

**2012 NEA Research Grant: “*Identifying pathways that enhance epidermal barrier integrity as new treatment approaches for Atopic Dermatitis*”.**

**Final Report, July 2013 - Anna De Benedetto, MD  
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Atopic Dermatitis (AD) is the most common inflammatory disorder of the skin, affecting up to 15 million Americans. AD is characterized by development of an immune response against otherwise innocuous environmental allergens. It is now widely accepted that skin barrier impairment is an essential feature of AD and allergic diseases in general. Dr. De Benedetto’s research is focused on characterizing the composition and function of epidermal tight junctions (TJ) in atopic dermatitis (AD). TJ are localized below the stratum corneum (outmost layer of the skin) and provide the skin with an additional barrier structure. TJs function as the “gate” for passage of small or even large molecules (ions and allergens). Our group recently demonstrated a TJ defect in the skin of AD subjects. Dr. De Benedetto’s NEA grant project focused on identifying skin barrier-enhancing drugs and beginning to understand their mechanism of action. The overriding hypothesis was that repairing the epidermal barrier defect may be effective approach to prevent and/or treat AD. We focused on drugs affecting two major pathways potentially relevant for barrier and these include drugs that activate the PPAR $\alpha$  and  $\gamma$  nuclear receptors and drugs that block histamine. The most novel observations were observed in the histamine blockade experiments. We observed that histamine, which is often found in high concentrations in AD lesions, significantly reduced epidermal TJ barrier function. Intriguingly, two recently published papers demonstrated that histamine also reduced expression of key stratum corneum barrier proteins (e.g. loricrin, filaggrin) (Allergy. 2013 Jan;68(1):37-47; J Invest Dermatol. 2013 Feb;133(2):469-78). Our studies further demonstrated that a compound that selectively blocks one of the Histamine receptor, namely H4R (JNJ7777120), could block the histamine-mediated inhibition of TJ function. More studies are underway to determine the H4R downstream signaling pathways that regulate TJ function. Blockade of histamine

induced barrier disruption may be an effective disease modification approach for AD subjects. Relatively selective H4R blockers are currently in various stages of development for a number of human conditions and therefore the possibility that these could be used to treat AD in the near future is quite promising.

Another aspect of Dr. De Benedetto's research focused on investigating the mechanisms that regulate expression and function of TJ in AD. We hypothesized that the TJ dysfunction observed in AD develops in response to the inflammatory cytokines found within AD lesions. Studies performed during this granting period demonstrate that the inflammatory cytokines (IL4, IL13 and IL17A) have divergent effects on TJ function and protein expression. IL17A induced a dose-dependent enhancement of TJ function and it also enhanced the expression of a key TJ component, Claudin-4. On the other hand, IL4, a crucial cytokine in AD development, blocked the IL17A-induced enhanced TJ integrity and prevented the enhancement in Claudin-4 expression. Importantly, Dr. De Benedetto was able to demonstrate for the first time, that Claudin-4 expression was reduced in AD skin as compared to controls. Furthermore, Dr. De Benedetto initiated an investigation of the mechanisms that regulate cytokines induced TJ function. We found that JAK1 or 3 signaling pathways mediate the barrier-enhancing effects of IL17A in human epidermis. A major target of JAK signaling is the phosphorylation and nuclear translocation of Signal Transducer and Activator of Transcription 3 (STAT3). IL17A enhanced STAT3 activation in the skin. Importantly, we found that IL4 reduced STAT3 phosphorylation and nuclear translocation both at baseline and in response to IL17A treatment, suggesting that IL4's attenuation of the IL17A barrier-enhancing effects is mediated by the JAK-STAT3 pathway. Importantly, currently JAK inhibitors have been tested for other skin disease.

Understanding what effects different cytokines have on TJ integrity and the mechanism(s) responsible for these actions is critical to develop new AD therapies aimed at barrier repair.

Dr. De Benedetto will continue to work on this project to clarify the mechanisms that induce TJ barrier defects and identify new therapeutics for AD subjects.

**Publications and Abstracts** obtained in 2012 with NEA support:

Kuo IH, Carpenter-Mendini A, Yoshida T, McGirt LY, Ivanov A, Barnes K, Gallo R, Borkowski A, Yamasaki K, Leung DY, Georas SN, **De Benedetto A**, Beck LA. Activation of epidermal toll-like receptor 2 enhances tight junction function – Implications for atopic dermatitis and skin barrier repair. J Invest Dermatol, 2012 Dec. PMID 23223142

Kuo IH, Yoshida Y, **De Benedetto A**, Beck LA. The cutaneous innate immune responses in atopic dermatitis. J Allergy Clin Immunol. 2013; Feb;131(2):266-78. PMID 23374259

**De Benedetto A**, Yoshida T, Kuo IH, Rafaels N, Boguniewicz M, Hata T, Schneider L, Hanifin J, Gallo R, Leung DY, Barnes KC, Beck LA. Connexin-26 is upregulated in atopic dermatitis. J Invest Dermatol. 2012 May, Vol. 132 (Issue S1):S52

**De Benedetto A**, Yoshida T, Kuo IH, Ivanov A, Beck L. Cytokines Milieu in Atopic Dermatitis Modulate Epidermal Tight Junctions Functions. TJ symposium, 1-4 Nov, 2012, Merida (Mexico)

**De Benedetto A**, Yoshida T, Kuo IH, Gestios S, Ivanov A, Leung DY, Beck L. Antagonistic effects of IL17A and IL4 on epidermal tight junctions. IID 2013 (accepted for poster presentation)

**Awards for 2012:**

**Center for Medical Countermeasures against Radiation – University of Rochester Pilot Project** (Principal Investigator).

**Dermatology Foundation Research Grant** (Principal Investigator).

**La Roche-Posay Foundation, 2013 Research Grant** (Principal Investigator).

**Dermatology Foundation, 2013 Research Career Development Award.**