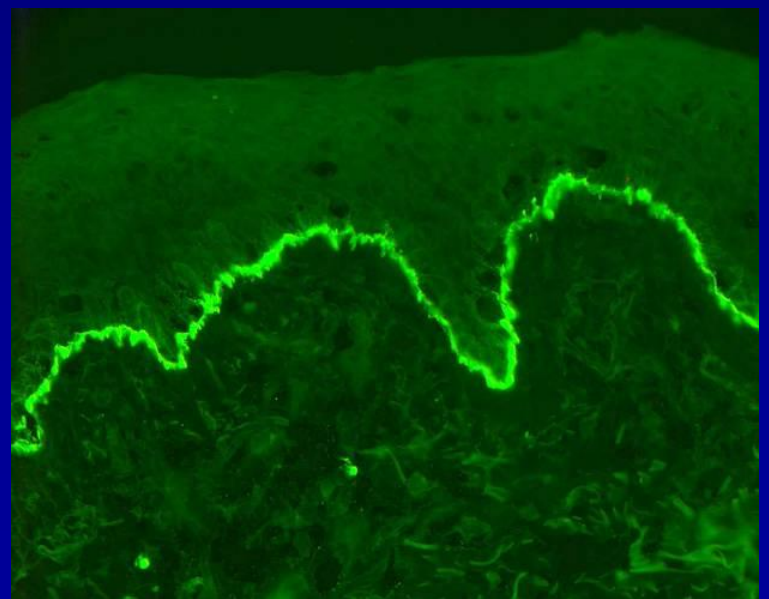
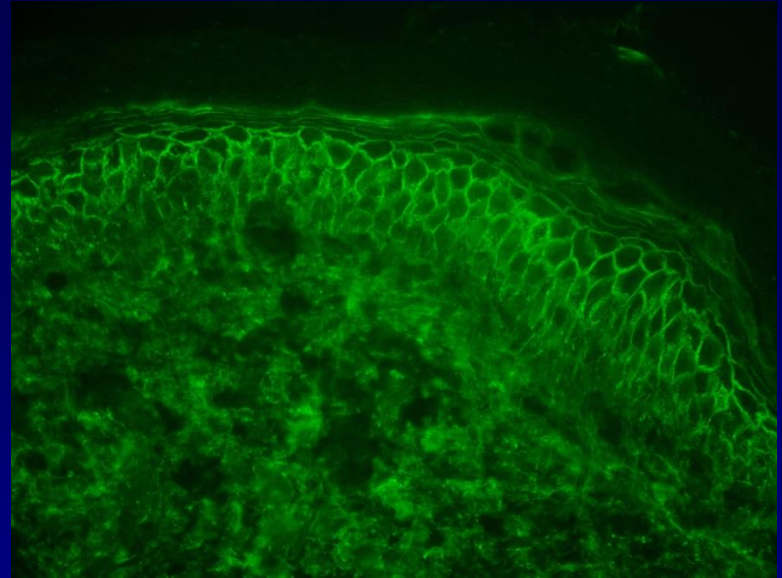
Robin Coombs (and colleagues) described the **Coombs antiglobulin reaction test**, used to determine if antibodies or complement factors have bound to red blood cell surface antigens *in vivo* causing hemolytic anemia

- **Waalder-Rose rheumatoid factor**
- **Hargraves' LE cell**
- **Witebsky-Rose induction of thyroiditis with autologous thyroid gland**

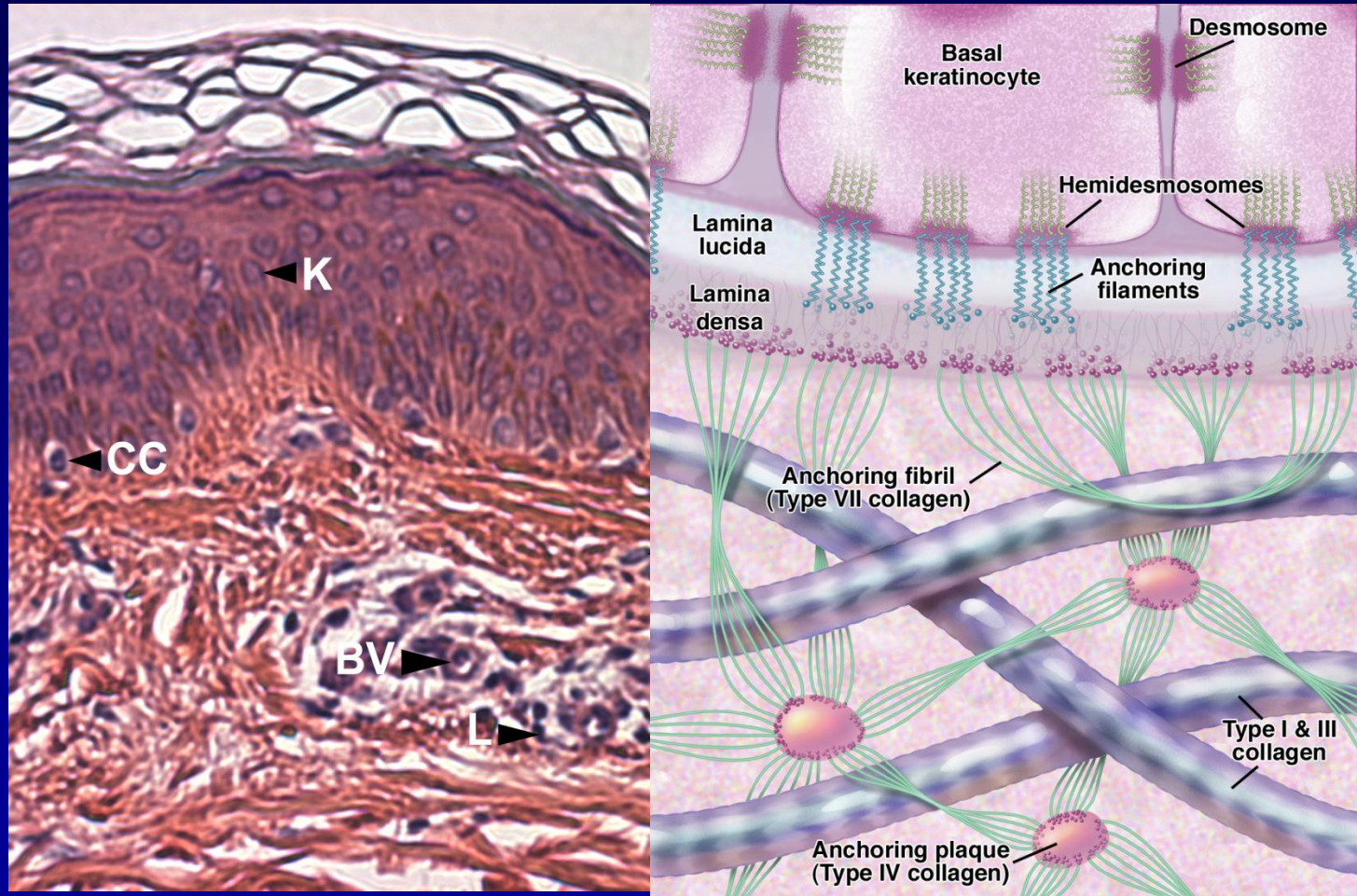
History

Mid 1960s

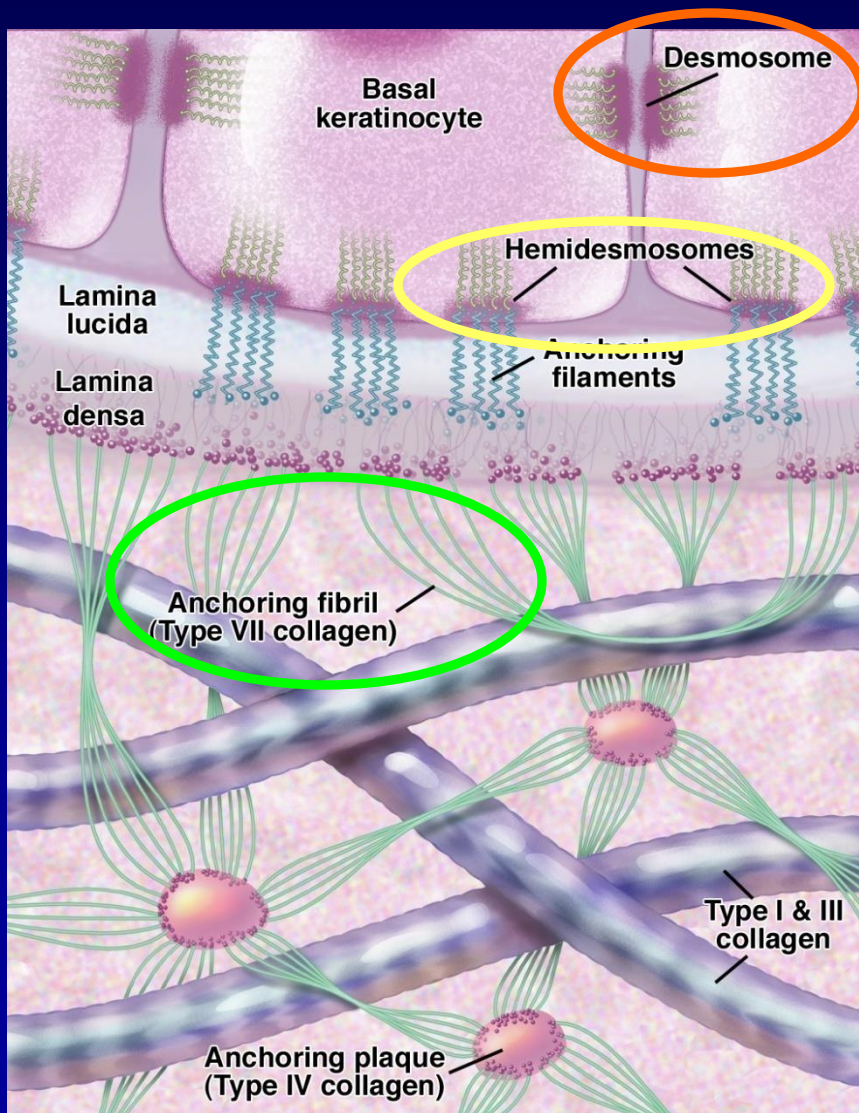
Ernest Beutner and Robert Jordon demonstrated IgG cell surface antibodies in pemphigus, autoantibodies in circulation and bound to the dermal-epidermal junction in bullous pemphigoid



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 & pseudoporphyria
- 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

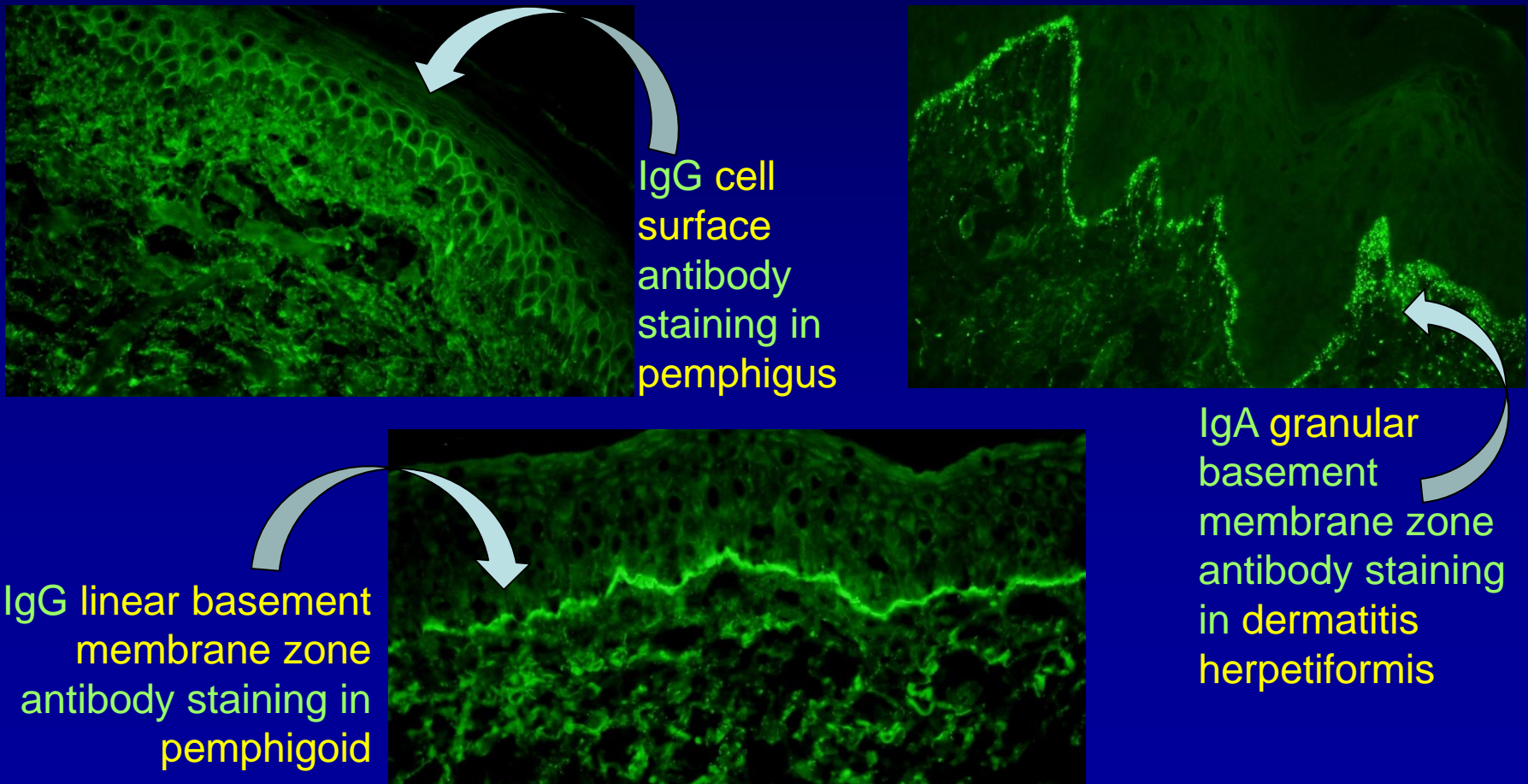
Immunodermatology Tests

- 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



Immunodermatology Tests

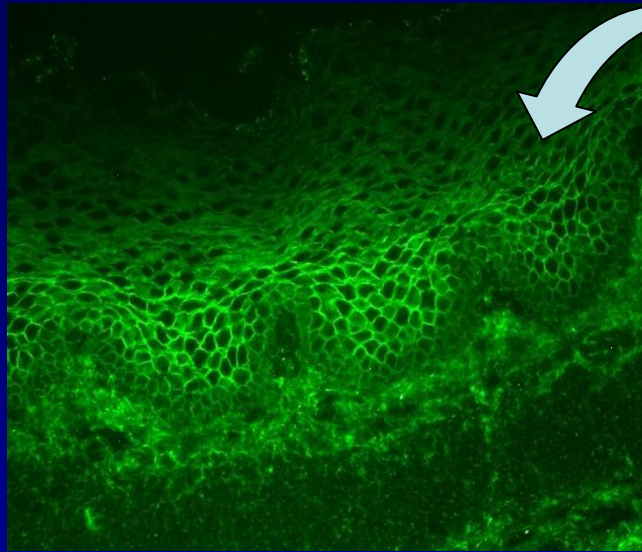
- Serum
 - Indirect immunofluorescence for circulating antibodies
 - Epithelial cell surface (pemphigus)
 - Basement membrane zone (pemphigoid, epidermolysis bullosa acquisita, linear IgA disease)
 - Cell surface, basement membrane zone and rodent substrates (paraneoplastic pemphigus)
 - Endomysium (dermatitis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

Immunodermatology Tests

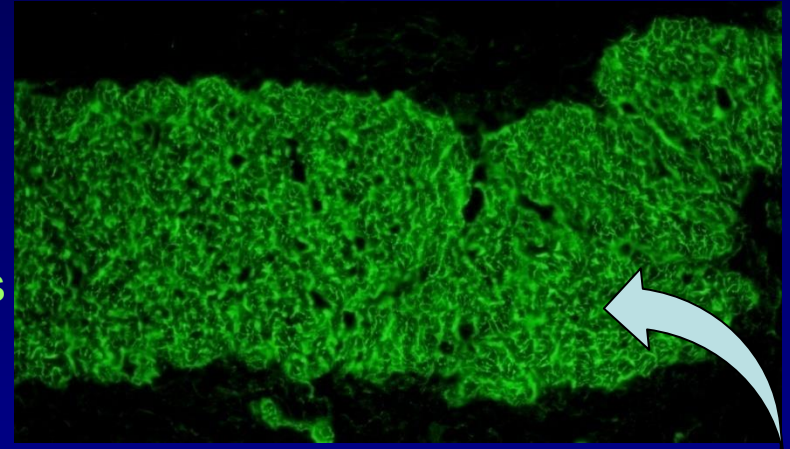
- Serum
 - Enzyme linked immunosorbent assays (ELISA)
 - IgG desmoglein 1 (pemphigus foliaceus) and IgG desmoglein 3 (pemphigus vulgaris)
 - IgG BP 180 and IgG BP 230 (bullous pemphigoid, mucous membrane pemphigoid, pemphigoid gestationis)
 - IgG collagen VII (epidermolysis bullosa acquisita)
 - IgG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

Immunodermatology Tests

- Serum
 - Indirect immunofluorescence

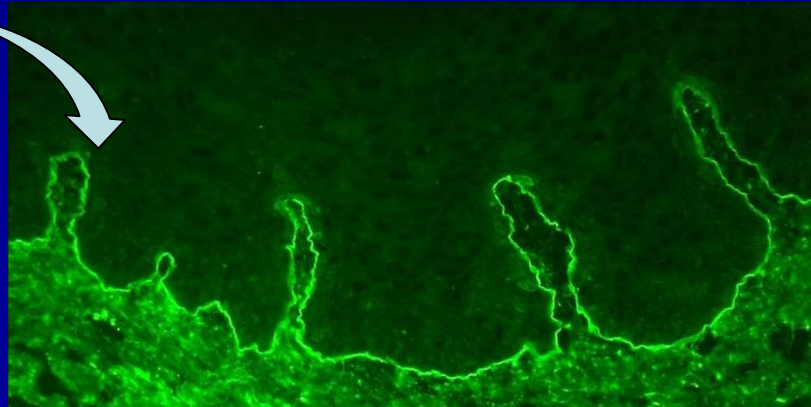


Serum IgG cell surface antibodies on monkey esophagus substrate in pemphigus



Serum IgA endomysial antibodies on monkey esophagus substrate in dermatitis herpetiformis

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



Immunodermatology Testing

DISEASES

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

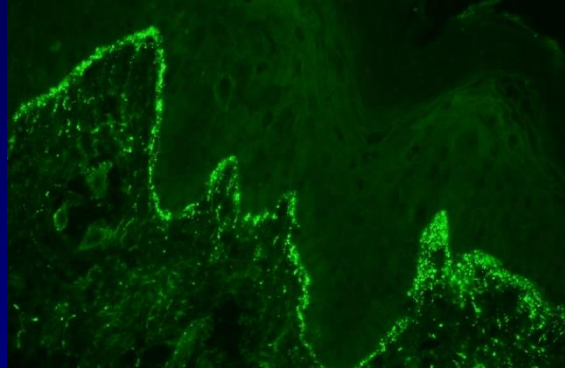
Location



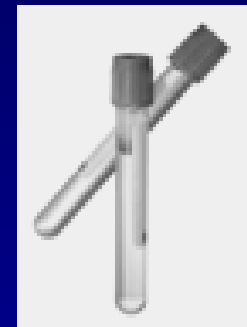
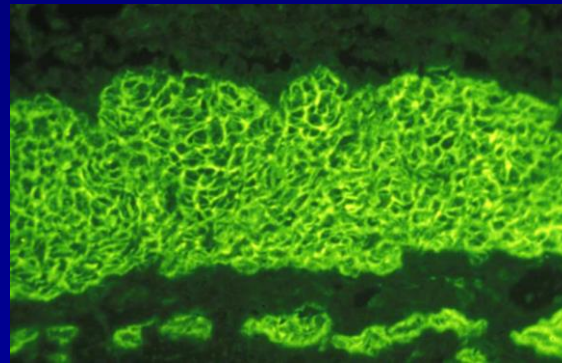
Immunodermatology Tests

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



Pemphigoid Variants



Inflammatory and desquamative gingivitis



Erythrodermic



Prurigo nodularis-like



Noninflammatory

Pediatric Pemphigoid



Infantile



Vulvar

Pemphigoid Variants

Atypical nonbullous presentations

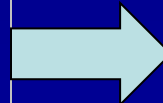
Ecematous lesions



Urticarial



Generalized pruritus
(with excoriations), no
primary lesions



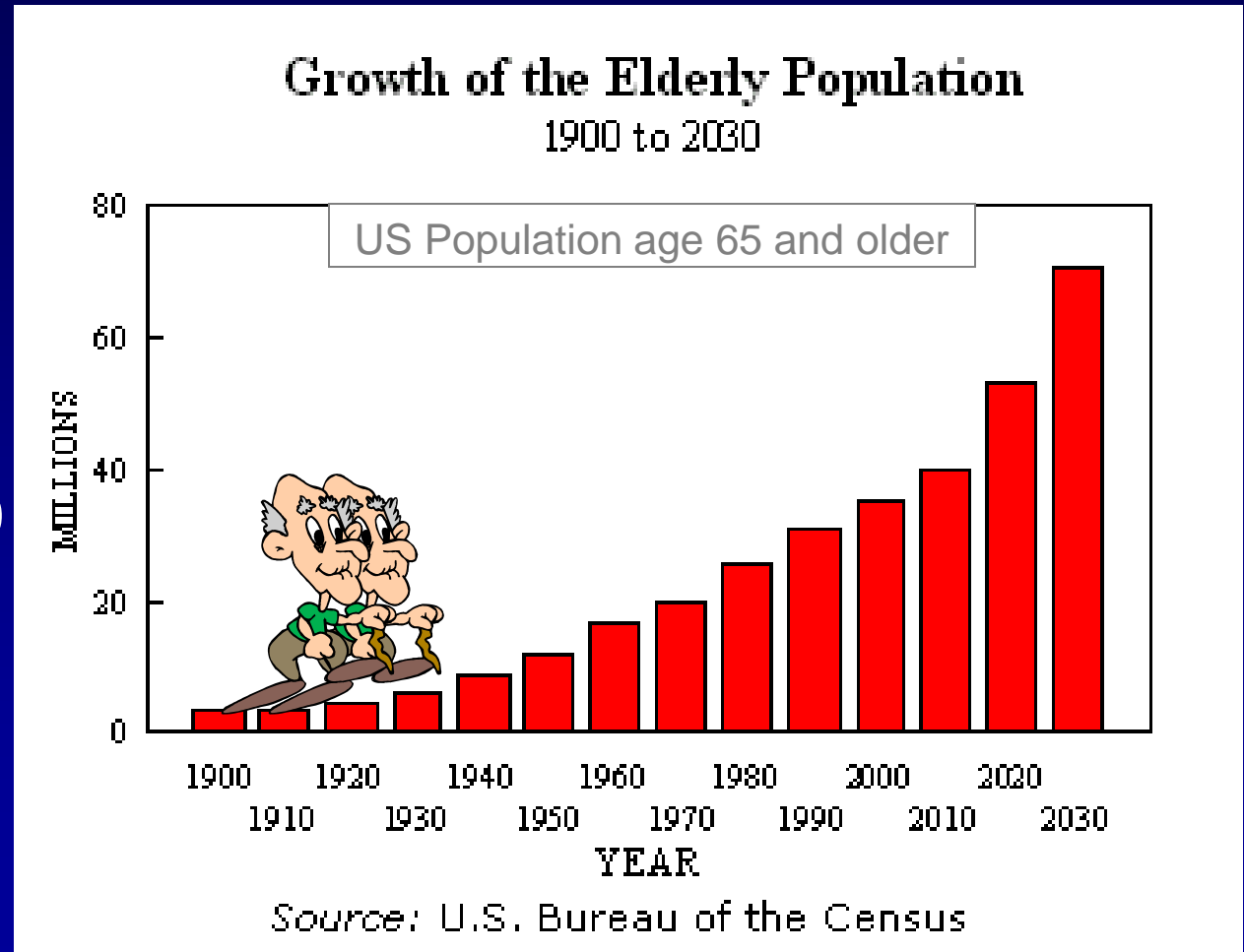
Bakker CV et al. Bullous pemphigoid
as pruritus in the elderly: a common
presentation. *JAMA Dermatol*
2013;149:950-3



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



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

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

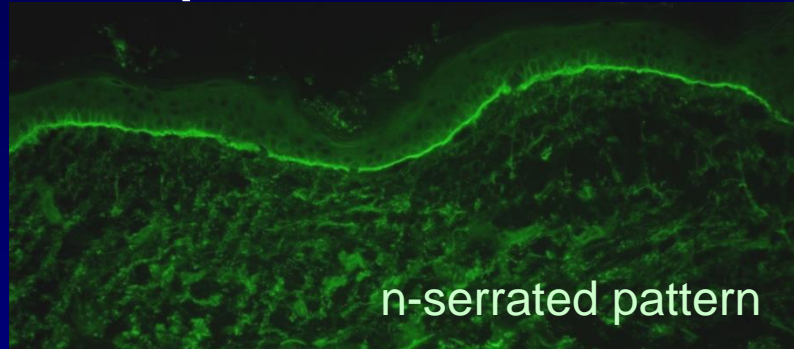
Pemphigoid Variants

- 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

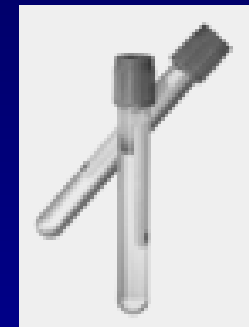
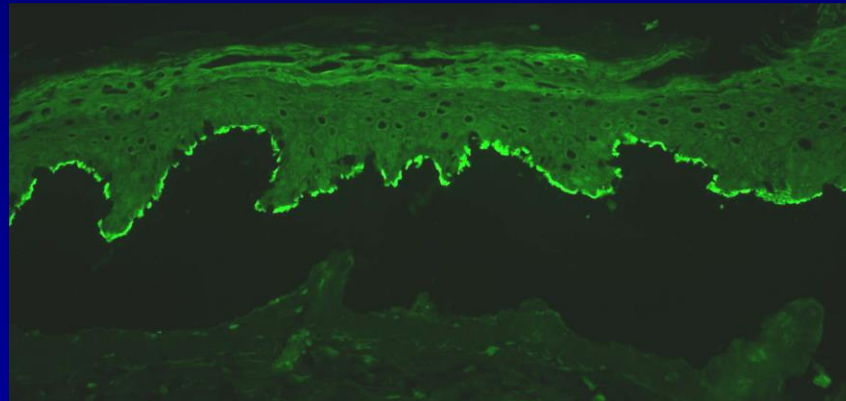
Immunodermatology Tests

Pemphigoid

- Tissue specimen



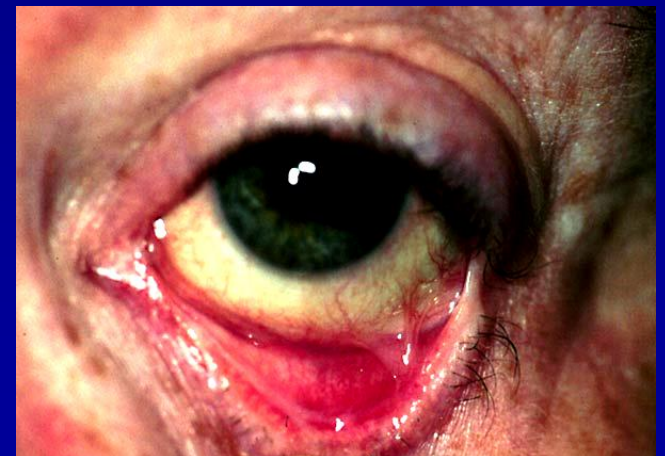
- Serum



- 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, epiligrin); anti-epiligrin cicatricial pemphigoid



Linear IgA Disease



Linear IgA Bullous Dermatitis

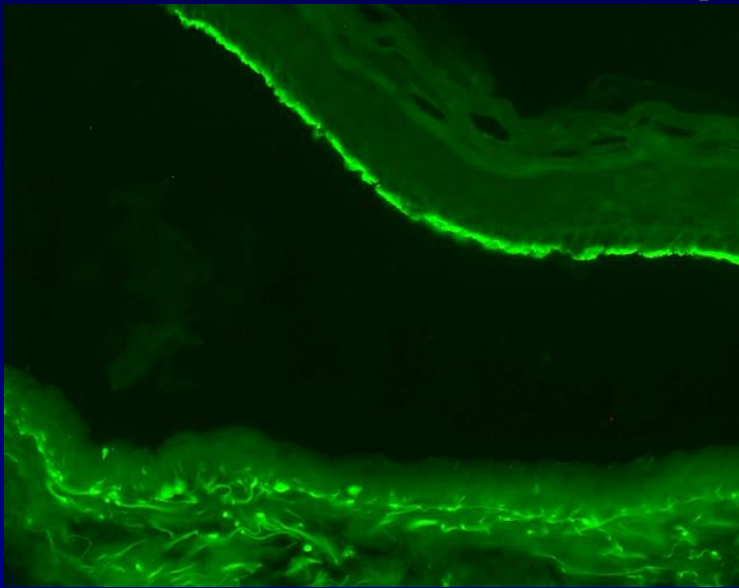


Chronic Bullous Disease of Childhood

- Tense bullae similar to pemphigoid
 - “String of pearls” or
 - “Cluster of jewels” sign

Linear IgA Bullous Dermatitis Immunopathology

Serum on human split skin substrate with anti IgA



Epidermal
localization

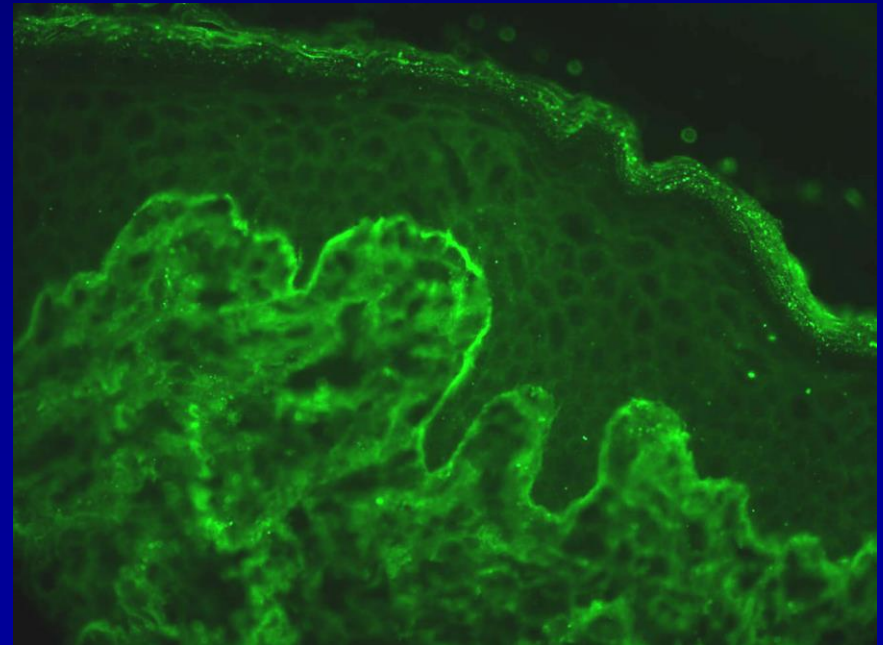
◀Roof of
separation:

IgA

basement
membrane zone
antibodies

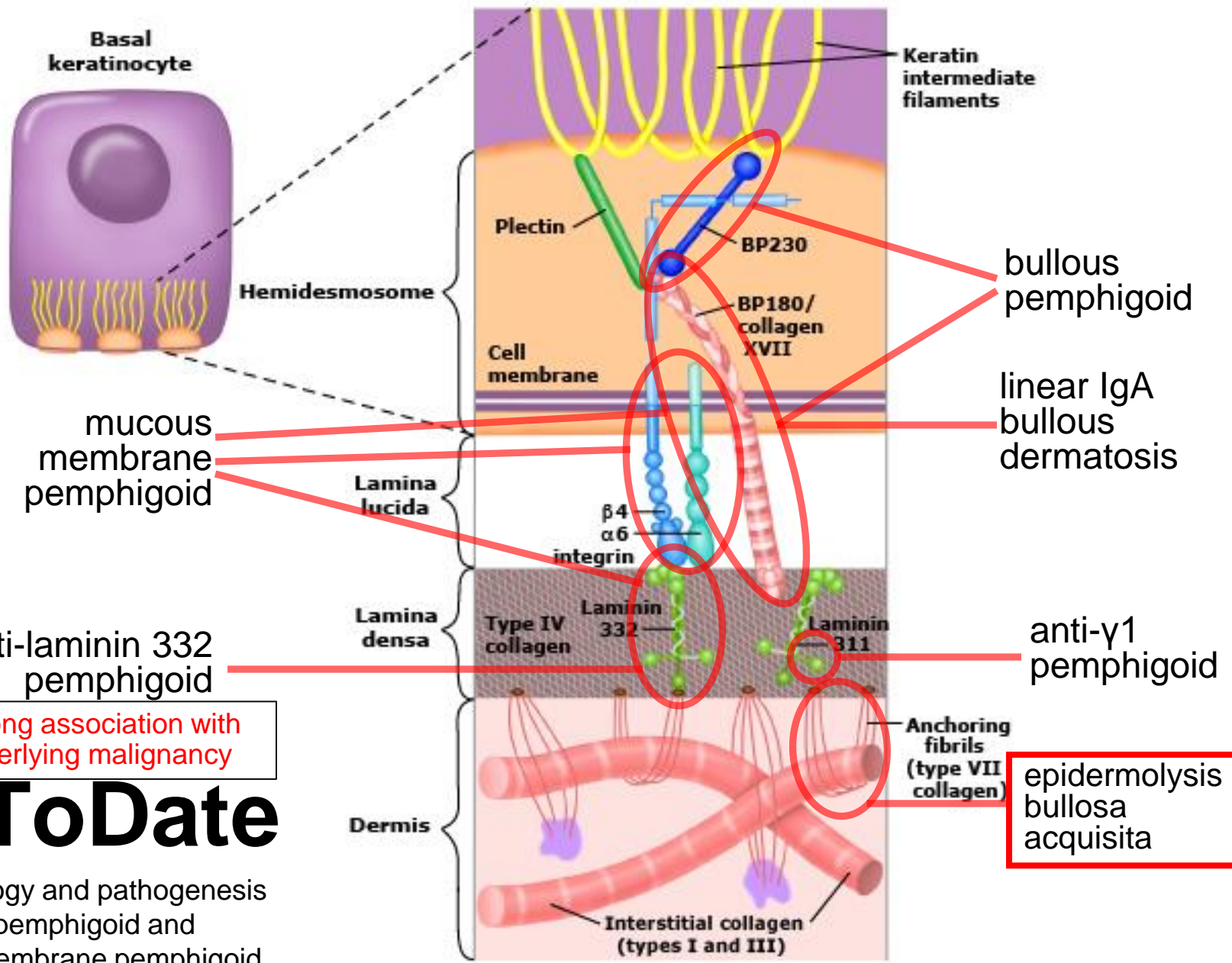
Direct immunofluorescence of
perilesional tissue with anti IgA

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



Strong association with underlying malignancy

UpToDate

Epidemiology and pathogenesis of bullous pemphigoid and mucous membrane pemphigoid

Epidermolysis Bullosa Acquisita



- Tense bullae
- Oral mucosal involvement common
- Occur in areas of trauma or friction

Epidermolysis Bullosa Acquisita

Immunopathology

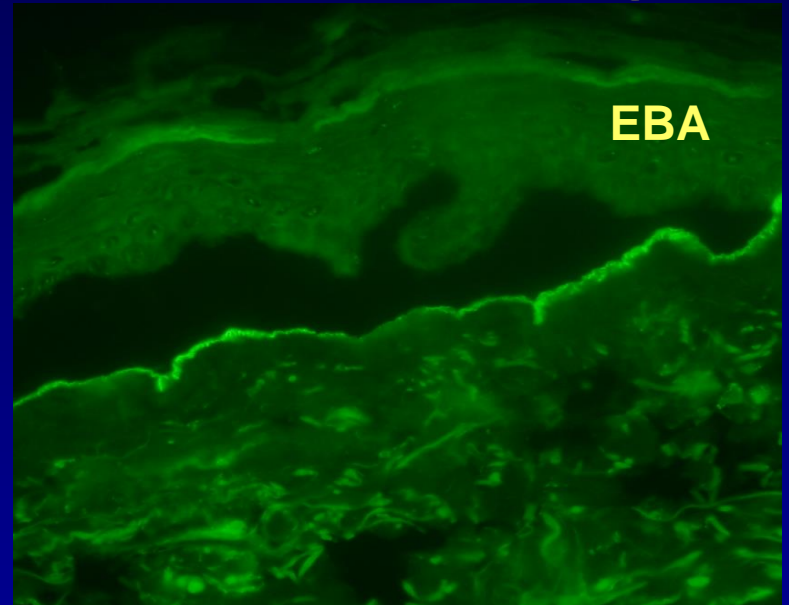
- Linear IgG and C3 BMZ, rarely IgA, IgM
 - Similar to pemphigoid but may be thicker, u-serrated pattern
- Indirect immunofluorescence of serum has IgG localized to dermal side of split skin
- Antibodies directed to Type VII collagen (anchoring fibrils)

Epidermolysis Bullosa Acquisita Immunopathology

Serum on human split skin substrate with anti IgG

Dermal
localization

Floor of
separation ►

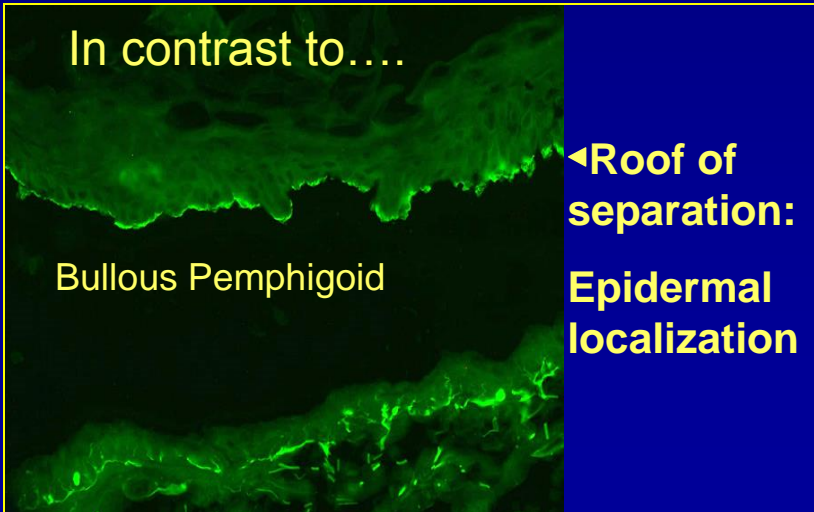


In contrast to....

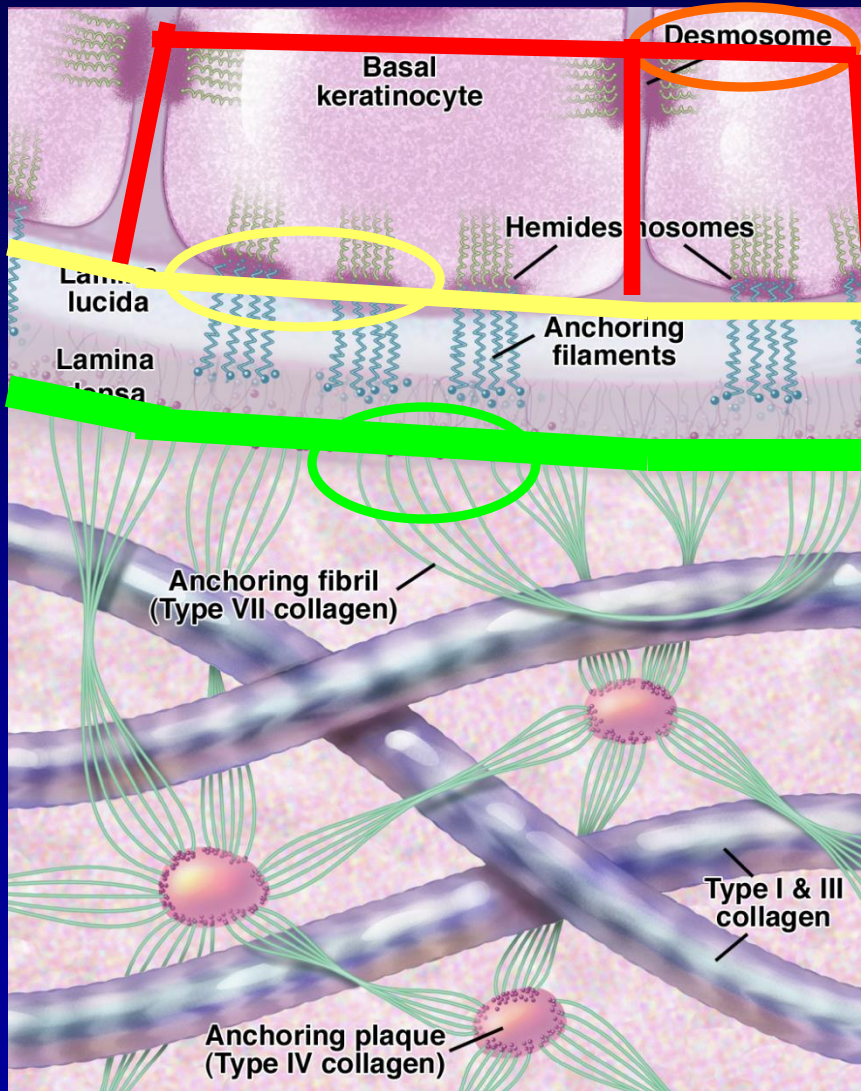
◀Roof of
separation:

Bullous Pemphigoid

Epidermal
localization



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 erythematosus
 - Fogo selvagem
 - Paraneoplastic pemphigus

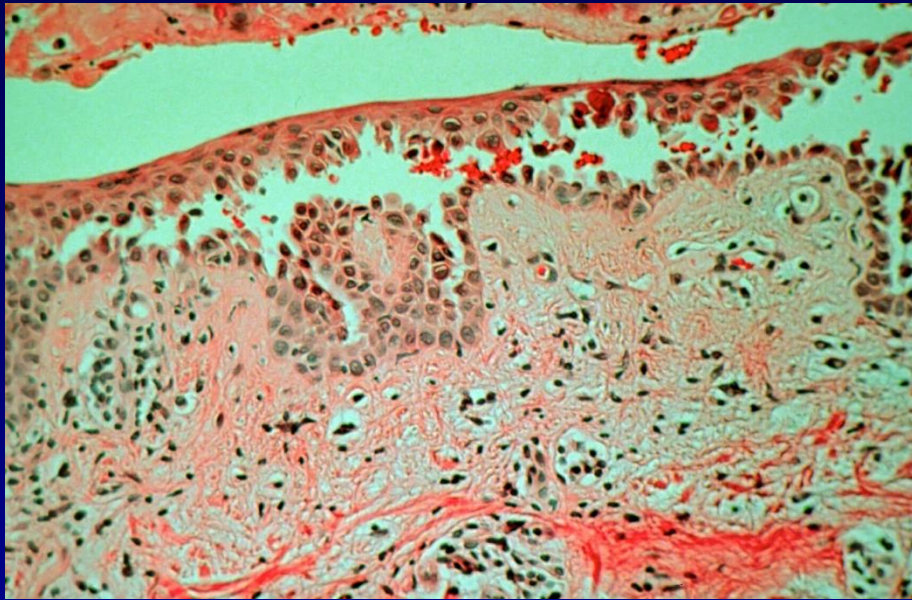
Pemphigus Vulgaris



- 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%

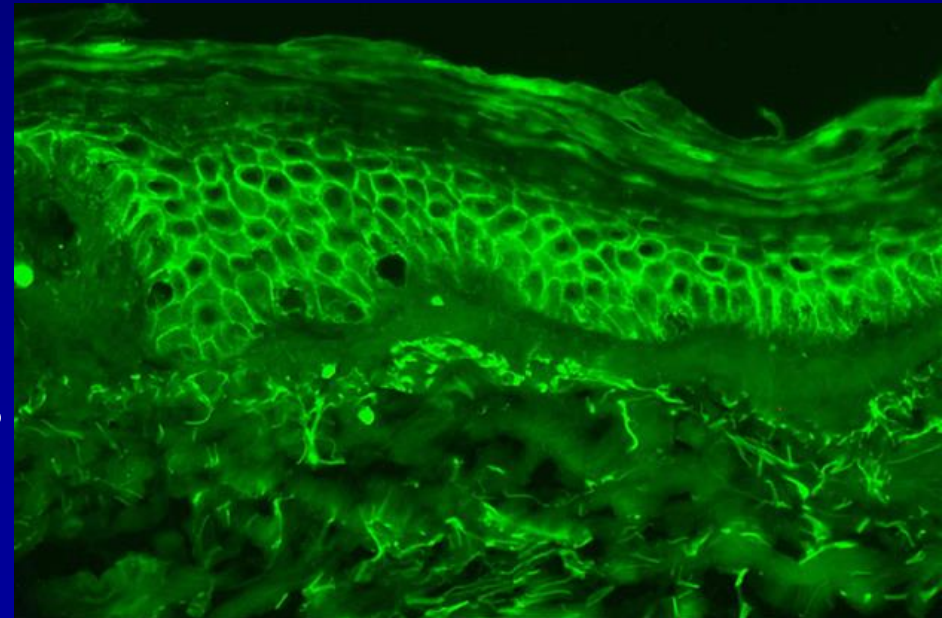
Pemphigus Vulgaris

Pathology and Immunopathology



- Acantholysis
- Dermal inflammation including eosinophils

- Epidermal cell surface IgG
- Serum cell surface and desmoglein 3 antibody titers correlate with disease activity



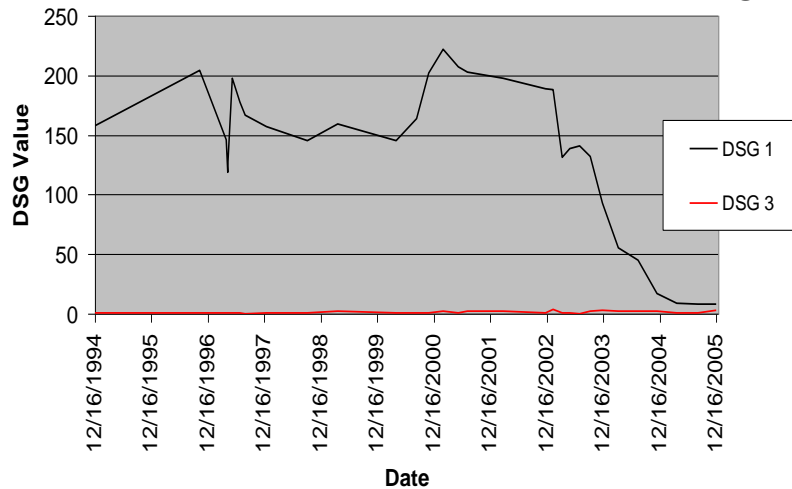
Pemphigus Foliaceus



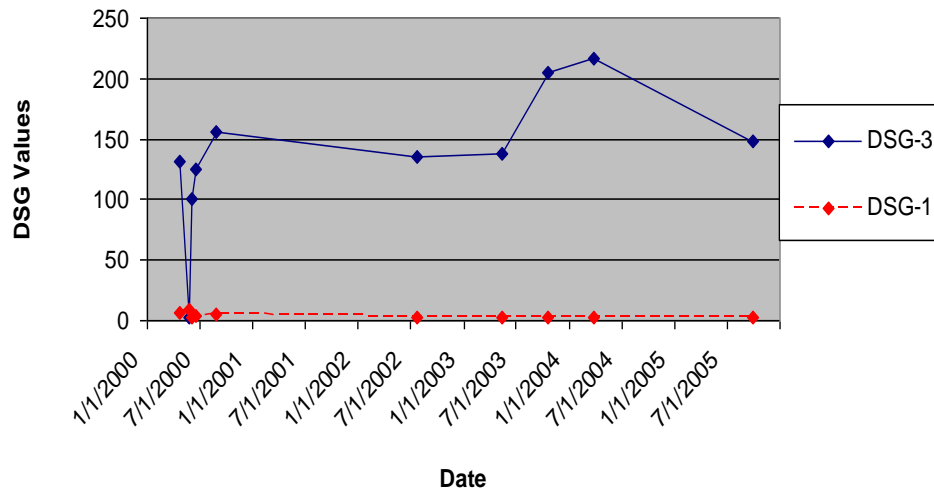
- Superficial cutaneous erosions
- Pattern of seborrheic dermatitis
- Mucosal involvement rare
- Cell surface and desmoglein 1 antibodies correlate with disease

Pemphigus

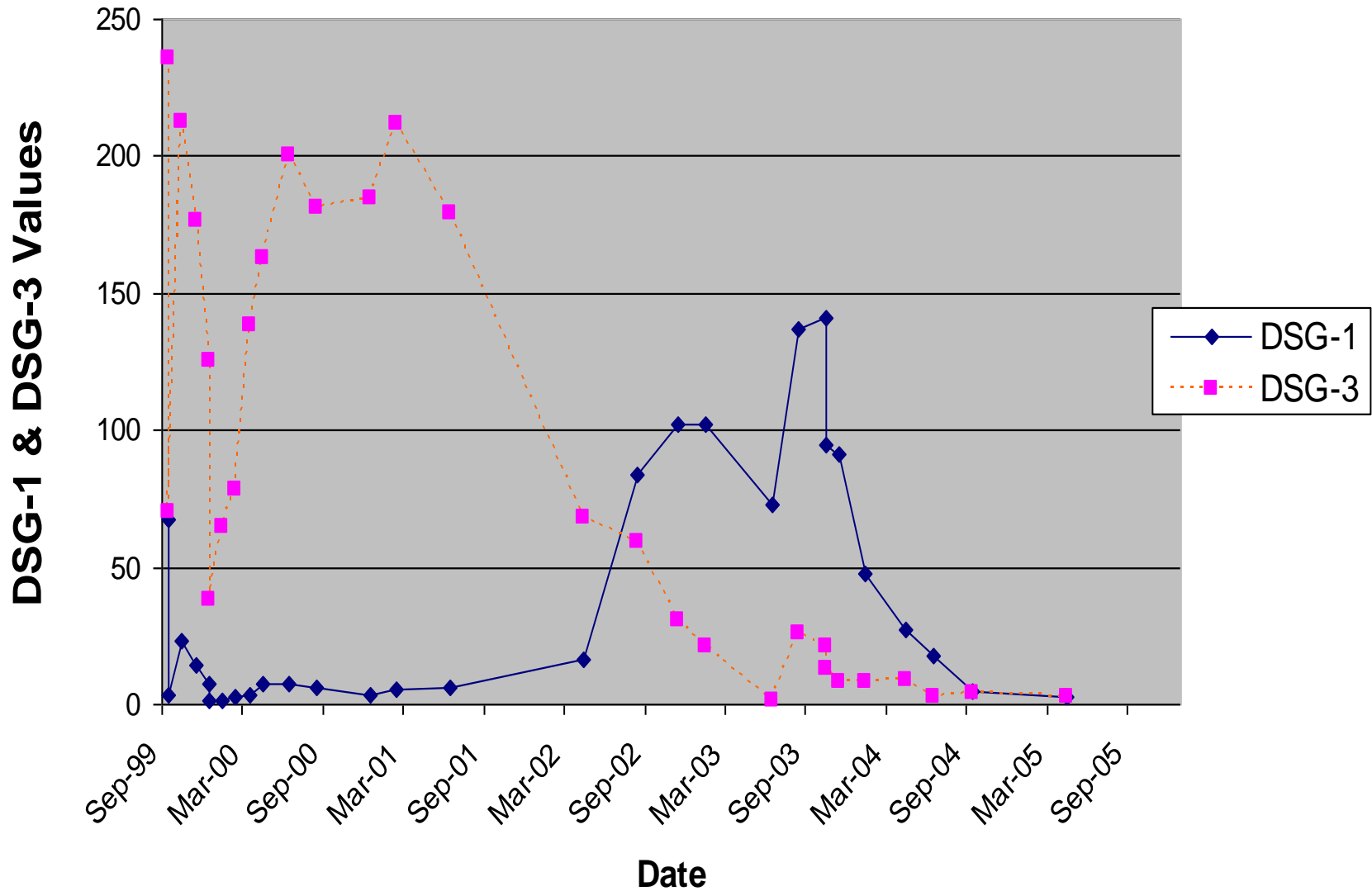
Pemphigus foliaceus



Pemphigus vulgaris



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
- Kalantari-Dehaghi M, Anhalt GJ, Camilleri MJ, et al. (2013) Pemphigus Vulgaris Autoantibody Profiling by Proteomic Technique. PLoS ONE 8(3): e57587. doi:10.1371/journal.pone.0057587

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

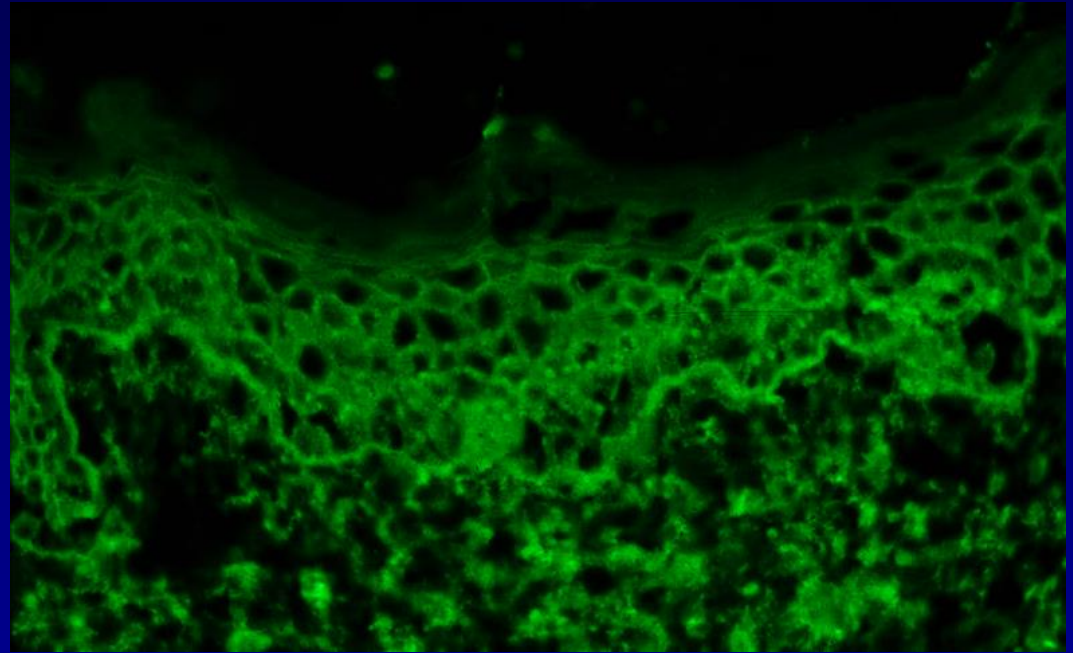
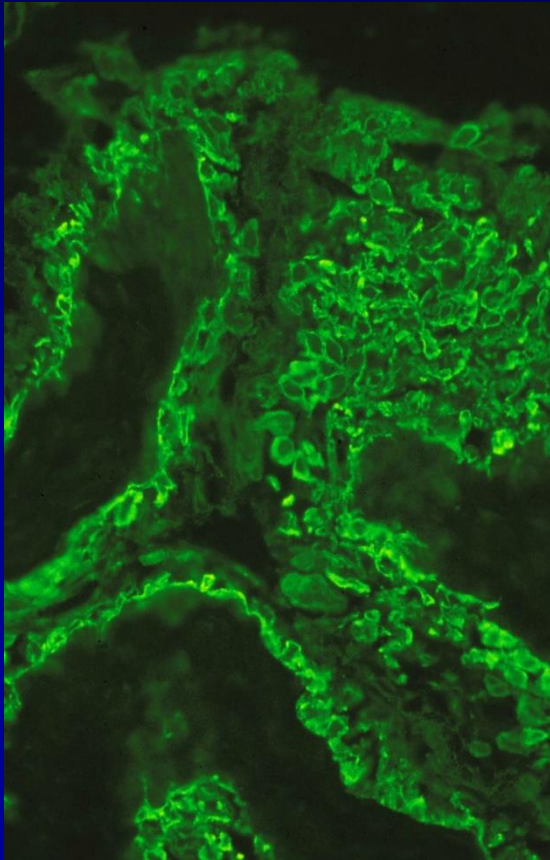


Paraneoplastic Pemphigus

- 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

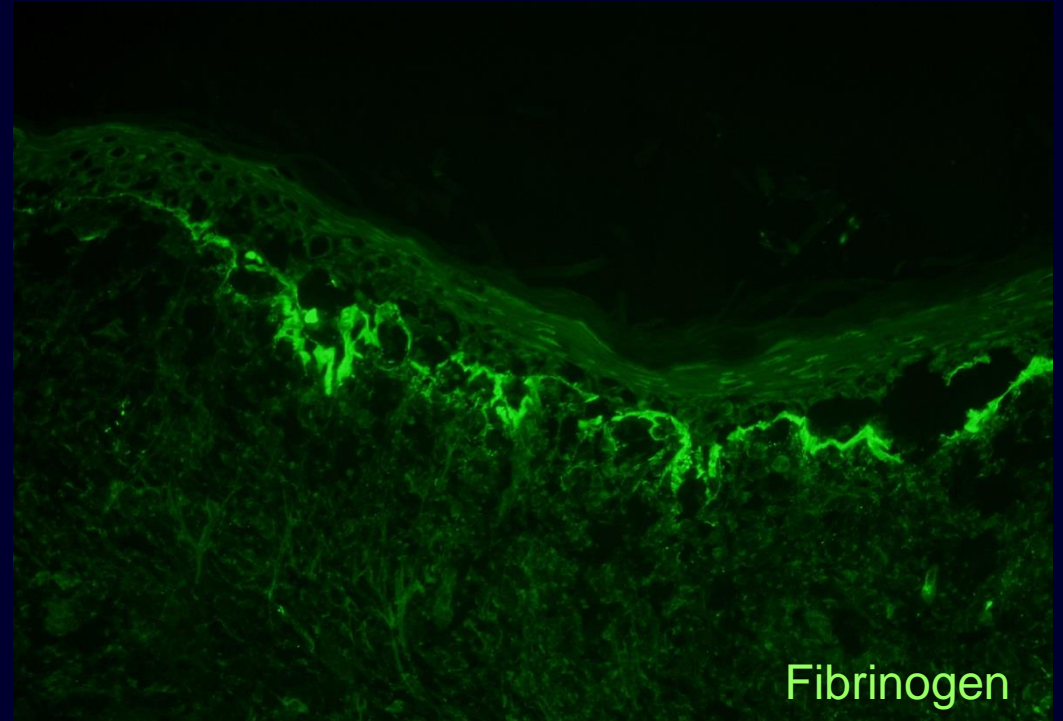
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- Urticaria
- Vasculitis (including Henoch-Schönlein purpura)



Lichen Planus



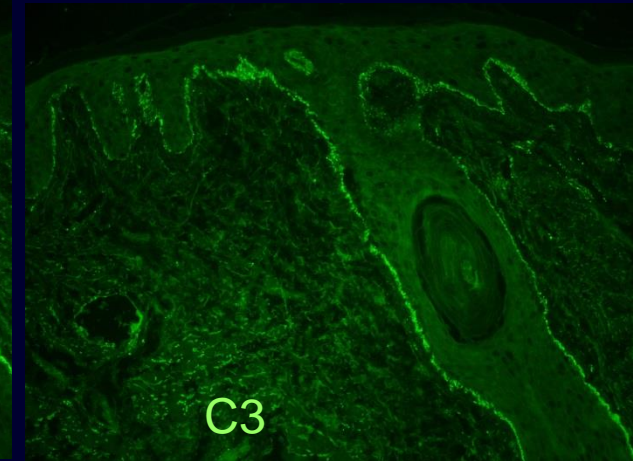
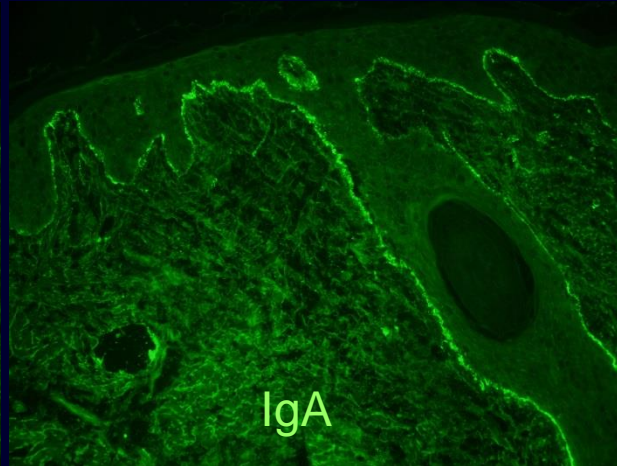
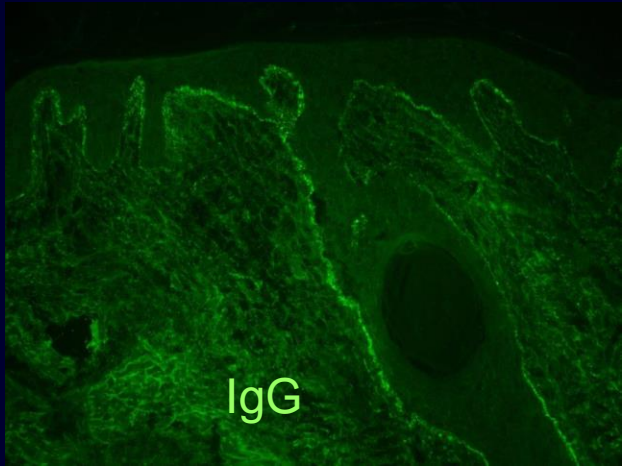
Nails and scalp



Fibrinogen

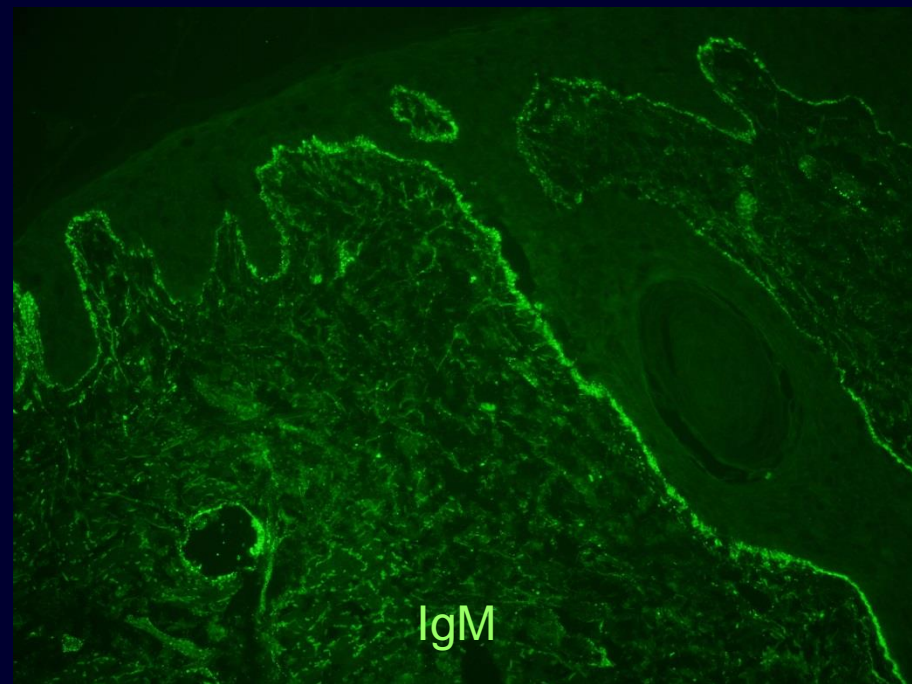


Lupus Erythematosus

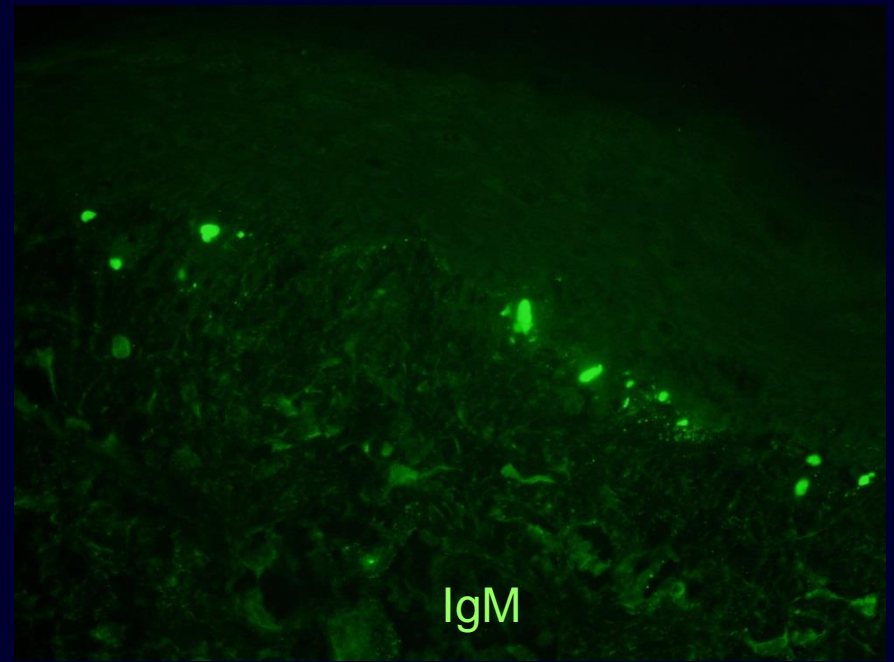
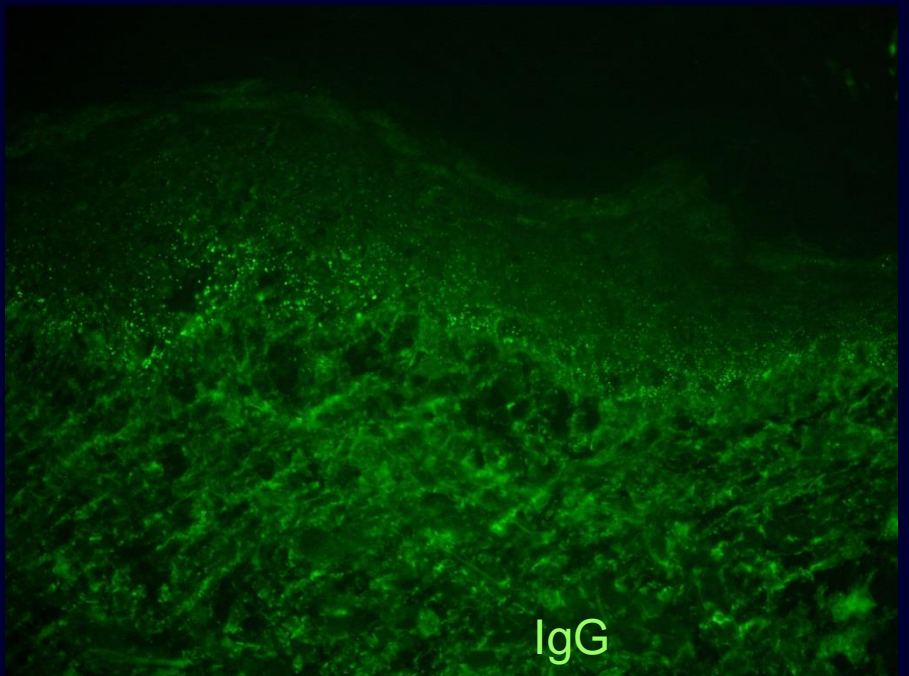


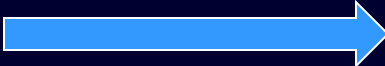
Granular immune deposits
along the BMZ

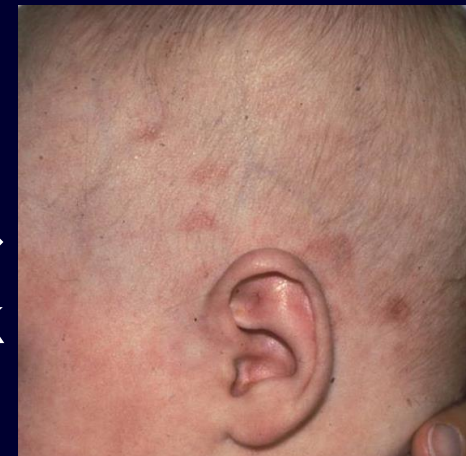
$IgM = C3 > IgG > IgA$



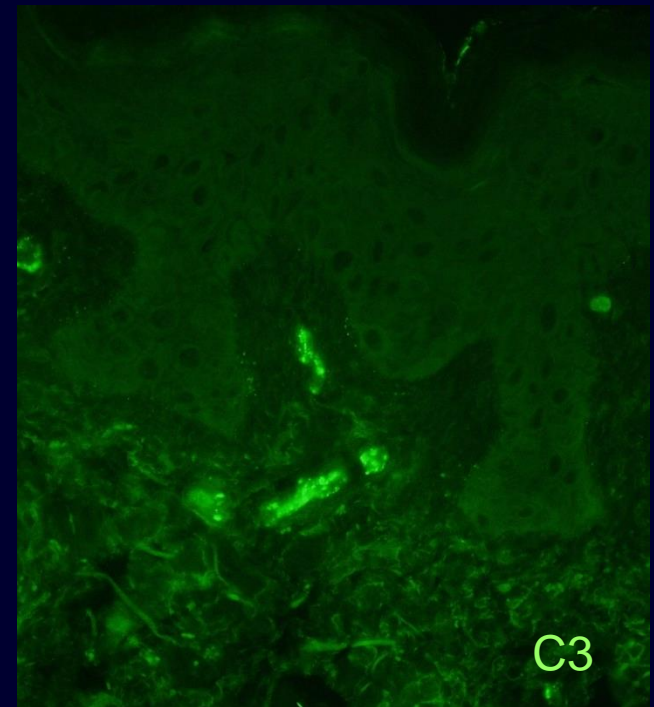
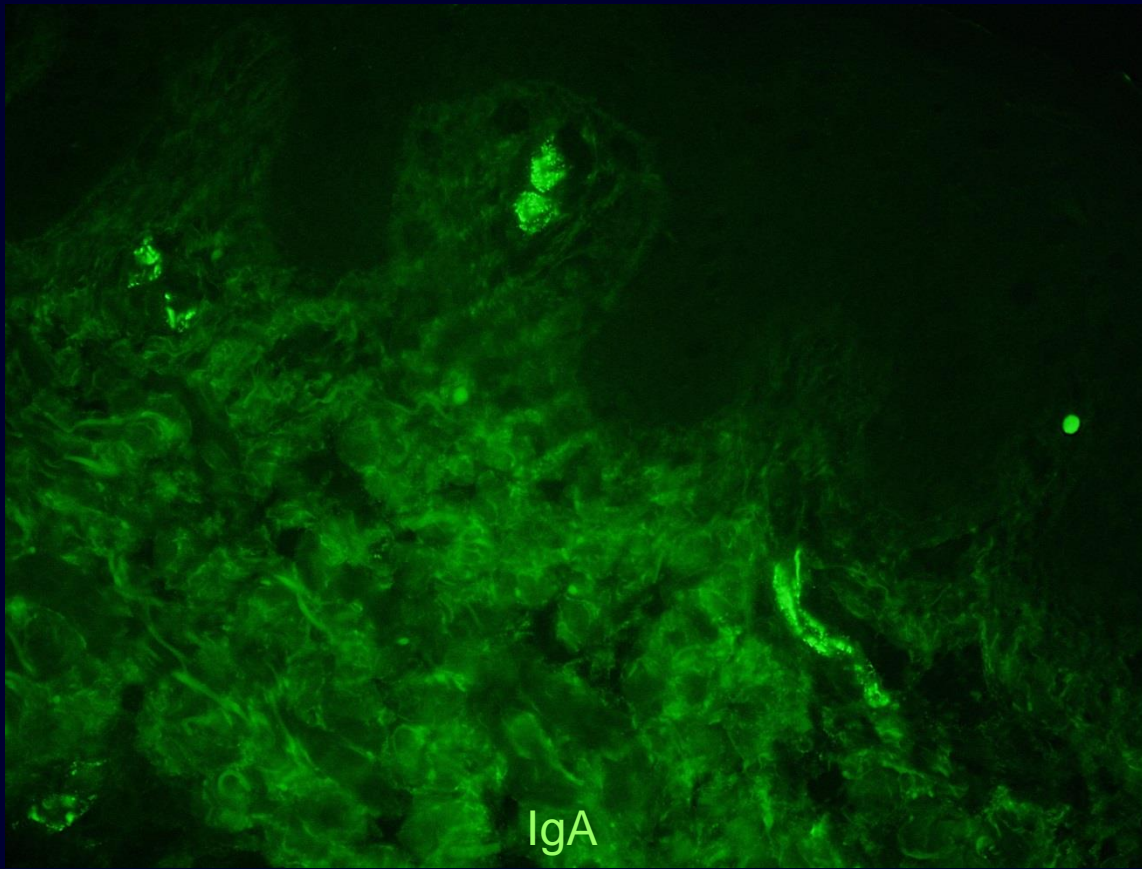
Subacute Cutaneous Lupus Erythematosus



- Drug induced
- Neonatal LE 
- Congenital heart block



Vasculitis



IgA Vasculitis
(Henoch Schoenlein Purpura)

Immunodermatology Tests are Helpful Diagnostically and in Monitoring Disease Activity

- Blistering Skin and Mucous Membrane Diseases
 - Pemphigoid (all types)
 - Pemphigus (all types, including paraneoplastic)
 - Epidermolysis bullosa acquisita
 - Linear IgA bullous dermatosis
 - Dermatitis herpetiformis & celiac disease
 - Porphyria & pseudoporphyria
- Other Immune-Mediated Diseases
 - Lupus erythematosus (all types)
 - Mixed / undefined connective tissue disease
 - Lichen planus & lichenoid reactions
 - Vasculitis (including Henoch-Schönlein purpura)
 - Eosinophil-associated diseases

Perilesional Tissue
AND
Serum

- Urticaria
- Drug reactions

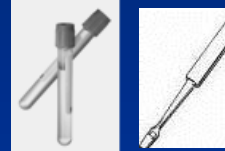
Lesional Tissue

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

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

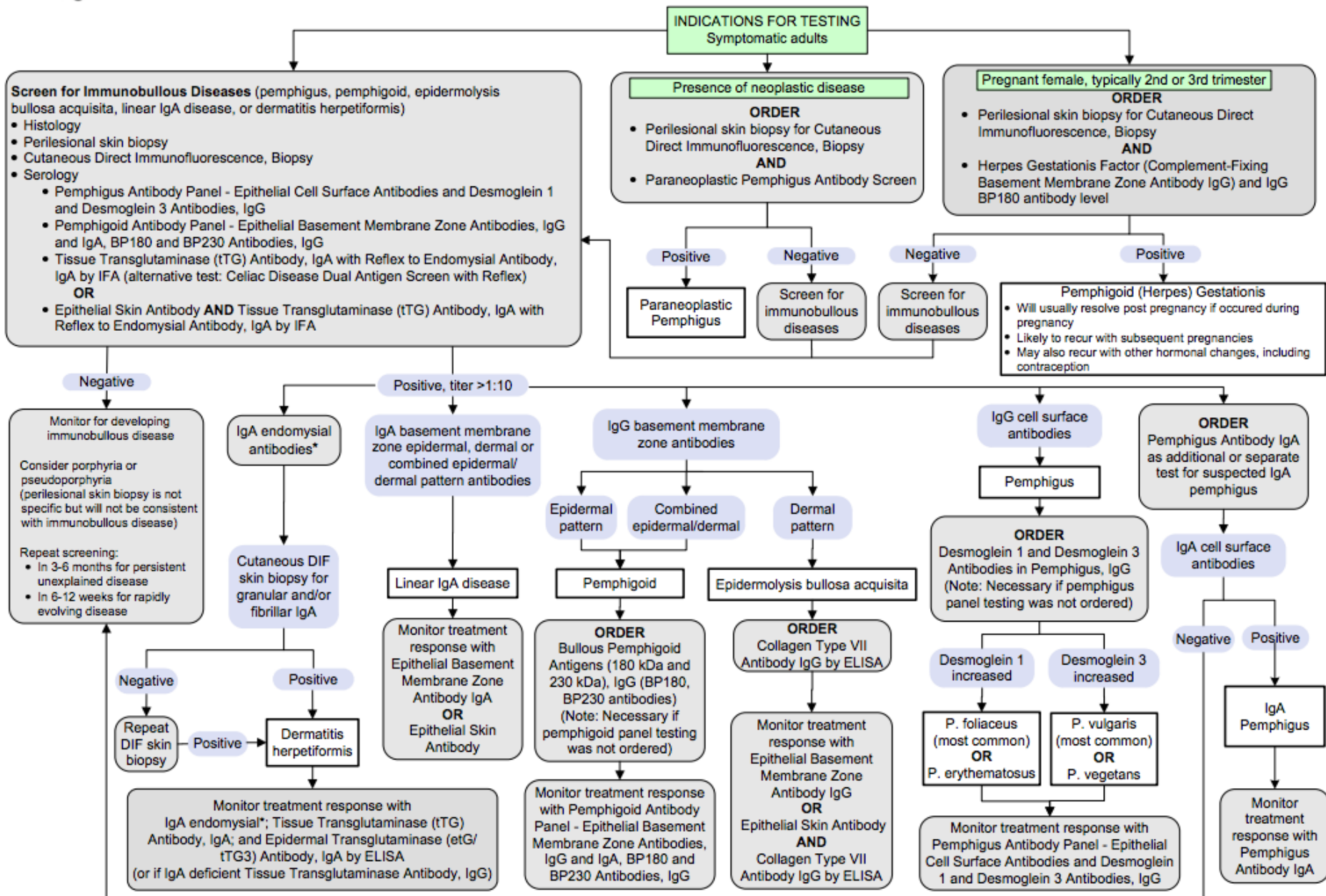
arupconsult.com/Topics/ImmunobullousSkinDz.html

- Toll free telephone number 1.866.266.5699

Marjorie Allen, Chief Technician, 11 years plus
Chase Myrick, Aubrey Curvin

Questions
about
specimen
procurement
and/or
patients?

Ask us



* 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.

Results 1 - 8 of 8 documents for your search ""Immunobullous Skin Diseases Testing Algorithm"".

Sort By: [Best Match](#) | [Title](#) Results per page:

- 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 pemphigus 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** ...

1

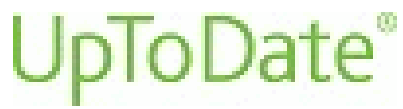
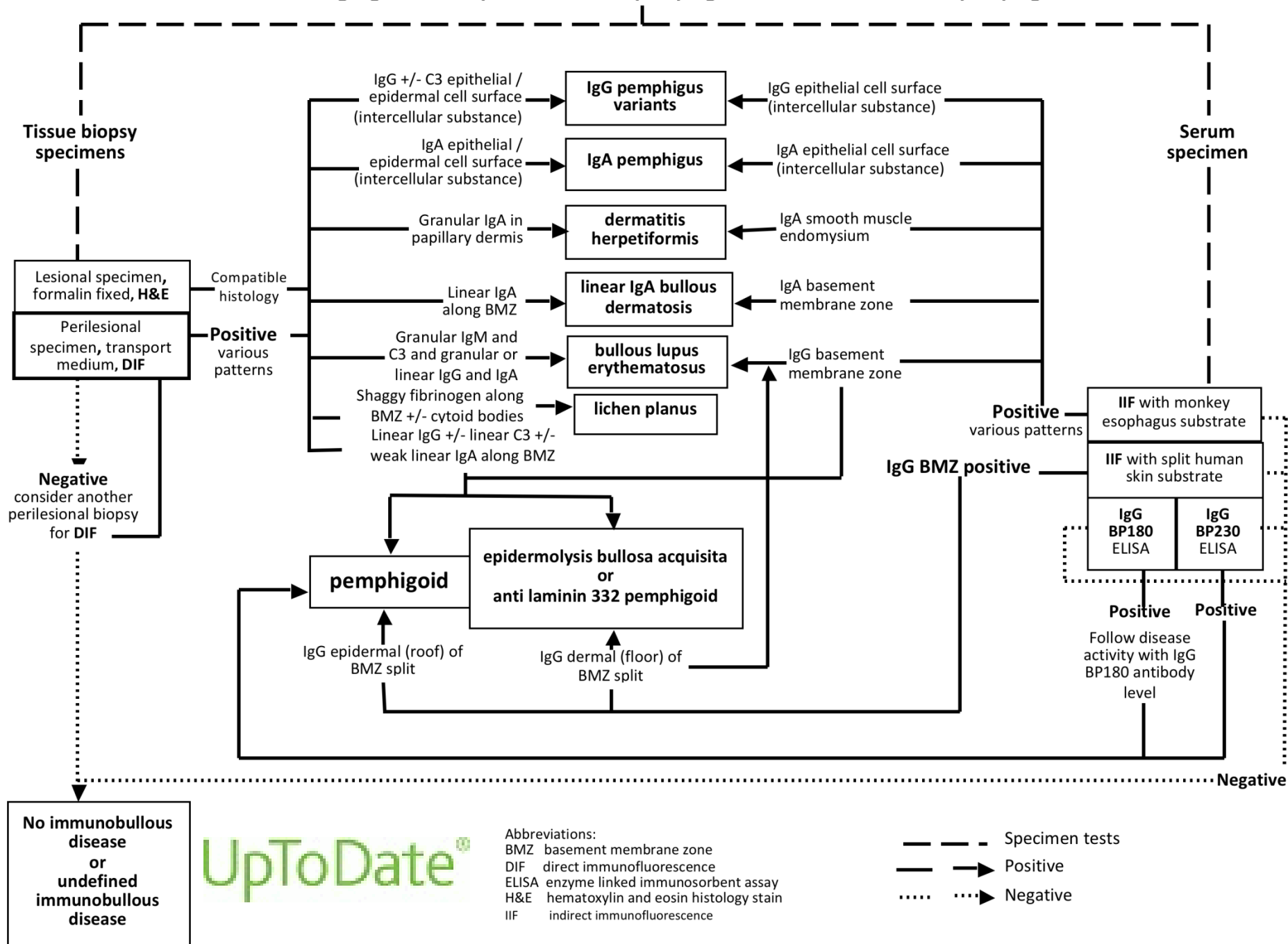
SEARCH AGAIN

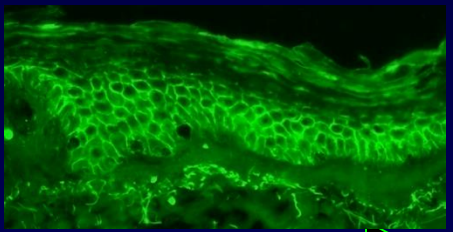
 Search within current results

ADVANCED SEARCH

all the words exact phrase at least one of the words but does not contain

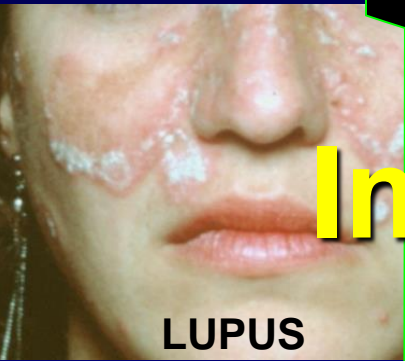
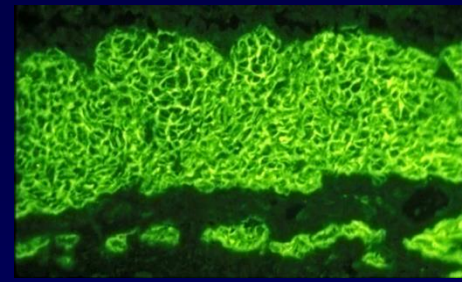
Clinical testing algorithm for possible bullous pemphigoid or mucous membrane pemphigoid





Pemphigus
Cell
Surface
Antibodies

IgA Endomysial
Antibodies in
Dermatitis
Herpetiformis



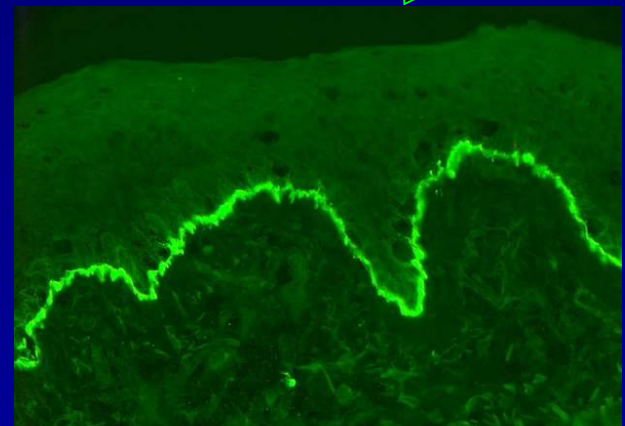
LUPUS



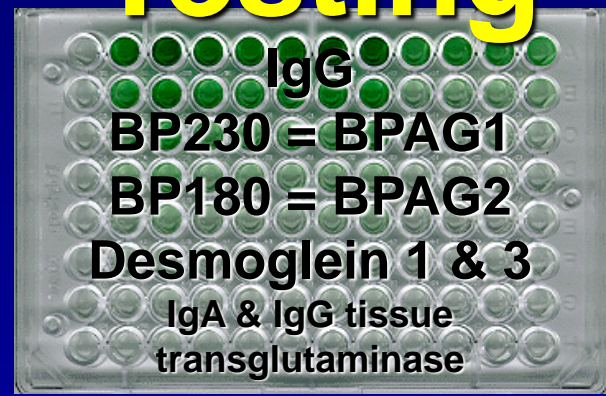
DERMATITIS
HERPETIFORMIS

The End

Immunodermatology Testing

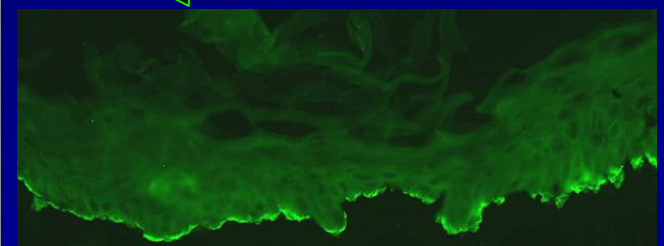


Pemphigoid BMZ Antibodies
Direct immunofluorescence
Skin biopsy



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

ELISA



Pemphigoid BMZ Antibodies
Indirect immunofluorescence
Serum