NEASE GRANT: FINAL REPORT

"**Characterization of Innate Immune Response Pathways to *Staphylococcus Aureus* in Keratinocytes from Atopic Dermatitis Patients**"

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Over the past year working on *Characterization of Innate Immune Response Pathways to Staphylococcus Aureus in Keratinocytes from Atopic Dermatitis Patients,* we confirmed some of our preliminary findings and we had the opportunity to work on new assay to better characterize the skin barrier impairment in Atopic Dermatitis (AD) subjects. Several line of evidences have identified in the impairment of the skin barrier function a key feature of AD pathogenesis. It is largely believed that a defect in skin barrier increases the susceptibility to microbes colonization, as well as penetration of allergens and toxins into the skin.

A high-throughput expression profiling study was performed on nonlesional epithelium (from skin blister roofs) of AD subjects (n=5) and nonatopic, healthy controls (NA; n=5). These profiles were generated using Illumina's Sentrix HumanRef-8 Expression BeadChips (>23,000 sequences) and analyzed using Gene Set Matrix Analysis. Although several gene clusters were , we decided to analyze all genes related to skin barrier function. Interestingly, the genes found in the Epidermal Differentiation Complex (EDC) were quite uniformly dysregulated. This region codes for proteins found in the stratum corneum, thought to be crucial for cutaneous barrier function. AD subjects had greater mRNA expression of *S100A* proteins (A8, A7, A9) and *Small Proline-Rich Proteins* (*SPRR1B, 2A*) compared to NA. In contrast, several *Late Cornified Envelope Proteins* (*LCE2B,2D,1B*) and *Loricrin* were downregulated in AD compared to NA. We have confirmed the dysregulation of some of these EDC genes at the protein level. Among the EDC genes, recently a particular interested has been direct on Filaggrin, which is a key protein in the stratum corneum. Its' function is critical for barrier function. Interestingly, several studies have shown that loss-of-function *FLG* mutations (i.e. lack of the protein expression in the skin) are associated with extrinsic Atopic Dermatitis (AD), early onset of eczema that persists into adulthood, and the coexistence of atopic asthma and AD. We

evaluate the Filaggrin expression in lesional and non-lesional skin samples from AD and non atopic controls. Preliminary data from ours and other laboratory (Leung Y D, Denver, CA) show defects in filaggrin expression in normal appearing (e.g. nonlesional) skin of AD. These findings suggest that local factors (namely, IL-4 and IL-13) might affected the expression of EDC proteins, and we can speculate that the filaggrin defect in AD could be also an acquired feature (Howell MD et al, 2007). Furthermore, we investigated whether mutations in *FLG* would have a direct impact on barrier function in other mucosal surfaces such as upper airway, oral mucosa and esophagus. Results from our study suggest that filaggrin is expressed in skin and oral mucosa only and is not expressed in upper or lower airway and esophageal epithelium and therefore is unlikely to play a direct role in barrier function at these mucosal surfaces (De Benedetto et al, 2008). We hypothesized that the skin is a key portal of entry for relevant allergens and irritants that can drive both skin and airway disease.

Additionally besides the stratum corneum integrity other structures have been shown to be critical for an effective barrier. Growing body of evidence suggests that intercellular junctions such as desmosomes, gap, adherens and tight junctions provide epithelial cells with mechanical support, polarity and form a primary barrier to the diffusion of fluid, electrolytes, macromolecules and pathogens. Several proteins involved in cell-to-cell junctions (adherens, gap and tight junctions) were indeed dysregulated in our arrays. Tight junctions (TJ) are lateral-apical intercellular connections observed between keratinocytes. Several study have shown a critical role of TJ in the regulation of epithelial permeability through the paracellular route. A defect in TJs would favor loss of water from inside to outside the skin as well as penetration of microbes, toxins and allergens into the skin. At the mRNA level our array data showed downregulation of *Claudin-1* and *23*, while *GJB2* (Connexin 26) was overexpressed in AD compared NA. We confirmed the downregulation of Claudin-1 in nonlesional AD skin biopsy compared to NA at the protein level. Interestingly, we noticed a Claudin-1 upregulation in lesional biopsies from AD subjects. These preliminary findings suggested that local factors can modulate Claudin-1 expression in keratinocytes. We then performed experiments (Western Blot and PCR) on primary human keratinocytes isolated from foreskin (PHFK) to evaluate

kinetic and modulation of intercellular junctions protein expression (such as Claudin-1, Occludin, ZO-1 and E-cadherin). Our preliminary results show that PHFK express Claudin-1 and Occludinonly when cultured in high Ca^{+2} media and the expression of Claudin-1 was upregulated after IL-4 and IL-13 stimulation. We also investigate the effect of bacteria components on TJ proteins expression, and found that Claudin-1 is upregulated after PGN (peptidoglycan, a component of gram positive bacteria wall [i.e. *S. aureus]*) stimulation. To investigate the functional role played by TJ in skin we performed experiments using Trans Electrical Epithelial Resistance (TEER) measurement, and FITC-dextran flux. These assays have been largely used by other groups to study epithelial resistance and ion transport in airway and intestinal epithelium. We evaluated barrier function in keratinocytes after inflammatory or microbial stimuli. Moreover, to better clarify the role played by Claudin-1 within the TJ function we performed Claudin-1 knockdown experiments using silent RNA technique. Our preliminary results show a reduction in TEER that is dose-dependent , suggesting a central role played by Claudin-1 in keratinocytes barrier function.

All together, our study suggest that an effective barrier in pseudostratified squamous epithelium may be determined by the relative expression of proteins important for formation of the cornified envelope (e.g. Filaggrin) and TJs. The barrier defect that results from dysregulation of these proteins probably results in greater penetration of allergens, irritants and microbes. We believe the compensatory increase in Claudin-1 or Filagrin observed in lesional AD skin may be due to the local actions of Th2 cytokines, allergen exposure or staphylococcal colonization. We believe what is more important is the study of barrier function in unaffected skin as this represents the baseline state - before allergens induce clinically obvious disease,

Drs. De Benedetto and Beck will continue to work on this project to better investigate the mechanisms that induce barrier defects in atopic dermatitis subjects.

Meeting presentation and publication:

De Benedetto A, McGirt LY, Brummet M, Cheadle C, Barnes KC, Beck LA. Altered Expression of Epidermal Differentiation Proteins in Atopic Dermatitis compared to Psoriasis or Nonatopic, Healthy Controls. The Journal of Allergy and Clinical Immunology January 2007 (Vol. 119, Issue 1 (Supplement), Page S280)

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Howell MD, Kim BE, Gao P, Grant AV, Boguniewicz M, Debenedetto A, Schneider L, Beck LA, Barnes KC, Leung DY.Cytokine modulation of atopic dermatitis filaggrin skin expression. J Allergy Clin Immunol. 2007 July;120 (1):150-5

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