Contributions on the Immunopathology of BP, PC and PF

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- AARD Scientific Advisory Board
- Fulltime employee: UNC School of Medicine
- Research supported by:
- No other disclosures
MILESTONES IN BULLOUS PEMPHIGOID (1970)

- Defining the BMZ & the site of injury
- Immunolocalization of BP autoantibodies
- Characterization of BP antigen(s)
- Pathogenicity of BP autoantibodies
- Mechanisms of autoantibody formation
- Etiology

- Detached epidermis
- Inflammatory cells

IgG & C3

IgG & C3 bound to the DEJ
BP autoantibodies bind Basal Cell Hemidesmosomes

AHA! Moment
BP staining depends on
BC membrane permeability

Immuno-EM
BP autoantibodies Recognize HDs

Indirect IF

Immuno-EM
BP antibody staining pattern depends on Cryosections

Milestones in BP Research

BP autoantibodies recognize Hemidesmosomal antigens

Mutasim et al, J Inv Dermatol 84:47-53, 1985
The Epidermal BP Antigens (by Immunoblotting)

BP230
Stanley (1981)

BP

BP180
Labib (1986)

Cell 24:897-903, 1981

J Immunol 136:1231-5, 1986
BP180 Antigen & Autoantibodies

G.J. Giudice

BP180 Mutation

Junctional EB (Blisters)

Giudice GJ & L.A. Diaz:

NH2

600

100

NC16A

1500

COOH

Basal Keratinocyte

Collagenous Domains

MCW-1 Epitope

BP180 Autosensitization

- Bullous Pemphigoid
- Herpes Gestationis
- Cicatricial Pemphigoid
- Linear IgA Dermatoses
- LP Pemphigoides

Diagnostic Markers

Labib, J Immunol 136:1231-5, 1986

31 Years (as of 2017)!

G.J. Giudice

BP180 Mutation

Junctional EB (Blisters)
BP180 ELISA

Herpes Gestationis
Milestones in Herpes gestationis Research

Herpes Gestationis sera recognize the BP180 antigen

J Clin Invest 81:2023-2026, 1988
COMPARISON OF HUMAN AND MURINE BP180

Z. Liu

RABBIT ANTI-MOUSE BP180 SERUM
The Bullous Pemphigoid Mouse Model
(Passive Transfer Anti-BP180 IgG)


IgG

C3
Milestones in Pemphigus Vulgaris

- As diagnostic markers
- In disease-pathogenesis
- Predictors of disease

Highlights: PV (Last 40 years)

A Civatte, (1943-France)

PV (Histology)

Jordon & Beutner, (1964-USA)

PV (Autoimmunity)


PV autoantibodies: The 70’

- Neonates from mothers with active PV may develop a transient PV neonatal
- Serum titers by IF roughly correlate with disease activity
- Removal of autoantibodies by plasmapheresis was beneficial
- PV Serum & IgG added to skin organ cultures induce acantholysis
Passive Transfer of Anti-epidermal Antibodies into Neonatal Mice

Testing the AHA Moment!

PV patient IgG

Circulating IgG
Tissue-bound IgG
Skin Lesions

G.J. Anhalt

Late 70’-Early 80’
Pemphigus Vulgaris
Animal Model

The Pemphigus Vulgaris Phenotypes

- Mucocutaneous PV: Dsg3 & Dsg1 Autoantibodies
- Mucosal PV: Dsg3 Autoantibodies

Expression of Dsg3 (mucosa & Epidermis): mPV

1. If hDsg3 is identical to mDsg3
   Human anti-Dsg3 antibodies should be pathogenic to mouse by passive transfer

2. If hDsg3 & mDsg3 are different
   Human anti-Dsg3 antibodies should not be pathogenic to mouse by passive transfer

Oral Mucosa

Epidermis

Human Mucosa Dsg3
Human Skin Dsg3
Murine Mucosa Dsg3
Murine Skin Dsg3
Mucosal PV - Current Studies

- **Generation of the hDsg3 Tg mouse**
  - Test expression of hDsg3 by IIF and IB
  - Passive transfer studies

- **Cross to Dsg3 knockout mouse** *
  - Test expression of hDsg3 by IIF and IB
  - Passive transfer studies

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* Koch and Stanley: J Cell Biology. 137:1091-1102, 1997

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D Culton
UNC Dermatology
IgG from mPV patients induce acantholysis in hDsg3Tg mucosa upon passive transfer.
Milestones in Pemphigus Foliaceus

- The PF/FS animal model (Roscoe)
- The antigens (Stanley)
- Endemic PF (Fogo Selvagem) (Diaz et al)

Vieira (1937)  Beutner, (1968)  

PF (Immunopathology)
Endemic Pemphigus Foliaceus (Fogo Selvagem)

Beutner, 1968

IgG

Dsg1

Vieira, 1937

Beutner, 1968
FS IgG4 Autoantibodies are Pathogenic

IgG4
Serun

B. Rock

Etiology of Fogo Selvagem

<table>
<thead>
<tr>
<th>Epidemiology</th>
<th>Serology</th>
<th>Immunogenetics</th>
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<tbody>
<tr>
<td>• Population at Risk</td>
<td>Autoantibody Response</td>
<td>HLA alleles</td>
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<tr>
<td>• Risk Factors</td>
<td>IgG</td>
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<td>IgM</td>
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Antigen Exposure

Normal Subject “at risk”

Incubation Time

FS Patient
Anti-Dsg1 Antibodies in Onchocerciasis, Leishmaniasis & Chagas


<table>
<thead>
<tr>
<th>Disease</th>
<th>FS/PF</th>
<th>Onchocerciasis</th>
<th>Leishmania</th>
<th>Chagas</th>
<th>SA-Blastomycosis</th>
<th>Leprosy</th>
<th>NHS</th>
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<tbody>
<tr>
<td>N^a</td>
<td>0/40</td>
<td>34/51</td>
<td>38/88</td>
<td>18/31</td>
<td>7/28</td>
<td>14/83</td>
<td>0/57</td>
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<tr>
<td>%</td>
<td>100</td>
<td>83</td>
<td>43</td>
<td>58</td>
<td>45</td>
<td>25</td>
<td>17</td>
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<td>95% CI</td>
<td>93-100</td>
<td>68-93</td>
<td>33-54</td>
<td>39-76</td>
<td>11-45</td>
<td>10-27</td>
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<td>P-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.32</td>
<td>0.80</td>
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</table>

Black fly (Simulid)  Kissing bug (Reduviid)  Sand Fly (Lutzomyia I)
IgG4 Anti-Dsg1 Antibodies

Hybridoma: IgG4 monoclonal abs

Phage Display: ScFv monoclonal abs

Sand Fly (Lutzomyia l)

Antigen Exposure

Normal Subject “at risk”

Incubation Time

FS Patient
Sand Fly Salivary Gland Antigens

- Valenzuela & Ribeiro, NIAID, Bethesda
- Xu et al, J Biol Chem, 286, 32383, 2011

Sand Fly (Lutzomyia l) → Salivary Glands → mRNA → cDNA Library → Homogenization in PBS → Recombinant Proteins → Soluble Antigens (SGLL)

Leishmaniasis is endemic in LV

- LJM11
- LJL17
- LJJ143
LJM11 is the major antigen of SCLL that are recognized by in FS sera.
The Environmental Etiology of FS

Strong experimental data supports the role of the sand fly antigen LJM11 as potential trigger of FS
Milestones on Pemphigus & Pemphigoid

1. 1950
   ♦ 100% mortality (PV)
   ♦ 10% spontaneous remission (endemic PF)
   <10% mortality

2. 1950
   Hench, introduce Steroids
   Rituximab & IVIg

   Vieira PF
   Civatte PV
   Lever BP
   Jordon-Beutner PV/PF/BP autoantibodies
   Anhalt et al PV Abs pathogenic
   Rituximab In PNP

FDA approval Rituximab lymphoma
CD20 identified Immunosuppressive drugs
Steroids RA
RA
PV/PF/BP