AAD 2017
Structure & Function of the Skin

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1. Patients with acquired, autoimmune blistering diseases have circulating autoantibodies directed against specific autoantigens in skin.

2. Such autoantigens often represent important structural proteins in skin.

3. Genes encoding such structural proteins are sometimes mutated in patients with inherited disorders characterized by blistering and/or skin fragility.
Pemphigus: Diseases of Cell-Cell Adhesion
Pemphigus Vulgaris
Pemphigus Vulgaris

Pemphigus Foliaceus
Pemphigus Foliaceus

Udey MC and Stanley JR: JAMA 282:572-576
A Desmosome

Adapted from JR Stanley
Pemphigus: Diagnostic Criteria

Integration of the Following Findings:

Clinical: Erosive lesions and/or flaccid blisters on mucous membranes and/or skin.

Histologic: Acantholytic blister formation within the epidermis.

Immunopathologic: Demonstration of in situ deposits of IgG autoAbs on the surface of keratinocytes and/or circulating IgG autoAbs vs. Dsg1 and/or Dsg3.
Design of Desmoglein ELISAs

- BV rDSG
- Patient IgG
- HRP-R/H IgG
Each pemphigus subtype has its own anti-desmoglein antibody profile.

<table>
<thead>
<tr>
<th></th>
<th>PF</th>
<th>PV</th>
<th>Normal</th>
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<tbody>
<tr>
<td>ELISA</td>
<td>index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\alpha Dsg1)</td>
<td>(\alpha Dsg3)</td>
<td>(\alpha Dsg1)</td>
<td>(\alpha Dsg3)</td>
</tr>
<tr>
<td>(\alpha Dsg1)</td>
<td>(\alpha Dsg3)</td>
<td>(\alpha Dsg1)</td>
<td>(\alpha Dsg3)</td>
</tr>
<tr>
<td>mucosal dominant</td>
<td>mucocutaneous</td>
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M Amagai 2006
Autoantibodies in patients with pemphigus interfere with the function of desmosomal cadherins.

The tissue distribution of Dsgs and the antigenic specificity of autoantibodies in patients with pemphigus determines the localization of blisters.
Distribution of Desmogleins 1 and 3 in Skin and Mucosa
Bullous Impetigo
Bullous Impetigo & SSSS

Staphylococcal exfoliative toxin A (ETA) causes blisters in bullous impetigo and Staphylococcal scalded-skin syndrome.

ETA causes loss of cell adhesion only in the superficial epidermis.

ETA has the structure of a serine protease.

ETA targets Dsg1 and creates a blister just below the stratum corneum.

Objective: To determine the prevalence of positive Dsg ELISA values during clinical remission. To ascertain how positive Dsg ELISA scores during remission compare with those during active disease.

Conclusions: Circulating anti-Dsg IgG autoantibodies are found in a considerable percentage of pemphigus patients in remission who previously had high levels of autoantibody production during active stages of disease.
Autoimmune Blistering Diseases?

Why is there not a perfect correlation between levels of IgG anti-Dsg autoAbs and disease activity in patients with pemphigus?
Human Monoclonal Autoantibodies Isolated By Phage Display

Pathogenic and nonpathogenic antibodies were isolated from pemphigus patients.

Epitopes defined by scFvs were blocked by autoantibodies from multiple pemphigus patients.

Genetic restriction of the PV autoantibody repertoire suggests that a limited number of antibody idiotypes cause disease.

Why do patients break tolerance to proteins in desmosomes and epidermal basement membrane?
Fogo Selvagem

Dermatology Clinics 29:413–418, 2011
Fogo Selvagem (FS) is mediated by IgG autoAbs vs. Dsg1. Clusters of FS overlap with those of leishmaniasis, a disease transmitted by sand fly (Lutzomyia longipalpis) bites. Salivary antigens from the sand fly (i.e., the LJM11 salivary protein) are recognized by IgG autoAbs from FS patients. Anti-Dsg1 monoclonal autoAbs derived from FS patients also cross-react with LJM11. Mice immunized with LJM11 generate anti-Dsg1 Abs. Thus, insect bites may deliver salivary antigens that initiate a cross-reactive IgG₄ autoAb response in genetically susceptible individuals and lead to subsequent FS.
Pemphigoid: Diseases of Cell-Matrix Adhesion

Bullous Pemphigoid, Pemphigoid Gestationis, Mucous Membrane Pemphigoid, p200 Pemphigoid, Linear IgA Dermatosis, Epidermolysis Bullosa Acquisita
Transmission Electron Microscopy

Basal Keratinocyte
LL
AF
af
HD
LD
Papillary Dermis
Epidermal Basement Membrane

- Basal keratinocyte
- Lamina lucida
- Lamina densa
- Sublamina densa
- Keratin IFs
- HD
- Anchoring filaments
- Anchoring fibrils
- Collagen
- Anchoring plaques
Basement Membranes

1. Substrate for cell attachment.
2. Template for tissue repair.
3. Matrix for cell migration.
4. Permeability barrier.
5. Substrata for modulation of epithelial cells.
<table>
<thead>
<tr>
<th>Family</th>
<th>Examples</th>
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<tr>
<td>Plakin</td>
<td>BPAG1; Plectin</td>
</tr>
<tr>
<td>Integrin</td>
<td>Integrin $\alpha_6 \beta_4$</td>
</tr>
<tr>
<td>Collagen</td>
<td>Collagens IV, VII, XVII</td>
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<tr>
<td>Laminin</td>
<td>Laminins 332, 311</td>
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</table>
Subepidermal Blistering Diseases: Diagnostic Criteria

Integration of the Following Findings:

Clinical: Erosive lesions and/or tense blisters on mucous membranes and/or skin.

Histologic: Blister formation within the epidermal basement membrane with or without an associated inflammatory infiltrate.

Immunopathologic: Demonstration of in situ deposits of IgG, C3, and/or IgA autoAbs in epidermal BM, and/or circulating autoAbs vs. epidermal BM and/or signature autoantigens characteristic of various subepidermal immunobullous diseases.
Bullous Pemphigoid
Bullous Pemphigoid Antigens 1 and 2

BPAG1: a 230 kD member of the plakin family of proteins.

BPAG2: a 180 kD type II transmembrane collagen.

IgG BPAG1 ELISA
IgG BPAG2 ELISA
Bullous Pemphigoid Antigen 2 (BPAG2)

Basal keratinocyte

NC16A, the immunodominant epitope of BPAG2
Passive Transfer of IgG from Patients with Bullous Pemphigoid to hBPAG2 “Humanized” Mice Yields Subepidermal Blisters With Clinical, Histologic, and Immunopathologic Features Like Those Seen in Patients.

Experimental Passive Transfer Studies in Neonatal Mice

Anti-NC16A IgG elicits complement activation, mast cell degranulation, and PMN-rich infiltrates in epidermal BM.

PMN-derived MMP-9 inactivates $\alpha_1$-protease inhibitor, allowing unrestrained activity of PMN elastase that contributes to subepidermal blister formation.

BP IgG dramatically decreased BP180 in cultured keratinocytes within 6 hours (with no change in $\alpha_6$ or $\beta_4$ integrin levels). Reduction of keratinocyte BP180 content increased detachment of treated cells from culture dishes subjected to vibration.


In conclusion, the COL17 depletion induced by BP autoAbs, and not complement activation, is essential for blister formation in an in vivo experimental model.
IgE Anti-BPAG2 Autoantibodies Contribute to the Pathogenesis of BP


Mapping the binding sites of anti-BP180 immunoglobulin E autoantibodies in bullous pemphigoid. JA Fairley et al, J Invest Dermatol 125:467-72, 2005

A pathogenic role for IgE in autoimmunity: bullous pemphigoid IgE reproduces the early phase of lesion development in human skin grafted to nu/nu mice. JA Fairley et al, J Invest Dermatol 127:2605-11, 2007


The Extracellular Domain of Type XVII Collagen is Proteolytically Cleaved by ADAMs (Sheddases of the Disintegrin-Metalloproteinase Family) to Yield Soluble Products
Model for the interaction of TACE and collagen XVII

The Extracellular Domain of Type XVII Collagen is Proteolytically Cleaved by ADAMs (Sheddases of the Disintegrin-Metalloprotease Family) to Yield Soluble Products
**BPAG2: Autoimmune and Inherited Blistering Diseases**

<table>
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<th>Protein</th>
<th>Structure</th>
<th>Immune</th>
<th>Inherited</th>
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<tr>
<td>BPAG2</td>
<td>HD-anchoring filament complexes</td>
<td>BP</td>
<td>GABEB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PG</td>
<td></td>
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<td></td>
<td></td>
<td>CP</td>
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<td></td>
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<td>LAD</td>
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Mucous Membrane Pemphigoid
MMP Autoantigens

Bullous pemphigoid antigen 2
Laminin 332 (aka, Laminin 5)
Integrin subunits $\alpha_6$ and $\beta_4$
Type VII collagen
Other yet to be characterized autoantigens
Laminin 332

[Laminin 5 (α3β3γ2),
epiligrin, kalinin, nicein,
GB3 antigen, BM600]
Anti-epiligrin cicatricial pemphigoid and relative risk for cancer.

Conleth A Egan MD, Zelmira Lazarova MD, Thomas N Darling MD, Carole Yee BSc, Timothy Cote MD, and Kim B Yancey MD
<table>
<thead>
<tr>
<th>Protein</th>
<th>Gene</th>
<th>Localization</th>
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<tr>
<td>Laminin 332</td>
<td><strong>LAMA3</strong></td>
<td>Lamina densa</td>
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<tr>
<td>$\alpha$ subunit</td>
<td><strong>LAMB3</strong></td>
<td>lamina lucida</td>
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<tr>
<td>$\beta$ subunit</td>
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<tr>
<td>$\gamma$ subunit</td>
<td><strong>LAMC2</strong></td>
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<td>Protein</td>
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<td>$\alpha_6$ integrin</td>
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<tr>
<td>$\beta_4$ integrin</td>
<td><em>ITGB4</em></td>
<td>HD-anchoring filament complexes</td>
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Epidermolysis Bullosa Acquisita
Type VII Collagen

[Diagram showing the structure of Type VII Collagen with labeled domains A, B, and C, and components NC1 and NC2.]
EBA: Passive Transfer Studies


Dystrophic Epidermolysis Bullosa

Dominant Dystrophic OMIM 131750, 131850, 131705

Recessive Dystrophic OMIM 226600
Emerging Therapies for Epidermolysis Bullosa

Administration of genetically modified fibroblasts.

Engraftment of ex vivo corrected keratinocytes.

Intradermal injection of allogeneic fibroblasts.

Bone marrow transfer can ameliorate disease in COL7A1 -/- mice.

Injection of recombinant type VII collagen corrects the disease phenotype in COL7A1 -/- mice.
Mol Ther 17:26-33, 2009.

Bone marrow transplantation for RDEB.
<table>
<thead>
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<th>Structural Target</th>
<th>Protein Target</th>
<th>Autoimmune Diseases</th>
<th>Genetic Diseases</th>
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<tr>
<td>Desmosomes</td>
<td>Dsg 1, 3, 4</td>
<td>PF, PV, PNP</td>
<td>Ectodermal dysplasias, skin fragility syndromes, altered epid differentiation</td>
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<tr>
<td></td>
<td>Dsc 3</td>
<td>PV, PNP</td>
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<tr>
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<td>Plakophilin I</td>
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<tr>
<td></td>
<td>Plakoglobin</td>
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<td>Desmoplakins</td>
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<tr>
<td>HD-anchoring</td>
<td>BPAG1 &amp; 2</td>
<td>BP, HG, MMP</td>
<td>AR-EB simplex</td>
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<tr>
<td>filament complexes</td>
<td>BPAG2</td>
<td>LAD</td>
<td>Junctional EB</td>
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<td>Laminin 332</td>
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<td>Junctional EB</td>
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<td>$\alpha_6\beta_4$ integrin</td>
<td>MMP</td>
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<tr>
<td>Anchoring fibrils</td>
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<td>EBA, BSLE</td>
<td>DDEB, RDEB</td>
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