Canine and human Atopic Dermatitis: researching the skin barrier with a One Health approach

Anna De Benedetto, M.D.
Assistant Professor
Director Clinical Trial Unit
adebenedetto@ufl.edu
Anna De Benedetto
F018 – Comparative Dermatology: Cases at the Intersection of Human and Veterinary Dermatology and the One Health Paradigm

DISCLOSURES

Allokos, Consultant
Novartis, Clinical Trial

The Dermatology Foundation has supported & advanced my research.
Goals for the presentation:

- Overview of Atopic dermatitis (AD): comparing canine and human AD

- Updates on the role of skin barrier in the pathogenesis of AD: canine and human evidences
Comparative Dermatology Laboratory

- Dr. Marsella has developed an experimental model of atopic dermatitis in dogs at UF which has been instrumental in studies on pathogenesis and studies to rapidly screen drugs with potential to be effective treatment options for both dogs and people.

- Dr. Santoro’s researches focus on the characterization and interaction of host innate immunity (antimicrobial peptides) and cutaneous microbes in atopic dermatitis.

- Dr. De Benedetto’s research focuses on the characterization of tight junction (TJ) composition and function in atopic dermatitis. Her projects aim to find skin barrier-enhancing drugs and begin to understand their mechanisms of action.

Mission
To improve the health and well-being of people and animals suffering from allergic/inflammatory skin disease
Atopic Dermatitis (AD - eczema)

- AD a common chronic, relapsing, inflammatory skin disease (in both people and dogs).
- Characterized by genetic predisposition, skin barrier disruption & an aberrant immune response (e.g. Th2 polarized) to environmental allergens.
- Pruritus and cutaneous infections are major drivers of the reduced quality-of-life associated with this disease.
Humans and dogs naturally develop Atopic Dermatitis (AD).

Close similarity between human and canine AD at the:
- pathogenesis,
- clinical,
- epidemiological and therapeutic levels.
### Canine AD is a Homologue of Human AD.

<table>
<thead>
<tr>
<th>AD</th>
<th>Dogs</th>
<th>Humans</th>
</tr>
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<tbody>
<tr>
<td>Age of onset</td>
<td>Young adults (1–3 years of age)</td>
<td>60% Early onset (&lt;2 years of age)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20% adult onset</td>
</tr>
<tr>
<td>Course of disease</td>
<td>Chronic progressive</td>
<td>Chronic and relapsing progression</td>
</tr>
<tr>
<td>Presence of IgE</td>
<td>80% of cases</td>
<td>70–80% of cases</td>
</tr>
<tr>
<td>Prevalence</td>
<td>20–30%</td>
<td>10% of children and 4% of adults</td>
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<tr>
<td>Association with other diseases</td>
<td>Food allergy, flea allergy</td>
<td>Food allergy, asthma, allergic rhinitis</td>
</tr>
<tr>
<td>Staphylococcal infection/colonization</td>
<td>Extremely common</td>
<td>Extremely common</td>
</tr>
<tr>
<td>Itch</td>
<td>Pruritus without lesions at onset</td>
<td>“The itch that rashes”</td>
</tr>
<tr>
<td>Progression of atopic march</td>
<td>Extremely rare</td>
<td>Common</td>
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</tbody>
</table>
AD pathogenesis

- Genetic & Acquired factors
- Wide spectrum of phenotype

Adaptive Immunity

- Th2
- Th17
- Th1

Itch

- TARC
- Eos
- IgE

Skin Barrier

- FLG
- TJ
- Lipids
- proteases

Microbiome

- ↑

Innate Immunity

- NK
- PMN
- Nuocytes
- TLR2
- AMP
- TLR2
- EDC

UF Health Dermatology

GAINESVILLE | JACKSONVILLE
Skin Barrier Defects in AD - Clinical Clues

- ↑ Risk of cutaneous colonization and infection.
- Xerosis (skin appears dry).
- ↓ Irritancy threshold.
- Epidermis is thicker than normal (H&E).
- Emollients are key in AD management.
Syndromes with AD features:

- Netherton Syndrome  
  SPINK5/LEKTI
- Peeling Skin Type B  
  CORNEODESMOSIN
- Ichthyosis Prematurity Syndrome  
  FATP4
- Severe dermatitis, multiple allergies and metabolic wasting (SAM) syndrome  
  DSG1
Skin Barrier is impaired in AD lesional & nonlesional area

- ↑ Transepidermal water loss (TEWL)
- ↓ Skin hydration
- ↑ pH

Multi probe System MPA5 – Courage & Khazaka
Skin Barrier is impaired in canine AD lesional & nonlesional area
Skin barrier impairment at birth predicts food allergy at 2 years of age.

Part of birth cohort study in UK; 1,903 infants recruited from 2009-2011. TEWL measured at day 2, 2 and 6 months. At 2 yo infants had food allergy screening.

- Day 2 upper-quartile TEWL was a significant predictor factor for Food Allergy (FA) at age 2 years (OR 4.1; 95% CI, 1.5-4.8). 75% of kids with FA TEWL in the upper quartile.

- Neonatal skin barrier dysfunction predicts FA at age 2.

- This data support the hypothesis of transcutaneous allergen sensitization for FA even in infants who do NOT have AD
A multicenter, multinational, 2-arm parallel-group, assessor-blind, randomized (1:1) controlled pilot trial of 6 months' duration (124 neonates at high risk for AD).

A statistically significant protective effect was found with the use of daily emollient on the cumulative incidence of atopic dermatitis with a relative risk reduction of 50% (relative risk, 0.50; 95% CI, 0.28-0.9; P = .017).

An investigator-blinded, randomized, controlled, parallel-group study (118 high risk neonates).

Emulsion-type moisturizer was applied daily during the first 32 weeks of life.

Approximately 32% fewer neonates who received the moisturizer had AD/eczema by week 32 than control subjects (P = .012).
The epidermis functions as a primary defense and biosensor to the external environment.

Both Barriers are impaired in AD

Stratum Corneum
Stratum Granulosum
Stratum Spinosum
Stratum Basale

Modified from Science 2010;329:1154
## Barrier defects in AD

<table>
<thead>
<tr>
<th>Barrier defects</th>
<th>Dogs</th>
<th>Humans</th>
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<tbody>
<tr>
<td></td>
<td>• decreased <strong>ceramides</strong></td>
<td>• decreased <strong>ceramides</strong></td>
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<tr>
<td></td>
<td>• discontinuous <strong>lipid lamellae</strong></td>
<td>• discontinuous <strong>lipid lamellae</strong></td>
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</table>
|                | • abnormal **filaggrin** 1 and 2 expression | • abnormal expression of **epidermal differentiation complex** components (including reduced expression of **filaggrin** and **loricrin**)
|                | • changes in expression of **tight junction** proteins | • impaired **tight junction** function and reduced expression of key components (claudin-1, -4 and 23) |
|                | • increased transepidermal water loss (**TEWL**) | • increased transepidermal water loss (**TEWL**) |
|                | • no documented mutation linked to AD. | • **FLG** null mutations |

*Vet Sci. 2017 Jul 26;4(3).*
Epidermal Barrier Hypothesis in AD

Th2 (and Th22/Th17) inflammation

Modified from JACI 2011; 127
FLG mutations are a major predisposing factor for human AD

- Ichthyosis Vulgaris (1/250; ± AD)
- 10% European ancestry are FLG mutation carriers
- AD subjects (±FLG) outgrowth the disease

Peanut Allergy (OR 5.3)

AD (OR 3.1)

Asthma (overall risk)

No increased risk

Asthma in the absence of atopic dermatitis

Asthma + AD (OR 3.3)

NEJM 2011;365:1315

Allergy 2013:68:37-47
Filaggrin (FLG) null-mutations

NEJM 2011;365:1315

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GAINESVILLE | JACKSONVILLE
FLG deficiency

- Reduced skin hydration
- Increased pH
- No effect on TEWL

- Disorganized keratin filaments
- Impaired lamellar body loading
- Abnormal lamellar bilayers

NMF: natural moisturizing factor
Tissue inflammation (e.g. cytokines) reduces FLG expression in AD

- Th2 cytokines (IL4, IL13 & IL31)
- Th17/22 cytokines (IL22 & IL17A)
- TNFα, IL25
- Aryl hydrocarbon receptor
Alterations of keratins, involucrin and filaggrin gene expression in canine atopic dermatitis.

Theerawatnasirikul S¹, Sailasuta A, Thanawongnuwech R, Surivaphol G.

Characterization of canine filaggrin: gene structure and protein expression in dog skin.

Kanda S¹, Sasaki T, Shiohama A, Nishifuji K, Amagai M, Iwasaki T, Kudoh J.

Increased filaggrin-metabolizing enzyme activity in atopic skin: a pilot study using a canine model of atopic dermatitis.

Fanton N¹, Santoro P², Cornegliani L¹, Marsella R².
Second line of defense, Tight Junctions (TJ), is compromised in AD.

Stratum Granulosum (Tight Junctions)

TJ: ↓CLDN1, ↓CLDN23 & ↓CLDN4^; ↓Resistance & ↑Permeability

2nd barrier

Langerhans Cell

Modified from Science 2010;329:1154

JACI 2011;127:773 and ^ unpublished
Tight junctions (TJ): primary barrier to the diffusion of fluid, electrolytes, macromolecules and pathogens via the paracellular pathway.

(Figure from Nastech Pharmaceutical Co, Inc.)
Claudin-1 is the most abundant claudin in skin and plays a role in barrier function.

**CLDN1:**

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<table>
<thead>
<tr>
<th>µg/cm²/min</th>
<th>TEWL</th>
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<tr>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>20</td>
<td>30</td>
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Claudin-1 gene mutations in neonatal sclerosing cholangitis associated with ichthyosis (NISCH) and alopecia.

*Gastroenterology* 2005;128(2):524
Defects in Tight Junction Function

Transepithelial Electrical Resistance (TEER)

FITC-Albumin Permeability

Significant Tight Junction Defects in non-lesional hAD Epidermis

DOGS: CLAUDIN1

CLAUDIN1

Nonatopic

Atopic Dermatitis

↓ CLDN 23
↓ CLDN 4
Dose-dependent role of claudin-1 in vivo in orchestrating features of atopic dermatitis.
Tokumasu R¹, Yamaga K², Yamazaki Y³, Murota H⁴, Suzuki K¹, Tamura A¹, Bando K⁵, Furuta Y⁵, Katavama I⁴, Tsukita S⁶.

The tight junction gene Claudin-1 is associated with atopic dermatitis among Ethiopians.
Asad S¹, Winge MC², Wahlgren CF³, Bilcha KD⁴, Nordenskiod M⁵, Taylan F⁵, Bradley M⁵,³.

Claudin-1 polymorphism modifies the effect of mold exposure on the development of atopic dermatitis and production of IgE.
Yu HS¹, Kang MJ¹, Kwon JW², Lee SY³, Lee E⁴, Yang SJ⁴, Jung YH⁴, Hong K⁶, Kim YJ¹, Lee SH¹, Kim HJ¹, Kim HY⁶, Seo JH⁷, Kim BJ⁸, Kim HB⁹, Hong SJ¹⁰.

Profile of skin barrier proteins (filaggrin, claudins 1 and 4) and Th1/Th2/Th17 cytokines in adults with atopic dermatitis.
Batista DJ¹, Perez L¹, Orfali RL¹, Zaniboni MC¹, Samorano LP¹, Pereira NV², Sotto MN¹, Ishizaki AS¹, Oliveira LM², Sato MN², Aoki V¹.

The role of glutathione S-transferase and claudin-1 gene polymorphisms in contact sensitization: a cross-sectional study.
Ross-Hansen K¹, Linneberg A, Johansen JD, Hersoug LB, Brasch-Andersen C, Menné T, Thyssen JP.
- How can we modulate keratinocytes TJ function?
- If we repair TJ integrity in vivo can we improve AD?
  • Can we reduced flares? Can we prevent sensitization?

In vitro and in vivo studies
We hypothesized that activation of PAR2 plays a role in TJ epidermal barrier impairment observed in atopic skin. Goal of this study was to investigate in-vitro the effect of PAR2 activation on keratinocytes TJ function and composition.
Background: AD and PAR2

✓ Protease Activated Receptor 2 (PAR2) is a G-protein-coupled receptor activated by proteases (e.g. dust mites, bacterial toxins).

✓ Increased proteases activity has been shown in Atopic Dermatitis skin (Voegeli et al., 2009).

✓ Activation of PAR2 has been associated to inflammation, altered epidermal barrier function and pruritus (Derian et al., 1997; Seeliger et al., 2003; Lee et al., 2010; Joo et al., 2016).

✓ PAR2 activation induced impairment of epithelial Tight Junctions (TJ) function; however, this had not yet been investigated in primary human keratinocytes (Wan et al., 1999; Kirschner et al., 2010; Lambrecht and Hammad, 2012).
PAR2 Activation Reduced Tight Junction Integrity

PAR2 = Agonist peptide SLIGKV-NH₂
REV = reverse peptide (control)

↓ TEER = ↓ TJ integrity
↑ Permeability = ↓ TJ integrity

PAR2 activation reduced and disrupted
Occludin and Claudin-1 membrane staining pattern
PAR2 activation reduced and disrupted Occludin/OCLD membrane staining pattern

Confocal Microscope: Nikon A1RMPsi at 20x;
Cell & Tissue Analysis Core – UF; NIH Grant # 1S10OD020026
PAR2 activation in canine AD

Effects of PAR2 antagonist on inflammatory signals and tight junction expression in protease-activated canine primary epithelial keratinocytes.

Kim HJ, Jeong SK, Hong SJ, Ahrens K, Marsella R.
PMID: 27306682
Similar articles

Regulation of epithelial cell tight junctions by protease-activated receptor 2.

Enjoji S, Ohama T, Sato K.
PMID: 24881651 Free PMC Article
Similar articles
Summary

- **PAR2** might contribute to epidermal barrier impairment of atopic skin (*dogs and people*), by reducing Claudin-1 expression and compromising TJ integrity.

- A strategy to selectively **block PAR2 downstream** signaling could result in much needed therapeutic interventions for AD in both *dogs and people* by targeting both barrier and inflammation/itch.
Conclusions

✓ The Skin is armed with **two** epidermal barrier structures:
  - Stratum Corneum (e.g. FLG, lipids)
  - Tight Junctions (e.g. CLDN1, OCL, ZO-1)

✓ Both barrier are damaged in AD subjects on genetic or/and acquired base.

*Treatments that repair the skin barrier function may prevent or stop the vicious cycle between epidermal barrier impairment and abnormal immune system in AD in both people and dogs.*
Section of Comparative Medicine.
President—Sir D’Arcy Power, F.R.C.S.

[October 26, 1927.]

What is Comparative Medicine?
By O. Charnock Bradley, D.Sc., M.R.C.V.S.

Abstract.—Inasmuch as it includes the study of disease in a considerable number of animals belonging to widely different species, there is some ground for regarding veterinary medicine as being comparative medicine. But this is held to be too narrow an application of the term.

There is better reason for the contention that human and veterinary medicine together compose comparative medicine. Notwithstanding marked differences between some of the diseases of man and those of the lower animals, the similarities and resemblances are much more numerous. Human and veterinary medicine are confronted with similar problems and employ similar means for their solution; and, taken together, they deal with a large group of animals sufficient to justify the contention that they are two branches of one medicine. But an even wider and more comprehensive conception of comparative medicine is suggested. It is held to embrace the study of disease processes in all animals (and possibly in plants also), in all conditions, and with the help of all available means. Its corpus contains elements that have been contributed, and are being contributed, from widely different sources. The physicist, the chemist, the physiologist, and others make discoveries that are susceptible of incorporation; and thus is accumulated a store of linked facts from which practitioners of human and veterinary medicine take what they need, and taking, give.

Saving Lives By Taking A One Health Approach

Connecting human, animal, and environmental health

More than half of all infections that people can get can be spread by animals. Diseases like rabies, Salmonella, and West Nile virus infections are examples of zoonotic diseases (or zoonoses)—diseases that can be shared between animals and people.
Thank you for your Attention!

adebenedetto@ufl.edu